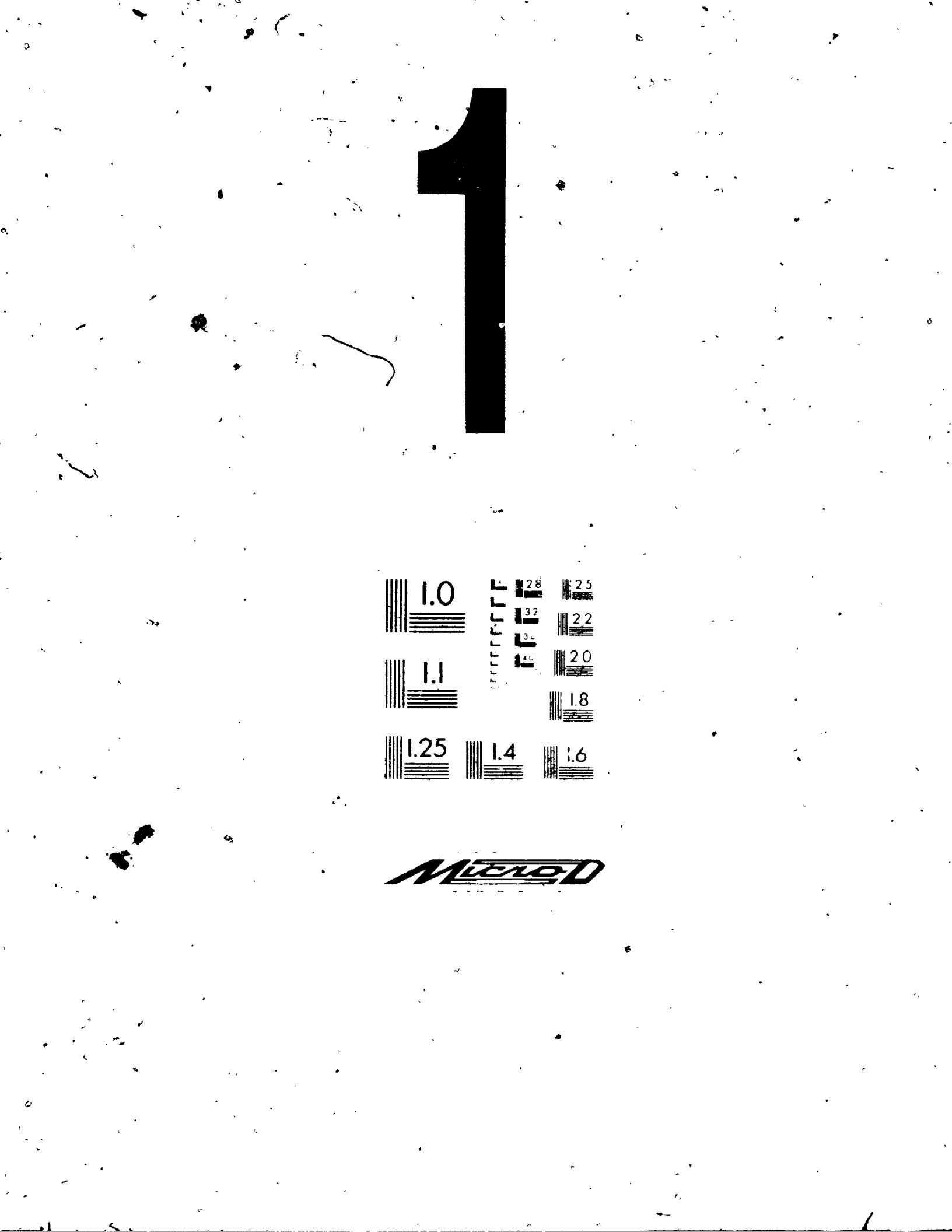
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dramatically reduced (See Exton et al, 1972). Replacement of a minimal amount of CORT in ADX rats reinstates the glycogenolytic effects of E and glucagon.

Recently, the effect of CORT on adrenergic-stimulated glycogenolysis has been further characterized. In the normal rat, the glycogenolytic effects of E in liver cells in vitro seem to be mediated mainly by stimulation of alpha-1 adrenergic receptors (eg. Chan & Exton, 1978; Sherline, Lynch & Glinsmann, 1972; Tolbert & Fain, 1974). However, in cells from ADX rats the glycogenolytic effect of alphal-adrenergic stimulation is reduced while the effect of beta-adrenergic stimulation is enhanced (Chan, Blackmore, Steiner & Exton, 1979). It has also been demonstrated that in the absence of CORT the alpha-1 adrenergic stimulation of hepatic glycogenolysis is shifted to a different pathway (eg. Hernandez-Sotomayor & Garcia-Sainz, 1984; Pushpendran, Corvera & Garcia-Sainz, 1984). Furthermore, it has been suggested that CORT may affect hepatic glycogenolysis in opposite ways depending on the time following administration of this steroid (Bialik & Roberts, 1985; Fleig et al, 1984). These issues are dealt with in more detail in the introduction and discussion for Experiments 2, 4, 5 and 6.

Neural vs Hormonal Control of Blood Glucose

Control of BG levels can occur by local (i.e. peripheral) mechanisms as well as by the central nervous system (CNS). The influence by the CNS may be by direct innervation of the adrenals, liver or pancreas or indirectly by controlling the release of hormones from the pituitary gland and/or adrenal cortex (eg. ACTH and CORT). It has been suggested

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ABSTRACT

This thesis represents an initial attempt to relate changes in glucose levels in the blood to a change in a very basic and important function of the brain, i.e. the ability to store and/or process information.

This work stems from previous studies which indicated that manipulation of brain norepinephrine and adrenal hormones (epinephrine and corticosterone) could modify the ability of rats to learn and/or remember an aversively-motivated, conditioned response. A number of physiological processes could be affected by alterations in the release of adrenal hormones or brain norepinephrine. The goal of the present work was to assess whether alterations in blood glucose levels might account for effects on avoidance behavior caused by manipulation of adrenal hormones and/or brain norepinephrine systems.

In a series of experiments it was demonstrated that depletion of brain norepinephrine could influence blood glucose levels, however, contrary to published views, it was established that central norepinephrine is not necessary for a stress-induced increase in blood glucose levels.

Another series of experiments confirmed that adrenal corticosterone was necessary for the blood glucose response to a very low dose of epinephrine. However, the role of corticosterone in the blood glucose response to a variety of stressors was, at most,

modulatory in nature.

On the other hand, it was clearly demonstrated that the adrenal medulla was required for the increase in blood glucose levels following several stressors, including footshock similar to that used in our aversively-motivated, learning paradigm. The increase in blood glucose levels, caused by footshock stress and by exogenous administration of E, was blocked by alpha-2 adrenergic receptor antagonists (phentolamine and yohimbine). Taken together, these data indicated that the stress-induced increase in blood glucose levels is mediated by an action of adrenal epinephrine on alpha-2 adrenergic receptors.

Finally, it was demonstrated that posttraining administration of glucose (by intraperitoneal injection) could enhance retention performance in an aversively-motivated discrimination task. Removal of the adrenal medullae (demedullation), which would eliminate the normal increase in blood glucose levels caused by the conditioning paradigm, caused a deficit in acquisition of the discrimination response. A very important finding was that posttraining administration of glucose enhanced retention performance in the demedullated rats.

It was concluded that the normal increase in BG levels during and following training in a shock-motivated discrimination task is an important physiological response which influences acquisition and retention performance.

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INTRODUCTION

Overview

The central issue in this thesis concerns physiological variables that influence information processing. Specifically, it is concerned with the involvement of the Hypothalamo-Pituitary-Adrenal (HPA) system and brain norepinephrine (NE) systems in modifying the acquisition and retention of aversively-motivated behaviors.

There is abundant evidence to indicate that hormones from the HPA system can alter the acquisition and retention of aversively-motivated behaviors (eg. de Wied, 1964; 1966; Martinez, Jensen & McGaugh, 1981; Sandman, Beckwith & Kastin, 1980) and a huge literature implicates central and peripheral adrenergic systems as important modulators of learning and memory processes (eg. Anlezark, Crow & Greenway, 1973; Stein, Belluzi & Wise, 1975; McGaugh, 1985; Gold, McCarty & Steinberg, 1982). For example, NE has been shown to reverse experimentally induced amnesia when administered directly into the brain (Stein et al, 1975) and NE or Epinephrine (E) have similar effects when injected systemically (McGaugh et al, 1982). Furthermore, hormones from the HPA system may interact with adrenergic systems to alter behavior. Initial studies indicated that the ability of rats to acquire a shuttlebox avoidance response is impaired by manipulation of the HPA system in combination with depletion of brain NE (eg. Ogren & Fuxe, 1974; 1977; Ogren, Archer, Fuxe & Eneroth, 1981; Roberts & Fibiger, 1977). However, other evidence indicated that the avoidance deficit is observed only

under certain circumstances (Wendlandt & File, 1979; Bialik, Pappas & Roberts, 1984a,b). Therefore, it is not clear from these studies exactly what aspects of the HPA system and brain NE systems were most intimately related to the change in behavior.

Glucose as a Potential Modulator of Conditioned Behavior

A number of physiological processes could be affected by manipulation of the HPA and/or NE systems, some of which could potentially influence avoidance behavior. The goal of the present work was to develop a framework within which to examine the extent to which a single physiological mechanism, alterations in blood glucose (BG) levels, can account for effects on avoidance behavior caused by manipulation of the HPA and/or brain NE systems.

An important finding that directed our research was that insults to the HPA and brain NE systems caused more severe impairments when testing was carried out in the dark rather than in the light phase of the animal's diurnal cycle (Bialik et al, 1984b). Rats, being nocturnal animals, are more metabolically active during the dark part of their cycle. Therefore, the behavioral deficit that was observed during the dark phase of the rats cycle might have been caused by an inability of the rats to mobilize enough stored energy (in the form of glucose, see pages 4-8) in order to supply the brain during a time of increased metabolic activity. Metabolic activity would be increased due to the phase of the diurnal cycle in which testing occurred and the additional

energy demands induced by the testing procedure. This formulation of the problem suggests that the control of BG level is the physiological variable that might be most closely related to avoidance performance. This suggestion led us to investigate further the presumed roles of adrenal hormones and brain NE in controlling levels of glucose in the blood.

Further investigations confirmed that, in rats, the increase in BG following brief footshock stress was mediated by release of E from the adrenal medulla (Bialik & Roberts, 1985). Our data also indicated that corticosterone (CORT) might function to dampen the increase in BG, since blockade of the footshock-induced increase in plasma CORT enhanced the rise in BG following brief footshock stress (Bialik & Roberts, 1985). We also found that lesions of central NE systems altered BG responses in some situations. Rats that were depleted of forebrain NE (by neonatal treatment with 6-hydroxydopamine) showed a more rapid increase in BG following footshock stress than sham lesioned rats (unpublished observations) and animals depleted of brain NE in adulthood showed an altered BG response to chronic dexamethasone treatment compared to intact controls (Bialik, Stevenson & Roberts, 1986). Furthermore, and possibly more importantly, brain NE is involved in mobilizing glucose from glycogen that is stored in cerebral tissue (Quach, Rose & Schwartz, 1978; Magistretti, Morrison, Shoemaker & Bloom, 1983), and CORT can influence this effect of NE (Roberts, Bialik, Back, Shoemaker & Magistretti, in preparation).

Taken together, these data led us to suggest that the combination

of impaired liberation of peripheral and central glycogen stores (caused by the lack of adrenal E and brain NE, respectively) would be expected to result in a greater behavioral disruption than either manipulation alone. Furthermore, manipulation of adrenal CORT might influence the behavioral effects of disruption of adrenergic systems by its influence on the liberation of peripheral and central glycogen stores.

Recent evidence has shown that moderate alterations in circulating glucose levels can affect the behavior of experimental animals and humans. Administration of glucose following classical conditioning trials enhances retention performance in rats (Messier & White, 1984; Messier, Blackburn & White, 1984) and posttraining administration of glucose enhances retention of an inhibitory avoidance response in rats (Hall & Gold, 1985; Gold, 1986). Conversely, insulin-induced hypoglycemia has been reported to impair T-maze learning in rats (Clayson, 1971), and moderate changes in BG levels have been related to some alterations in cognitive functioning in diabetic patients (Holmes, Hayford, Gonzalez & Weydort, 1983).

Our studies indicated that after several days of treatment with dexamethasone (DEX), rats develop severe hyperglycemia and are impaired in the acquisition of an active avoidance response. Surprisingly, both of these effects of DEX are attenuated by lesions of central NE systems (Bialik et al, 1986; Bialik et al, 1984a).

In summary, manipulation of adrenal hormones and brain NE can affect the supply of glucose to the brain from both peripheral and central stores, and these effects may have important implications for

the control of some aversively-motivated behaviors. The present research project focuses on the involvement of adrenal hormones and brain NE in the mobilization of energy stores in the periphery, as well as behavioral consequences of changes in BG levels.

The majority of the experiments (Experiments 1-9) relate to the involvement of adrenal hormones and brain NE in altering BG levels in response to various challenges. These experiments are expected to yield information concerning the supply of glucose to the brain from the blood. The final two experiments demonstrate that manipulation of BG levels alters the acquisition and retention of an aversively-motivated "Y-maze" task (Experiments 10 and 11). [Studies will be reported elsewhere which investigate the involvement of adrenal hormones and brain NE in the mobilization of glucose from glycogen stores in the brain, as well as possible behavioral consequences of these effects.]

The background information and rationale for these experiments follow.

Control of Blood Glucose

The importance of glucose for a biological organism is indicated by the fact that the greatest proportion of the chemical reactions in cells are concerned with making the energy in foods into glucose so as to be available to the various machinery of the cell (eg. See Guyton, 1976). There is little doubt that the central nervous system controls the

selection and ingestion of macronutrients (fat, protein and carbohydrate). Furthermore, it is likely that some of the brain nuclei and neurochemicals that are involved in controlling macronutrient intake are also involved in controlling the liberation of glucose from endogenous stores of protein, fat and carbohydrate. Although it is very likely that the brain's control of BG levels will not be completely understood without considering the organism's macronutrient intake, and vice versa, the present thesis is limited to discussing the mobilization of glucose from endogenous sources. An important issue though, which will be considered later, is the effect of withholding all food (i.e. fasting) on the BG response to a stressor (See Experiments 3d & 3e).

Storage of glucose as glycogen occurs mainly in the liver and muscle, with the liver being the dominant organ (5-8% of organ weight can be made up of stored glycogen). When glycogen is broken down into glucose (i.e. glycogenolysis) in the liver it passes immediately into the general circulation to be used by the cells of the body. Glucose is released by liver cells but not by most other cells (including brain cells) because in the liver the metabolic product of glycogen, glucose-6-phosphate, is converted to glucose by a phosphatase. Glucose-6-phosphate cannot pass through the cell membrane while glucose can move relatively freely through the cell membrane and into the bloodstream (eg. Guyton, 1976). Thus, it is thought that it is the process of glycogenolysis in the liver that allows the organism to quickly supply the cells of the body with adequate glucose to perform their various functions (See the discussion for Experiment 3d though).

There is currently some debate concerning the relative contributions that adrenal hormone and direct neural stimulation of the liver have in mediating the rapid changes in BG levels that occur following the application of various stressors. However, before discussing these issues, the basic processes involved in glycogen storage and breakdown are described.

Gluconeogenesis and Glycogenolysis

There are two main ways in which BG levels can be elevated from endogenous stores. One way is through the liberation of glucose from glycogen stored in the liver, and to a lesser extent in other tissues (glycogenolysis). The other way is through the synthesis of glucose from metabolites of fat (eg. glycerol) and protein (amino acids), a process called gluconeogenesis.

Gluconeogenesis. Gluconeogenesis is the name of the process responsible for the synthesis of glucose from lactate, pyruvate, glycerol and some amino acids. The only organs capable of carrying out gluconeogenesis are the liver and kidney, with the liver being the more important of the two. Regulation of gluconeogenesis can occur directly, by influences on liver enzymes, or indirectly, by influences on the availability of substrates. The predominant action of glucocorticoids is thought to be on the liberation of substrates (eg. fatty acids and amino acids) from peripheral tissues (lymph, skin and adipose tissue) for use by the liver in the process of gluconeogenesis (eg. Long, Katzin & Frey, 1940; Munck, 1971). Presumably, the fat and protein wasting and the

resulting hyperglycemia seen in rats receiving chronic dexamethasone treatment (See Experiments 7 and 8) is largely a result of the increased catabolism of peripheral tissues caused by this glucocorticoid.

Glycogenolysis. Glycogenolysis is the process by which glycogen is broken down to yield glucose molecules. The liver is the organ which possesses the greatest stores of glycogen and when glucose molecules are liberated from glycogen in the liver they immediately pass out of the cell. This process of glycogenolysis in the liver allows the organism to quickly supply the cells of the body with adequate glucose to perform their various functions. Most of the experiments discussed in this paper are concerned with the rapid rise in BG caused by a stimulus, and therefore, presumeably relate to the release of glucose from glycogen stores in the liver.

Liver (i.e. hepatic) glycogenolysis is thought to be controlled by direct neural innervation and by circulating hormones. The hormones that are considered to play the most important roles are E, glucocorticoids (CORT, being the major glucocorticoid in the rat), glucagon and insulin. Insulin promotes glycogen synthesis by stimulating synthetase activity and blocking the effects of E, while glucagon and E promote glycogenolysis by stimulating the conversion of phosphorylase b to phosphorylase a (Exton, Friedman, Wong, Brineaux, Corbin & Park, 1972; Fleig, Noether-Fleig, Roeben & Ditschuneit, 1984).

The effect of CORT has traditionally been thought to be permissive. That is, CORT alone has no affect on glycogenolysis, however, in the absence of CORT the glycogenolytic effects of E and glucagon are

dramatically reduced (See Exton et al, 1972). Replacement of a minimal amount of CORT in ADX rats reinstates the glycogenolytic effects of E and glucagon.

Recently, the effect of CORT on adrenergic-stimulated glycogenolysis has been further characterized. In the normal rat, the glycogenolytic effects of E in liver cells in vitro seem to be mediated mainly by stimulation of alpha-1 adrenergic receptors (eg. Chan & Exton, 1978; Sherline, Lynch & Glinsmann, 1972; Tolbert & Fain, 1974). However, in cells from ADX rats the glycogenolytic effect of alphal-adrenergic stimulation is reduced while the effect of beta-adrenergic stimulation is enhanced (Chan, Blackmore, Steiner & Exton, 1979). It has also been demonstrated that in the absence of CORT the alpha-1 adrenergic stimulation of hepatic glycogenolysis is shifted to a different pathway (eg. Hernandez-Sotomayor & Garcia-Sainz, 1984; Pushpendran, Corvera & Garcia-Sainz, 1984). Furthermore, it has been suggested that CORT may affect hepatic glycogenolysis in opposite ways depending on the time following administration of this steroid (Bialik & Roberts, 1985; Fleig et al, 1984). These issues are dealt with in more detail in the introduction and discussion for Experiments 2, 4, 5 and 6.

Neural vs Hormonal Control of Blood Glucose

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that hepatic neural regulation of BG (i.e. direct innervation of the liver) is fine-tuned and rapid and therefore suitable for emergency situations while hormonal regulation (eg. innervation of the pituitary, pancreas and adrenal medulla) is more stable and suitable for modifying neural regulation (eg. Shimazu, 1981). This hypothesis is challenged later.

With respect to the control of BG levels by the CNS, many investigators believe that the ventral medial (VMH) and lateral hypothalamus (LH) act in a reciprocal manner in the regulation of carbohydrate metabolism (eg. Ban, 1975; Shimazu, 1981). The VMH is considered to be part of the sympathetic nervous system and through connections with the splanchnic nerve, stimulation of this nucleus increases BG levels and decreases liver glycogen content (Shimazu, Fukuda & Ban, 1966), presumeably by a direct action of stimulating phosphorylase a activity in the liver (Shimazu, Matsushita & Ishikawa, 1978). The LH, on the other hand, is considered part of the parasympathetic system (Shimazu et al, 1966) and through connections with the vagus nerve, stimulation of the LH causes an increase in synthetase 1 activity in the liver (Shimazu et al, 1978) which increases glycogen synthesis and less glucose would be available for release into the circulation.

Recently, however, this reciprocal relationship between the VMH and LH in the control of BG has been called into question. Atrens, Sinden, Penicaud, Louis-Sylvestre and LeMagnen (1984) have found that there is no clear anatomical distinction between medial and lateral hypothalamic

sites from which electrical stimulation produces increases or decreases in BG levels. Furthermore, there was a tendency to find more sites which caused increases in BG in ventral-lateral areas of the hypothalamus than in ventral-medial areas.

These authors also demonstrated that stimulation of both the VMH and LH increased energy utilization, as measured by oxygen consumption and respiratory quotient, and the VMH had the greater effect (Atrens, Sinden, Penicaud, Devos & Le Magnen, 1985). This increase in metabolic rate should also be reflected in a more rapid disappearance of glucose from the blood. The involvement of the adrenal medulla in the BG response to electrical stimulation of the hypothalamus is addressed in Experiment 6.

An extension of the neural hypothesis is that, following application of a stressor, BG levels are positively correlated with NE turnover in the hypothalamus and that hypothalamic NE system(s) stimulate glucose release from the liver by activating a direct neural pathway (Smythe, Grunstein, Bradshaw, Nicholson & Compton, 1984). A role of hypothalamic NE in altering BG levels is supported by reports that direct application of NE into the hypothalamus can cause increases in BG (Steffens, Damsma, van der Gugten & Luiten, 1984; de Jong, Strubbe & Steffens, 1977; Shimazu & Matsushita, 1979). In Experiments 8 & 9, the involvement of central NE systems in the BG response to different stimuli is investigated.

Evidence used to support the notion of neural, as opposed to hormonal, control of BG responses to stress is presented by Shimazu and

coworkers and Smythe and coworkers. Shimazu (1967; Shimazu & Amakawa, 1968) reported that the increase in phosphorylase a activity in the liver following stimulation of the splanchnic nerve is still observed in adrenalectomized (ADX) rats. Inasmuch as activation of liver phosphorylase a mediates the physiological BG response to a stressor, BG levels should increase in response to stimulation of the VMH or splanchnic nerve in ADX rats. However, it has been reported that the increase in BG following VMH or splanchnic stimulation is altered in ADX animals. It was reported that the BG response following VMH stimulation was abolished by adrenal demedullation (Booth, Coons & Miller, 1969) or ADX (Himsworth, 1970), while another report claimed that ADX caused only a slight reduction in the BG response to VMH stimulation (Frohman & Bernardis, 1971). As well, in ADX calves the increase in BG release from the liver following low frequency stimulation (i.e. 1 cps) was abolished while the response to higher frequencies was not affected (Edwards, 1972).

Reports of the BG response of ADX animals to various stressors also do not yield consistent results. For example, 2-deoxyglucose (2-DG) increases BG levels by inhibiting glycolysis in brain cells (this causes glucose starvation in brain cells, i.e. neuroglycopenea) and the BG response of rats to intraperitoneal administration of 2-DG was reported to be unaffected by ADX (Smythe et al, 1984). Other reports, however, claim that the response to intraperitoneal or intraventricular administration of 2-DG was totally abolished by ADX (Frohman, Muller & Cocchi, 1973) or adrenal demedullation (Granneman & Friedman, 1984).

Furthermore, 3-O-methylglucose causes hyperglycemia by lowering brain glucose levels (it inhibits the transfer of glucose from the blood into brain tissue) and this hyperglycemic effect was abolished by denervation of the adrenal medulla (Himsworth, 1968).

While some investigators do not acknowledge that these discrepant views exist, others do. In the discussion of a recent paper, one group of investigators (Tordoff et al, 1984) correctly conclude that whether neural or hormonal regulation of hepatic glucose release predominates following application of a specific stressor remains to be determined.

Taken together, these reports indicate that there is no clear concensus on the mediational role of adrenal hormones in the BG response(s) to various stressors. There are a number of issues that, when considered, might help to put these discrepant findings into perspective. These issues are discussed below, and include; 1) the use of different species and sex of experimental animal; 2) the use of anesthetized vs unanesthetized animals; 3) the use of in vitro or in situ methods for measuring liver enzymes or glucose release from the liver vs in vivo determination of BG levels; 4) the use of fasted vs non-fasted animals; 5) the type of stimulus used to induce changes in BG or liver enzymes; 6) possible time dependent effects of glucocorticoids on liver enzymes; and 7) diurnal rhythms of hepatic glucose metabolism. These issues are discussed with relevance to the major goal of the first section of the present paper, which is to elucidate the physiological mechanisms that are involved in altering BG responses to various stressors in freely moving, conscious rats.

Interpretation of Data Relating to the Control of Blood Glucose

A potential source of confusion is that adrenergic stimulation has different effects on hepatic enzymes involved in glycogenolysis in male and female rats (Studer & Borle, 1982; 1983). However, the studies discussed below were carried out on male rats and the present experiments used male rats.

The major research groups that study the involvement of the CNS in the control of BG use different species of animals as experimental subjects. Shimazu's group used rabbits as their experimental subject, Lautt used cats, and others, including Smythe, used rats. All of the relevant behavioral work, which is to be discussed later, was carried out in rats.

Lautt and coworkers have used the anesthetized cat and rat to identify the neural connections from the brain to the liver that are involved in altering BG levels (For reviews see Lautt, 1980; 1983). Stimulation of the hepatic nerves in the anesthetized cat increases BG levels and this effect is blocked by the alpha-adrenergic receptor antagonist, phentolamine, but not by the beta-adrenergic antagonist, propranolol (Lautt, 1979). Furthermore, the BG increase caused by surgical trauma is attenuated by hepatic denervation and by ADX, but is totally blocked only by the combination of hepatic denervation and ADX (Lautt & Cote, 1977). Another important point is that these authors found that, in fasted rats, neural input to the liver was the major mediator of the hyperglycemic response to surgical trauma (Lautt & Cote, 1977). The type of adrenergic receptor mediating this stress response

was not determined. In summary, Lautt has demonstrated that, in the anesthetized cat, stimulation of the hepatic nerves can increase BG levels and that this effect is blocked by phentolamine. Furthermore, both the adrenal gland and the hepatic nerves mediate the BG response to surgical trauma in the anesthetized cat and rat.

In rabbits, Shimazu and co-workers have identified neural connections from the VMH to the liver via the splanchnic nerve that, when stimulated, promote the conversion of phosphorylase b to a. As well, this group has data indicating that activation of a neural pathway from the LH to the liver via the vagus nerve promotes synthetase activity. The effects of stimulation are blocked by alpha-adrenergic receptor antagonists and are unaltered in rabbits that have had their adrenals and pancreas removed. On the other hand, the effects of E on liver enzymes involved in glucose metabolism are blocked by beta-receptor antagonists (Shimazu & Amakawa, 1968). In summary, Shimazu's group has very convincingly demonstrated that, in the anesthetized rabbit, the VMH and the LH can affect liver enzymes independent of other glucoregulatory hormones.

In unanesthetized rats, a reciprocal relationship between LH and VMH stimulation on BG has not been confirmed (eg. Atrens et al, 1984). In fact, these investigators found that stimulation in ventrolateral areas more consistently produced increases in BG than stimulation in ventromedial areas. An additional critical finding was that stimulation could produce opposite effects depending on whether the animal was anesthetized or not (Atrens et al, 1984; Dubuc, Leshin & Willis, 1982).

In Experiment 7, it was determined whether hypothalamic stimulation increased BG levels in awake rats in the absence of the adrenal medulla. The type of adrenergic receptor that is involved in these effects is unknown for the rat (Lautt, 1980).

In awake normal rats, 2 hours of immobilization stress caused hyperglycemia which is attenuated by phentolamine and not affected by propranolol (Nakhooda, Sole & Marliss, 1981). In a strain of diabetic rats, phentolamine did not affect the immobilization stress-induced increase in BG levels while propranolol attenuated the rise in BG levels (Nakhooda et al, 1981). It is interesting that ADX can also affect the relative contribution of alpha- and beta-adrenergic stimulation to changes in liver enzymes in vitro (See below).

Based on in vitro experiments, it has been established that E increases hepatic glycogenolysis mainly through stimulation of an alpha-1 adrenergic receptor (eg. Chan & Exton, 1978; Sherline et al, 1972; Tolbert & Fain, 1974). However, in ADX rats the effect of alpha-1 adrenergic stimulation is reduced while the effect of beta-adrenergic stimulation is enhanced (Chan et al, 1979). Similar studies have not been done to see if these effects on liver enzymes are reflected in BG levels. The effects of alpha-adrenergic stimulation by clonidine and phenylephrine, and of beta-adrenergic stimulation by isoproterenol in normal, demedullated and ADX rats were determined in Experiment 2. In addition, the ability of alpha- and beta-adrenergic blockers to inhibit the increase in BG caused by E or footshock was determined in Experiment 3.

Time of day is another variable that can influence the degree of hyperglycemia observed following a specific manipulation. It has been reported that the hyperglycemic effect of intracerebroventricular 2-DG is greater when this drug is administered during the light period rather than the dark period (Yamamoto, Nagai & Nakagawa, 1984). Furthermore, the ability of liver cells (hepatocytes) to incorporate exogenous glucose into glycoen in vitro is affected by the time of day that the cells are obtained from the rat (Walker, 1977).

Another issue (that may be related to the previous point) is that one of the major effects of CORT is to promote glycogen synthesis (eg. Hems & Whitten, 1980; Schudt, 1979; 1980). Since glycogen stores in the liver are decreased by glucocorticoid deprivation, the altered BG response in ADX rats might be partially due to the low level of liver glycogen. Furthermore, acute increases or decreases in CORT levels may have very different influences on BG levels than more prolonged changes in CORT levels. The traditional effect of CORT (or any glucocorticoid) on hepatic glycogenolysis is considered to be to enhance the effect of E and glucagon. Recently, however, it has been reported that the synthetic glucocorticoid, dexamethasone, can inhibit glucagon-induced glycogenolysis in hepatocytes from rat (Fleig et al, 1984). As well, we have reported that blockade of the footshock-induced increase in CORT levels by injection of Metopirone enhanced the BG increase observed following footshock stress (Bialik & Roberts, 1985). Therefore, when manipulating CORT levels, different effects are expected depending on whether the manipulation is acute or of longer duration. The short-term

effect of glucocorticoids seems to be to attenuate BG increases while the long-term effect seems to be to maintain or enhance BG increases, by ensuring sufficient glycogen content in the liver. This issue is dealt with in Experiments 4 and 5.

Finally, a stressor probably causes increases in BG by activating neural connections from the brain to both the liver and the adrenal medulla (eg. Lautt & Cote, 1977; Sourkes, 1983). However, the relative contribution that each of these pathways makes to the overall BG response may be dependent on the specific stressor employed (eg. Tordoff et al, 1984) or the intensity of the stressor (See Experiments 1a and 1b).

Importance of Blood Glucose for Normal Information Processing

The importance of normal glucoregulation to psychological processes is not generally accepted. However, there are indications that altered glucoregulation may play a role in abnormal psycho-physiological states such as drug addiction (eg. alcoholism; Zito, Vickers, Telford & Roberts, 1984), depressive states (Russell & Johnson, 1981; Gerber, Choki, Brunswick, Reivich & Fraser, 1983; Storlien, Higson, Gleeson, Smythe & Atrens, 1985) and impaired learning and memory (Gold, 1986; Holmes et al, 1983). For the purposes of the present paper my interest in glucoregulation is focused on the potential relevance of glucoregulation to normal and disturbed information processing.

I will ultimately be testing the hypothesis that any manipulation,

not only manipulation of adrenal hormones, that affects glucoregulation following footshock stress is expected to affect the storage of information related to the experience of the footshock. If this is so, storage of the information required to avoid the environment where the footshock was administered should be modified by manipulations of posttraining glucose levels. Furthermore, I will hypothesize that glucose affects the storage of information, at least in part, by acting as a cue that is associated with performance of the conditioned response (See page 45). Although this is the explicit hypothesis that will guide future work, it is possible that the effects of altered glucoregulation on information processing is the result of increases or decreases in general efficiency of the brain.

There is evidence in the literature that alterations in the availability of the glucose supply to the brain might mediate some of the effects of hormones on information processing. For example, Gold and colleagues have investigated extensively the effects of E on the modulation of information storage and retrieval (eg. Gold & McGaugh, 1975; Gold & van Buskirk, 1976; McGaugh, 1985) and have recently reported that the facilitatory effects of posttraining E injections on retention of a passive avoidance response can be mimicked by posttraining injection of glucose (Hall & Gold, 1985; Gold, 1986). Furthermore, behaviorally effective, posttraining injections of E were found to increase BG to comparable levels as found following behaviorally-effective doses of glucose (Hall & Gold, 1986).

Another group of investigators have reported that both ADX and

adrenal demedullated rats are impaired on a retention test of a passive avoidance response (Borrell, de Kloet, Versteeg & Bohus, 1984b). Posttraining administration of E restored normal retention behavior in a dose-dependent fashion in both operated groups. An important observation was that demedullated rats required higher doses of E than ADX rats in order to reach normal retention performance. In demedullated rats it is possible that the release of CORT (in response to the footshock of the training session) had the opposite effect on BG levels than E did, the result being lower BG levels. In support of this view, these investigators reported data showing that when ADX rats were administered CORT 60 minutes prior to "training plus posttraining E injections" CORT decreased the efficacy of E in restoring retention performance (Borrell, de Kloet & Bohus, 1984a).

Taken together, these data are highly suggestive that E affects retention of a passive avoidance response by its effect of increasing BG levels. Furthermore, CORT may modulate the effect of E on retention performance by its effect(s) on BG levels or glucose utilization in the brain. These suggestions received support from our initial studies aimed at determining the role of E and CORT in the physiological BG response(s) to a mild stressor, such as the footshock that rats experience during avoidance conditioning.

We found that removal of the entire adrenal gland or just the adrenal medulla completely abolished the initial (less than 20 minutes) increase in BG observed following brief footshock stress (Bialik & Roberts, 1985). More importantly, it was also found that inhibition of

CORT synthesis, by an acute injection of Metopirone, significantly enhanced the hyperglycemic response to brief footshock stress (Bialik & Roberts, 1985). These results clearly indicated that the stress-induced hyperglycemia caused by brief footshock stress was mediated by release of E from the adrenal medulla. Furthermore, the release of CORT from the adrenal cortex appeared to attenuate this rise in BG. These data indicate that CORT may cause a reduction in the BG response to E, and might also provide an explanation for why CORT reduced the efficacy of E in restoring passive avoidance performance in ADX rats (Borrell et al, 1984a). The interactive effects of CORT and E on BG levels are investigated further in Experiment 5, and the effects of changes in BG levels on conditioned avoidance performance are studied in Experiments 10 and 11.

As discussed above, the mechanisms that control BG following various stressors are not generally agreed upon. There are arguments as to the importance of direct neural vs hormonal stimulation of hepatic glycogenolysis for the glycemic response to different stressors and agents that induce hyperglycemia. Furthermore, there is some uncertainty as to the importance of alpha- and beta-adrenergic receptors for the BG changes to various stressors. Therefore, the first 6 studies determine the relative contribution of adrenal hormones for the glycemic responses to various stimuli. A direct stimulatory effect of E is confirmed while a modulatory role of CORT is suggested. The effects of E are found to be mediated mainly by an interaction with alpha-2 adrenergic receptors (Experiments 2 & 3). That brain NE systems can influence the glycemic

responses to these stimuli is demonstrated in Experiments 8 and 9. Finally, it is demonstrated that manipulation of BG levels affects the ability of rats to learn, and remember, a discriminated avoidance response (Experiments 10 & 11).

EXPERIMENT 1

Experiment 1 determined the contribution of adrenal hormones to the hyperglycemic response caused by 2-DG. This drug stimulates an increase in BG levels through its effect of inhibiting glycolysis in brain cells. The brain responds by signalling the body to increase the release of glucose from the liver. Smythe et al (1984) reported that 2-DG increased BG levels in ADX rats, and therefore the signal from the brain to the liver was by direct neural communication and not due to the release of adrenal hormones. However, these investigators did not directly compare the glycemic response to 2-DG in ADX vs sham operated rats. This issue is re-investigated in Experiment 1a and 1b.

It has recently been suggested that the two major adrenal hormones, E and CORT, may mediate opposite effects on the glycemic response to brief footshock stress (Bialik & Roberts, 1985). Therefore, the respective roles of E and CORT in mediating hyperglycemia were investigated by comparing the BG responses to 2-DG in rats that were depleted of endogenous E and CORT (ADX), depleted of only E (DEMED) or left intact (SHAM).

Experiment 1a

Method

Subjects. Subjects were 18 male Wistar rats weighing 200-225 grams on delivery (Woodlyn Farms, Guelph, Ontario). Rats were maintained on a reversed 12-hr light/dark cycle (lights on at 1800 hr). After at least two weeks of acclimatization to the animal quarters, rats were randomly assigned to one of three groups. Groups were designated according to treatment as adrenalectomized (ADX), adrenal demedullated (DEMED) and sham operated (SHAM). Five days following the completion of the BG tests, 2-DG induced changes in plasma CORT levels (serum was collected 30 minutes following injection of 2-DG) were determined in 4 rats from each of the operated groups.

Surgical Procedures. Adrenalectomies and adrenal demedullations were performed using the dorsal approach. Briefly, an incision was made in the skin on the dorsal surface and the abdominal wall was cut inferior to the lowest rib. For adrenalectomies, the adrenal capsule was clamped, and the adrenal gland and some surrounding tissue were removed. For adrenal demedullations, the adrenal capsule was clamped, an incision was made in the adrenal cortex and the medulla was extracted. The abdominal wall and fascia were sutured, and the skin incision was closed with wound clips. Both adrenals were located and clamped but not removed in the sham operated animals. All rats were allowed 7 days to recover from surgery.

Drug Injections. 2-deoxy-D-glucose (Sigma) was administered in a

saline vehicle at a dose of 500 mg/kg ip.

Blood Glucose. Blood glucose was measured from approximately 100 ul of blood collected from a small incision made in the tail. The values were determined using Dextrostix (Miles Laboratories Ltd., Ames Division, Rexdale, Ontario) which were read using a glucometer (Miles Laboratories). Baseline BG values were determined immediately prior to the administration of 2-DG. Subsequent determinations were made 15, 30, 45, 60 and 120 minutes later.

Plasma Corticosterone. Four untreated and 4 2-DG injected rats (30 min. post injection) were decapitated for the CORT assay. Approximately 3 ml of trunk blood was collected into ice-cooled, heparin-rinsed glass centrifuge tubes. The blood was immediately centrifuged in a table-top centrifuge (International Equipment, Model CL). This procedure yielded approximately 1.0 ml of serum (or plasma) which was then pipetted into 1.5 ml plastic tubes with caps. The samples were immediately placed in a freezer until assayed for total (i.e. bound plus free) plasma CORT concentration by a modification of the fluorescence method of Givner and Rochefort (1965). The iso-octane step was eliminated, chloroform replaced by methylene chloride, and 0.2 ml of plasma was analyzed in duplicate for each sample.

Results

Blood Glucose. Figure 1a shows that both ADX and demedullation attenuated the increase in BG caused by administration of 2-DG. Analysis of Variance performed on these data revealed a significant interaction

between surgery and time of BG determination [$F(10,65)=26.87$, $p<0.001$]. Post-hoc analyses with the Newman-Keul's test revealed that the BG levels among groups did not differ at 15 minutes post-injection, but at each time point from 30 to 120 minutes following the injection of 2-DG both ADX and demedullated rats had BG levels lower than those of sham operated rats.

Plasma Corticosterone. Plasma CORT levels in samples collected 30 minutes following 2-DG (500 mg/kg ip) were lower in ADX rats ($X=8.0 \pm 0.8$ ug/dl) than in demedullated ($X=54.0 \pm 6.4$) and sham operated rats ($X=87.6 \pm 7.8$).

Discussion

Removal of the entire adrenal gland or just the adrenal medulla was found to attenuate the increase in BG observed following injection of 2-DG. However, these animals did show an initial rise in BG levels that was equivalent to sham operated controls at 15 minutes post-injection. Although Smythe et al (1984) did not compare the BG response to 2-DG in ADX and SHAM rats, they did report that ADX attenuated the hyperglycemic response to 2-DG in a letter to Lancet that was published at a later date (Grunstein, Smythe & Storlien, 1985).

The present data indicate that while the largest part of the BG increase following administration of 2-DG is mediated by adrenal E, there is still an early component of the BG response that is independent of adrenal hormones. This early component may be mediated by direct neural input to the liver as suggested by Shimazu (1981). The suggestion

of direct neural stimulation of BG release from the liver is supported by studies utilizing a different stressor and a different species. The hyperglycemic response to surgical trauma in cats has been shown to be attenuated by either ADX or hepatic denervation, but the response was eliminated only in animals receiving both ADX and hepatic denervation (Lautt & Cote, 1977).

The intensity of stress caused by 500 mg/kg of 2-DG or surgical trauma would appear to be orders of magnitude greater than what would be experienced in a behavioral situation. We have already shown that depletion of adrenal E alone completely eliminates the increase in BG levels caused by behaviorally-relevant footshock stress (Bialik & Roberts, 1985). The next experiment investigated the BG response of E-depleted rats to a lower dose of 2-DG.

Experiment 1b

The dose of 2-DG that was used in the first experiment was an extremely high dose and was used only in order to make a direct comparison to the data reported by Smythe et al (1984). The next experiment determined the effect of surgically removing or denervating the adrenal medulla on the BG response to a lower dose of 2-DG.

Method

Subjects. Subjects were 19 male Wistar rats weighing 200-225 grams on delivery (Charles River Farms, St. Constant, Quebec). Rats were

maintained as described for Experiment 1.

Surgical Procedures. Demedullation and sham operations were performed as described in Experiment 1. Denervation of the adrenal gland was accomplished by surgically removing the adrenal gland and then replacing it in the fatty tissue from where it was taken. The gland was sutured to this tissue to promote revascularization. This procedure usually leads to deterioration of the adrenal medulla but not the adrenal cortex (See Bennett, Liang & McGaugh, 1985; Liang, Juler & McGaugh, 1986). To ensure that the adrenal cortex was functional, animals were exposed to footshock stress prior to being sacrificed and blood was collected and analysed for CORT levels.

Drug Injections. An injection of 125 mg/kg ip of 2-DG was given immediately following the determination of baseline BG levels. Determinations of BG were made again 20, 40, 60 and 120 minutes later.

Results and Discussion

Plasma CORT levels were found to be increased following footshock in both DEMED ($X=29.2 \pm 3.6$ ug/dl) and DEN ($X=31.1 \pm 8.1$ ug/dl) rats, compared to non-shocked, intact rats (14.4 ± 3.1 ug/dl). Although the adrenal cortex was functional in the DEMED and DEN rats, CORT levels in these groups were lower than in SHAM operated rats ($X=47.5 \pm 3.3$ ug/dl), indicating that the adrenal cortex probably was slightly damaged during the operations.

Analysis of Variance performed on the BG data indicated a significant interaction between Surgery and Time of BG determination

[$F(6,48)=2.94$, $p<0.05$]. Further analyses, using the Newman-Keul's procedure, indicated that only the SHAM operated group showed an increase in BG levels and that the DEMED and DEN groups had significantly lower BG levels at each time point tested (See Figure 1b).

In conclusion, the first two experiments indicate that the increase in BG levels caused by 2-DG is mediated mainly by the release of E from the adrenal medulla. In fact, it is only when very high doses of this drug are used that there is a non-adrenal component to the BG response.

EXPERIMENT 2

In the first experiments, the largest proportion of the BG response to 2-DG was accounted for by the release of a substance from the adrenal medulla. Previously, it was shown that demedullation abolished the rise in BG caused by brief footshock stress (Bialik & Roberts, 1985). In vitro data have indicated that E increases hepatic glycogenolysis mainly through stimulation of an alpha-1 adrenergic receptor (Chan & Exton, 1978; Sherline et al, 1972; Tolbert & Fain, 1974). Furthermore, in tissue from ADX rats the glycogenolytic effect caused by alpha-1 adrenergic stimulation was found to be reduced while beta-adrenergic stimulation of glycogenolysis was facilitated (Chan et al, 1979). Similar studies have not been done to determine if the effects of ADX on adrenergic-stimulation of liver enzymes are reflected in BG levels. In fact, it is not clear which adrenergic receptor mediates the increase in BG levels

observed following injection of E.

In the next two experiments an attempt was made to determine which adrenergic receptor(s) is involved in the BG increase induced by E. In Experiment 2, it was determined whether the increase in BG levels caused by E could be mimicked by injection of alpha- and/or beta-adrenergic receptor agonists. The adrenergic agonists that were used are thought to be specific to a certain class of receptors (See Szabadi, Bradshaw & Nahorski, 1985). Phenylephrine (PHENYL) was used to stimulate alpha-1 receptors, clonidine (CLON) for alpha-2 receptors, and isoproterenol (ISO) for beta receptors. The BG response to these adrenergic agonists, and to E, were determined in intact and ADX rats. Furthermore, DEMED rats were used in some cases in order to rule out the possibility of a non-specific release of E from the adrenal medulla accounting for any observed differences in responses between ADX and intact rats.

Method

Subjects. Subjects were 66 male Wistar rats weighing 200-225 grams on delivery (Charles River Farms, St. Constant, Quebec). Rats were maintained as described for Experiment 1. Groups were designated according to treatment as ADX, DEMED, or SHAM operated. Six rats from each of the operated groups received one of the four drug treatments (i.e. E, CLON, ISO or both CLON & ISO). Each group of six rats received all of the doses of the drug, except that only the lowest dose was used when CLON and ISO were administered together. Furthermore, a complete dose response curve was obtained for PHENYL in intact and ADX rats.

Surgical Procedures. Operations (i.e. ADX, DEMED, SHAM) were performed as described in Experiment 1. Animals were allowed one week to recover from surgery prior to testing.

Drug Injections. All drugs were injected intraperitoneally and the doses were as follows; CLON - 0.01, 0.05, 0.1 and 0.5 mg/kg; ISO - 0.01, 0.05, 0.1 and 0.5 mg/kg; E - 0.01, 0.05, 0.1 and 0.5 mg/kg. For the combined treatment with CLON and ISO the 0.01 dose was used. Due to the lack of an effect at all but the highest dose of PHENYL, an additional higher dose was used. Therefore, the doses of PHENYL that were tested were 0.05, 0.1, 0.5 and 1.0 mg/kg ip. At least three days were allowed between each drug testing day.

Blood Glucose. Determinations of BG were made as described for Experiment 1. Levels were determined before drug injections and again 20, 40, 60 and 120 minutes later.

Plasma Corticosterone. Plasma CORT was determined as described for Experiment 1. Following completion of the BG experiments, rats were sacrificed and blood collected 20 minutes following administration of the 0.1 mg/kg dose of E, CLON and ISO, and the 0.5 mg/kg dose of PHENYL.

Results and Discussion

Plasma Corticosterone. Each of the adrenergic agonists caused an increase in CORT levels in SHAM and DEMED rats but not in ADX rats (See Figure 2a). It should also be noted that DEMED rats had lower CORT levels than SHAM rats, which probably indicates that the adrenal cortex was slightly damaged during removal of the adrenal medulla.

The interesting finding here is that each of the adrenergic agonists caused an increase in CORT levels that was roughly equivalent to the increase that we observe following footshock stress (See Figure 2b). At least in the case of CLON, the stimulation of CORT secretion is independent of the normal hormonal control by adrenocorticotropin (ACTH) from the anterior pituitary gland, since CLON reduces ACTH levels in the blood while increasing CORT levels (Smythe, Bradshaw, Gleeson, Grunstein & Nicholson, 1985).

Blood Glucose. In general the results indicated that the SHAM group showed the largest increase in BG levels, the DEMED group the second largest and the ADX group had the lowest levels following injection of each drug. Based on published *in vitro* data using specific adrenergic agonists it was expected that the hyperglycemia caused by ISO would be greater in ADX than SHAM and DEMED rats and hyperglycemia caused by E, CLON and/or PHENYL would be attenuated in ADX rats compared to SHAM and DEMED rats.

Clonidine. Analysis of Variance performed on the data indicated a significant main effect of Dose of CLON [$F(3,45)=35.62$, $p<0.001$], Surgery [$F(2,15)=18.05$, $p<0.001$], Time of BG determination [$F(4,60)=28.54$, $p<0.001$], as well as a significant interaction between Surgery and Dose of CLON [$F(6,45)=3.64$, $p<0.005$]. In Figure 2d, it can be seen that CLON caused a rapid and large increase in BG levels and that levels were still elevated 2 hours following the injection. Further analyses using the Newman-Keul's procedure indicated that the higher the dose of CLON the greater the BG response (See Figure 2d), and that SHAM

rats had higher BG levels than DEMED rats and both groups had higher levels than ADX rats (See Figure 2d). Analysis of the interaction effect indicated that only the SHAM rats had a smooth dose-response curve, while ADX rats responded similarly to the highest two doses, and DEMED rats responded similarly to the middle two doses (See Figure 2c). Furthermore, there were no differences among groups at the 0.01 mg/kg dose, while ADX rats had significantly lower BG levels than SHAM rats at each of the other doses, and DEMED rats had significantly lower BG levels than the SHAM group at the 0.10 and 0.50 mg/kg doses. The ADX and DEMED groups did not differ at the 0.10 dose.

The important points to notice in Figure 2c are 1) the BG response in ADX rats reached asymptote at the 0.1 mg/kg dose and 2) ADX rats had lower BG levels than the other groups of rats only at the higher doses, at the lowest dose there was no difference among groups in the BG response to CLON. Both of these findings might be a reflection of the lower level of stored glycogen in the liver of these rats, rather than to an effect of CORT on adrenergic stimulation of glucose release from the liver. At low doses when the glycogen supply is not taxed, ADX does not influence the glycemic response to CLON. The maximum increase in BG occurs at 0.1 mg/kg and even with a dose that is 5 times higher no additional increase is observed. Therefore, it seems likely that the limited supply of stored glycogen imposed a ceiling on the dose response curve to CLON in ADX rats.

In summary, the BG increase following injection of CLON was greatly reduced in ADX rats. However, the BG differences that were observed

between the ADX group and the DEMED and SHAM groups may be explained entirely by the reduced supply of glycogen stored in the liver of these animals. Furthermore, that all groups showed similar increases in BG levels at the lowest dose of CLON supports the notion that depletion of CORT does not affect increases in BG levels caused by alpha-2 adrenergic stimulation when glycogen stores in the liver are not taxed.

Phenylephrine. The lowest two doses of PHENYL that were tested (0.05 and 0.1 mg/kg) did not cause a significant change in BG levels in intact rats, therefore the dose of 1.0 mg/kg was also tested. Analysis of Variance carried out on these data indicated a significant 3-way interaction effect, Surgery x Dose x Time of BG determination [$F(6,105)=2.43$, $p<0.05$]. It can be seen in Figure 2e, that only the 0.5 and 1.0 mg/kg doses of PHENYL caused significant increases in BG levels in intact rats while no dose of PHENYL caused a significant increase in BG levels in ADX rats.

In summary, even when high doses of PHENYL were used to stimulate alpha-1 adrenergic receptors, only a relatively small increase in BG levels were observed. This increase was eliminated by ADX, as would be predicted by in vitro data showing that alpha-1 stimulation of glucose release from the liver is greatly attenuated in ADX rats (eg. Chan et al, 1979; Hernandez-Sotomayer & Garcia-Sainz, 1984; Pushpendran et al, 1984).

Isoproterenol. Analysis of Variance performed on the data indicated main effects due to Surgery [$F(2,55)=3.41$, $p<0.05$] and Time of BG determination [$F(3,165)=41.96$, $p<0.001$]. as well as a significant

interaction between Surgery and Time of BG determination [$F(6,165)=3.37$, $p<0.005$]. Further analyses with the Newman-Keul's procedure indicated that ADX rats had lower BG levels than SHAM operated rats (See Figure 2f). Analysis of the Time main effect indicated that BG levels were significantly elevated at 20, 40 and 60 minutes following administration. In Figure 2f, it can be seen that the increase in BG caused by ISO was smaller and of shorter duration than that caused by CLON (Figure 2c).

Analysis of the Surgery by Time interaction effect indicated that both ADX and DEMED groups had lower BG levels than the SHAM group 60 minutes following the injection of ISO, while levels were not significantly different at other time periods (See Figure 2f).

One of the most important findings here is that there was not a significant effect of the dose of ISO that was used. A dose of 0.01 mg/kg raised BG levels to the same level as a dose of 0.5 mg/kg. The reason for this may be that ISO directly stimulated insulin release from the pancreas (See Halter, Beard & Porte, 1984), which would counteract the increase in BG levels caused by direct stimulation of hepatic glucose release by ISO. These two opposing effects of ISO would be intensified at higher dose levels, but the net effect on BG levels would be similar. The differences in the magnitude and time course of the BG responses to ISO and CLON are probably largely due to the effect of ISO to stimulate insulin release and of CLON to inhibit insulin release (See Halter et al, 1984; Metz, Halter & Robertson, 1978).

In summary, there was no indication that the effect of ISO on BG

levels was enhanced in ADX rats, as was predicted on the basis of in vitro studies. Therefore, the global BG response to beta-adrenergic stimulation does not reflect the effects of beta-adrenergic stimulation of liver enzymes involved in glycogenolysis or gluconeogenesis. This may be due to beta-adrenergic stimulation of insulin release which dominates BG levels under conditions of increased beta-adrenergic stimulation.

Clonidine plus Isoproterenol. Analysis of Variance performed on the data indicated only a main effect of time [$F(3,54)=32.73$, $p<0.001$]. It can be seen in Figure 2h, that the low doses of these two drugs produced a large increase in BG levels by 20 minutes and levels had still not returned near baseline at 2 hours. Furthermore, this effect appears to be a simple summation of the effects of the two drugs. There were no significant effects due to surgical treatment.

Epinephrine. In Figure 2i, it can be seen that E increased BG levels by 20 minutes following injection, and levels were still elevated at 2 hours. In Figure 2i, it can be seen that there was a significant effect of the dose of E that was administered [$F(3,180)=82.74$, $p<0.001$]. Further analyses using the Newman-Keul's procedure, indicated that the increase in BG caused by the 0.01 and 0.05 doses were not different while the 0.1 and 0.5 mg/kg doses produced successively greater increases (See Figure 2i). The main effect of surgery was also significant [$F(2,60)=14.75$, $p<0.001$]. Further analyses using the Newman-Keul's test indicated that ADX rats had significantly lower BG levels following the injection of E than either DEMED or SHAM rats, while the latter two groups did not differ. (See Figure 2i).

There was also a significant interaction between the dose of E and surgery [$F(6,60)=2.38$, $p<0.05$]. Analysis of this interaction effect indicated that ADX rats had lower BG levels than SHAM and DEMED rats only at the 0.01 and 0.5 mg/kg doses (See Figure 21).

The most important finding was that ADX eliminated the increase in BG caused by 0.01 mg/kg (ip) E. At this low dose the reduced glycogen content in the liver of ADX rats would not be taxed. Therefore, this effect would be consistent with a reduced stimulation by E of glucose release from glycogen stores in the liver. Furthermore, ADX abolished the increase in BG caused by the specific alpha-1 adrenergic agonist PHENYL. Taken together, these data indicate that at low doses (i.e. 0.01 mg/kg ip) E stimulates BG increases by an action on alpha-1 receptors and this effect is absent in ADX rats. At higher doses, E probably acts at alpha-2 and/or beta receptors to increase BG levels, and ADX has little or no effect on the BG response. At the highest dose of E (i.e. 0.5 mg/kg ip), very large increases in BG levels are obtained in intact rats, while ADX rats demonstrate much lower BG levels. This latter effect is probably due, at least in part, to the reduced hepatic glycogen stores of ADX rats.

General Discussion for Experiment 2

The data from this experiment clearly show that CLON was the most effective agent for increasing BG levels. Furthermore, BG levels were still markedly increased two hours following injection of this drug. It

is not known if the effect of CLON is directly on the liver, since it is known that CLON lowers blood levels of insulin (Smythe et al, 1985), presumeably by a direct effect of inhibiting the release of this peptide from the pancreas (Metz et al, 1978). On the other hand, ISO is thought to stimulate the release of insulin (Halter et al, 1984), which probably accounts for the short duration of the increase in BG levels that is caused by this drug. Furthermore, the lack of a dose response relationship for ISO on BG levels is probably due to the fact that the higher the dose the greater is the stimulation of glucose release from the liver and the greater is the release of insulin from the pancreas.

The effect of PHENYL on BG levels in intact rats was not predicted by the effects of this drug on hepatic glucose release. In vitro work done on dispersed liver cells indicated that the release of glucose from these cells following treatment with E was mediated mainly through alpha-1 adrenergic receptors (Chan & Exton, 1978; Sherline et al, 1972; Tobert & Fain, 1974). However, in the present studies, the effect of E was most closely mimicked by the combination of CLON and ISO (See Figure 2k), with PHENYL being much less potent.

The <u>in vitro</u> data did however, predict the effects of ADX on the BG increase caused by stimulation of alpha-1 receptors by PHENYL. In vitro work indicated that CORT was necessary for alpha-1 stimulation of hepatic glucose release and the present results indicate that the increase in BG levels induced by PHENYL was abolished in ADX rats. Furthermore, the increase in BG levels caused by a low dose of E was abolished by ADX. Taken together, these data indicate that a low dose of

E stimulates a rise in BG levels mainly through an alpha-1 adrenergic receptor in the liver, whereas higher doses of E act predominantly on alpha-2- and/or beta-adrenergic receptors to stimulate large increases in BG levels.

In conclusion, the effect of adrenergic agonists on BG levels in intact rats could not be predicted by the action of these drugs on hepatic glucose release. On the other hand, the BG response of ADX rats to low doses of E and to PHENYL was predicted by the effects of E and adrenergic agonists on hepatic BG release.

EXPERIMENT 3

In the next series of experiments the effects of alpha- and beta-adrenergic receptor blockade on the glycemic responses caused by E and footshock stress were determined. Yohimbine (YOH) was used to block alpha-2 adrenergic receptors, phenoxybenzamine (PHENOXY) for alpha-1 receptors, phentolamine (PHENT) for both alpha-1 and alpha-2 receptors, and propranolol (PROP) for beta-adrenergic receptors. It has been reported that 2 hours of immobilization stress causes hyperglycemia which is attenuated by phentolamine and not affected by propranolol (Nakhooda et al, 1981). Furthermore, we have previously shown that release of adrenal E can account for the BG response to brief footshock stress and it is believed that E stimulates glucose release from the liver mainly by stimulation of alpha-1 adrenergic receptors (eg. Chan & Exton, 1978). However, in Experiment 2 it was found that stimulation of

alpha-2 adrenergic receptors with CLON (or CLON plus ISO) most closely mimicked the BG response observed following E administration. Therefore, it was expected that only blockade of alpha-2 adrenergic receptors would attenuate the BG increase caused by E and footshock.

Experiment 3a

Experiment 3a investigated whether alpha- or beta-adrenergic receptors mediated the increase in BG caused by injection of E and by brief footshock stress.

Method

Subjects. Subjects were 32 male Wistar rats weighing 200-225 grams on delivery (Charles River Farms, St. Constant, Quebec). Rats were maintained as described in previous experiments.

Procedure. The experiment was run in two separate phases. Each agent used to produce hyperglycemia constituted one phase of the experiment. In the first phase animals were administered E (0.1 mg/kg ip) and in the second phase they received footshock stress. In the first phase rats were divided into four groups (eight rats each) which were designated according to adrenergic-antagonist administration as saline (SAL), PHENT, PROP and PHENT+PROP. In the second phase the groups of eight rats received different antagonist treatments, such that no group of eight rats received the same antagonist treatment more than once. The dose of PHENT and PROP was 1.0 mg/kg ip.

Phase One: Epinephrine. Baseline BG determinations were made 30 minutes following injection of the adrenergic-antagonist(s). Immediately following the baseline BG determination, E was administered (0.1 mg/kg ip) and BG values were determined again 20, 40, 60, and 120 minutes later.

Phase Two: Footshock stress. For the administration of footshocks, rats were placed in a shock box that was enclosed in a sound-attenuating wooden chamber. Air circulation was provided by exhaust fans which also emitted 65-db (SPL) background noise. The boxes were made of black plexiglass (measuring 82 x 35 x 15 cm) with a grid floor made of steel bars (2 mm in diameter). The grids were connected by neon bulbs to permit scrambling of the shock. An AC electric current (0.5 mA) was delivered through the grid floor. Animals received one shock every 20 seconds for 5 minutes. A microprocessor controlled the presentation of the shocks. Determinations of BG were made immediately prior to the presentation of the footshocks and again 20, 40 60 and 120 minutes later.

Results and Discussion

Epinephrine. Analysis of Variance performed on the BG data indicated a main effect due to adrenergic antagonist treatment [$F(3,24)=8.16$, $p<0.001$]. Animals treated with PROP had BG levels 97% of VEH-treated controls, while the PHENT and PHENT+PROP groups had BG levels which were only 39% and 30% of controls, respectively (See Figure 3a).

The interaction effect between adrenergic antagonist treatment and time of BG determination was also statistically significant [$F(9,72)=2.52$, $p<0.05$]. It can be seen in Figure 3b that the PHENT-treated groups had much lower BG levels than the VEH- and PROP-treated groups 20, 40 and 60 minutes following injection of E ($p<0.05$, Newman-Keul's), while there were no differences among groups at 2 hours.

Clearly, these data indicate that E increases BG levels largely by stimulating alpha-adrenergic receptors. On the other hand, beta-adrenergic receptors appear not to be involved in the BG response to E.

Footshock. A priori t-tests indicated that BG levels were significantly higher in rats treated with VEH or PROP than in rats administered PHENT or PHENT+PROP (See Figure 3c). In fact, BG levels were not increased in either of the PHENT-treated groups. These data indicate that the footshock-induced increase in BG levels is mediated by stimulation of alpha-adrenergic receptors.

Previously, we had shown that the same amount of footshock that was administered here caused an increase in BG levels that was mediated by release of E from the adrenal medulla (Bialik & Roberts, 1985; See Figure A). Now we have shown that blockade of alpha-adrenergic receptors largely attenuates the BG response to both E and footshock stress. Therefore, the increase in BG caused by the amount of footshock administered in the present studies is mainly the result of stimulation of alpha-adrenergic receptors by E which is released from the adrenal medulla.

Experiment 3b

Since the results from the previous experiment indicated that alpha-adrenergic receptors were responsible for the increase in BG following administration of E, the next experiment determined whether it was the blockade of alpha-1 or alpha-2 receptors that was responsible for this effect. YOH and PHENOXY were used to block alpha-2 and alpha-1 receptors, respectively.

Method

Subjects. Subjects were 36 male Wistar rats previously used 3 weeks before in a pilot experiment for the "Y-maze" task that was used in Experiment 10 (no drug treatment was given at that time). Rats were maintained as described in previous experiments and weighed 300-350 grams at the time of the experiment.

Procedure. The experiment was run in two identical replications, except that a different adrenergic antagonist was used in each replication. Each replication consisted of 9 rats pretreated with a vehicle injection and 9 rats given YOH or PHENOXY. All rats were injected with E (0.10 mgkg ip) 30 minutes following the pretreatment. Levels of BG were determined at the time of the E injection and again 20, 40, 60 and 120 minutes later.

Results and Discussion

Analysis of Variance carried out on these data did not indicate any significant effects due to YOH [$F(1,16)=4.05$, $p>0.05$] or PHENOXY pretreatment [$F(1,16)=1.23$, $p>0.05$]. However, there was a tendency for YOH to reduce and PHENOXY to enhance the increase in BG caused by E.

Experiment 3c

The previous experiment did not adequately answer the question of whether alpha-1 or alpha-2 (or both) receptor blockade was responsible for the effect of PHENT in attenuating the increase in BG caused by E in Experiment 3a. Therefore, in the next experiment higher doses of the receptor antagonists were used to attempt to block the BG response to E. All groups were administered E following treatment with different adrenergic receptor blockers. Treatment groups were aimed at blocking beta receptors (PROP), alpha receptors (PHENT), only alpha-1 receptors (PHENOXY) or only alpha-2 receptors (YOH).

Method

Subjects. Subjects were 30 male Wistar rats weighing 200-250 grams on delivery (Charles River Farms, St. Constant, Quebec). Rats were maintained as described in previous experiments.

Procedure. All rats were injected with E (0.10 mg/kg ip) 30 minutes following administration of one of the 5 adrenergic antagonist

treatments. Levels of BG were determined at the time of the E injection and again 20, 40, 60 and 120 minutes later.

Results and Discussion

Analysis of Variance performed on the data indicated a significant interaction between Drug Pretreatment and Time of BG determination [$F(12,75)=8.09$, $p<0.001$]. Inspection of Figure 3e reveals that both drugs that blocked alpha-2 receptors (PHENT and YOH) significantly attenuated the BG increase caused by E, while blockade of beta receptors (PROP) had no effect and blockade of alpha-1 receptors (PHENOXY) significantly enhanced the BG increase.

In conclusion, it is clear that in vitro data, which indicate that the E-induced release of glucose from the liver is predominantly mediated by alpha-1 receptors, can not be used to predict the BG response to adrenergic agonists. Furthermore, it has been shown that the BG response to E in intact rats is mediated predominantly by alpha-2 adrenergic receptors. It is not obvious in which organ these alpha-2 receptors are located, but it is most likely that the effect is a combination of stimulation of hepatic glucose release and an inhibition of insulin release from the pancreas.

In addition, the effect of the alpha-1 adrenergic blocking agent, PHENOXY, of greatly potentiating (162% at 40 minutes) the E-induced increase in BG levels was surprising given the extensive publication of in vitro data indicating that E stimulated glucose release from the liver by interacting with the alpha-1 adrenergic receptor. Since

systemic injections of PHENOXY would block both central and peripheral alpha-1 adrenergic receptors, it must be considered whether the effect of PHENOXY reported here was mediated by an effect on alpha-1 receptors in the brain (See Experiment 9).

Experiment 3d

Our previous data (Bialik & Roberts, 1985) indicated that release of E from the adrenal medulla was responsible for the increase in BG observed following brief footshock stress. However, it has been reported that there is a direct neural component from the hypothalamus to the liver that is responsible for increases in BG under some circumstances. For example, following injection of 2-DG there is still an early increase in BG that is observed in ADX or adrenal demedullated rats (Grunstein et al, 1985; Experiment 1).

It has been reported that the increase in BG caused by surgical trauma is attenuated by hepatic denervation and by ADX, but is totally abolished only by the combination of hepatic denervation and ADX (Lautt & Cote, 1977). An additional finding, which may be important, is that these authors found that neural innervation of the liver mediated almost the entire hyperglycemic response to surgical trauma in 24 hr fasted rats (Lautt & Cote, 1977). If this is also the case for footshock-stress, then we have a way of differentially studying neural and hormonal mediation of stress-induced BG responses.

The next experiment investigated the effect of depleting adrenal E

on footshock-induced hyperglycemia in 24-hr food-deprived rats. It was expected that hyperglycemia would be observed in E-depleted rats, although the increase in BG would be smaller than in intact rats.

Method

Subjects. Subjects were the 19 rats from Experiment 1b which were used to determine 2-DG induced hyperglycemia. These rats had received DEMED, DEN or SHAM operations approximately one month previously. More than two weeks was allowed between the completion of Experiment 1b and the beginning of testing.

Procedure. Rats were food deprived for 24 hours prior to testing. Footshock was administered and BG levels determined as described for Experiment 3a.

Results and Discussion

The footshock-induced increase in BG levels in 24 hr food-deprived, intact rats (See Figure 3f) was similar to that observed in non-deprived rats (i.e. food removed 2 hr prior to testing) (See Figure A). This is surprising since it is thought that rats fasted for 24 hr have very low levels of glycogen stored in their livers, therefore this increase in BG levels would be due to increased gluconeogenesis (eg. Arneric, Chow, Bhatnagar, Webb, Fischedr & Long, 1984). Gluconeogenesis is generally thought to be a slower process than glycogenolysis, since the latter requires the liberation of substrates from peripheral tissue, the transport of these substances to the liver, the conversion to glucose in

the liver and then the release into the circulation, whereas glycogenolysis only requires the conversion of liver glycogen to glucose and the subsequent release of the glucose (See page 6).

Furthermore, it was expected that the footshock-induced increase in BG would not be totally abolished by DEMED or DEN, as would be predicted by Lautt's data in anesthetized rats following 24 hours of food deprivation. However, it is clear from Figure 3f that both DEMED and DEN completely abolished the increase in BG caused by footshock stress in 24 hour food-deprived rats.

It can be concluded that the release of E from the adrenal gland can account for the entire increase in BG levels observed following brief footshock stress. Furthermore, it appears that E can account for footshock-induced hyperglycemia regardless whether the increase in BG levels is due mainly to increased glycogenolysis (i.e. in non-deprived or 2 hr food-deprived rats) or to increased gluconeogenesis (i.e. in 24 hr food-deprived rats).

Experiment 3e

The previous experiment demonstrated that adrenal E mediated the increase in BG caused by footshock stress in 24 hour food-deprived rats. The next experiment determined if adrenal demedullation could also eliminate the BG response to a low dose of 2-DG in 24-hour food-deprived rats.

Method

Subjects. Subjects were 18 rats that had been tested two weeks earlier in a "Y-maze" task in Experiment 10. They had received saline or glucose injections at that time.

Surgical Procedures. Adrenal demedullations and sham operations were carried out as described in previous experiments. Rats were tested approximately 10 days following surgery.

Drug Injections. All rats were food deprived for 24 hours prior to the injection of 2-DG. All rats received a single injection of 125 mg/kg ip 2-DG immediately following the determination of baseline BG levels. BG levels were determined again 20, 40, 60 and 120 minutes later.

Results and Discussion

Analysis of Variance performed on the data indicated a significant Surgery x Time interaction [$F(3,48)=34.33$, $p<0.001$]. Inspection of Figure 3g shows that BG levels of the DEMED and SHAM groups were not different at 20 minutes, but that levels were significantly lower in DEMED rats at 40, 60 and 120 minutes. It is clear that the largest part of the BG increase caused by 2-DG is due to the release of E from the adrenal gland. However, unlike for footshock stress, there was a small but significant increase at 20 minutes. This initial increase may have been mediated by direct neural innervation of the liver as suggested by Lautt (See above). On the other hand, the role of another hormone (such as glucagon) in mediating this initial rise in BG can not be ruled out.

General Discussion for Experiment 3

In summary, it is very clear that the release of adrenal E can account for the increase in BG following a behaviorally-relevant stressor (i.e. footshock) as well as most of the increase in BG following injection of 2-DG in non-fasted rats. Similar results were obtained in 24 hour fasted rats. Furthermore, the data indicated that E stimulated alpha-2 adrenergic receptors to cause the increase in BG following footshock stress in non-fasted rats. It is expected that similar effects would be observed in 24-hour fasted rats, but this is not known. There is evidence that the E-stimulated increase in BG in non-fasted rats is due to enhanced glycogenolysis, whereas the rise in BG in fasted rats is due to enhanced gluconeogenesis (Arneric et al, 1984). Inasmuch as these two processes are mediated by different adrenergic receptors, the increase in BG following E administration would be altered differently by alpha- and beta-adrenergic receptors.

It is also not known which receptors (adrenergic or non-adrenergic) mediate the non-adrenal component of the BG increase observed following high doses of 2-DG (Experiment 1a) or a low dose of 2-DG in fasted rats (Experiment 3e). [There is some evidence in rabbits that different adrenergic receptors mediate hormonal vs direct neural stimulation of glucose release from the liver (Shimazu & Amakawa, 1968).] Preliminary data in our lab (Smythe, Roberts & Bialik, in preparation) indicate that adrenergic receptor blockade does not influence the non-adrenal component of the BG increase following a high dose of 2-DG. Given that

sympathetic innervation of the liver is thought to be mainly adrenergic, our preliminary evidence indicates that the non-adrenal component of the BG increase observed following administration of 2-DG is not mediated by direct innervation of this gland. There is other evidence to support this view. Vagotomy combined with coeliac ganglionectomy, a surgical procedure that would eliminate the neural connections from the brain to the liver but not the adrenal gland (eg. See Mayer, 1980, pg. 58), had no effect on the increase in BG levels following 200 mg/kg 2-DG (Granneman & Friedman, 1984).

It is unlikely that neural connections from the brain to the liver play a significant role in the BG responses to footshock stress or 2-DG administration. The non-adrenal component to the 2-DG induced hyperglycemia may be mediated by another hormone, and glucagon from the pancreas would be the logical first choice to investigate as the mediator of this non-adrenal, non-neural effect.

In conclusion, the increase in BG levels following brief footshock stress or 2-DG administration is mediated by an action of adrenal E on alpha-2 adrenergic receptors. Direct neural input to the liver plays, at most, a minimal role in the BG response to these stressors.

EXPERIMENT 4

Recently, Munck, Guyre and Holbrook (1984) have developed the hypothesis that one function of CORT, when it is released in response to stress, is to dampen acute responses to stress. That is, CORT is

hypothesized to function to restore homeostasis in many systems following perturbations caused by stress. One situation that is relevant to the present topic, is the role of CORT in altering the BG response observed following various stressors.

It has been suggested that the hyperglycemic response to stress might function to protect the brain from some physiological effect(s) that occurred as a consequence of the stress. For example, Baum and Porte (1980) have reported that animals which are prevented from developing hyperglycemia during hypoxia had more severe damage to brain tissue than animals which showed a normal hyperglycemic response to this stressor. Recently, Sapolsky (1985; 1986) found that acute administration of a high dose of CORT exacerbated the damage caused by a number of neurotoxic substances (Sapolsky, 1985; 1986).

On the other hand, an exaggerated hyperglycemic response to stress may also produce adverse effects, both morphologically (eg. Pulsinelli, Waldman, Rawlinson & Plum, 1982; Johansen & Diemer, 1986) and behaviorally (eg. Gold, 1986; Hall & Gold, 1986). Furthermore, we have previously reported that inhibition of the stress-induced release of CORT potentiates the BG increase caused by footshock stress (Bialik & Roberts, 1985) and causes a deficit in acquisition of an active avoidance response (Bialik, Roberts & Pappas, 1984a). In the next two experiments, the effect of CORT on the stress-induced increase in BG was investigated further.

As mentioned above, inhibition of the stress-induced release of CORT, by injection of Metopirone (MET), causes an enhancement of the BG

increase normally observed following brief footshock stress (Bialik & Roberts, 1985). In the next experiment, the specificity of this effect was investigated. First, it was determined whether the stress-induced release of E and CORT are necessary for Metopirone to have its effect. That is, would Metopirone augment the hyperglycemic response to an injection of E? The injection of E should not be stressful, yet both E and CORT levels in the blood would be increased (See Experiment 2). Second, it was determined if Metopirone would alter BG levels observed following exogenous administration of glucose, and therefore rule out an interaction between CORT and E on specific glucoregulatory mechanisms.

Method

Subjects. The subjects were 48 male Wistar rats weighing 200-250 grams on delivery (Charles River Farms, St. Constant, Quebec). Rats were maintained as described for the previous experiments. An additional 30 rats were used for the CORT determinations. These rats had been tested in a "Y-maze" avoidance task (Experiment 10) three weeks previously.

Procedure. Rats were randomly assigned to one of 6 groups with 8 rats per group. Groups were designated according to treatment as Metopirone plus Epinephrine (MET+E), vehicle plus E (VEH+E), MET plus glucose (MET+GLUC), VEH+GLUC, MET plus saline (MET+SAL) and VEH+SAL.

Drug Injections. Administration of MET occurred 1 hr prior to the injection of E (0.05 mg/kg), GLUC (100 mg/kg) or SAL. Metopirone was dissolved in a vehicle of 40% propylene glycol and 60% saline and 1 ml/kg was injected ip at a concentration of 50 mg/ml. The other drugs

were prepared in a vehicle of saline and 1 ml/kg was injected ip at the concentrations reported above.

Blood Glucose. Levels of BG were determined prior to Metopirone injection and again at the time of E or GLUC administration and 20, 40, 60 and 120 minutes later.

Plasma Corticosterone. Due to the high probability of non-specific fluorescent materials in the blood following Metopirone treatment, a radioimmunoassay was used to determine plasma CORT levels (RIA kit from Diagnostics, Montreal, Quebec). Rats were randomly assigned to one of 6 groups with 5 rats per group. Three of the groups were pretreated with MET and three with VEH and 90 minutes later one MET and one VEH group received either E (0.05 mg/kg), GLUC (500 mg/kg) or VEH. For each rat, CORT determinations were carried out on 200 ul of blood collected prior to drug pretreatment (MET or VEH), prior to drug injections (E, GLUC or VEH), and again 30 and 60 minutes later. Blood samples were collected in 0.5 ml plastic tubes which were frozen until they were analyzed.

Results and Discussion

Blood Glucose. Analysis of Variance performed on the data indicated main effects due to drug pretreatment (i.e. MET or VEH) [$F(1,42)=4.43$, $p<0.001$] and drug administration (.e. E, GLUC or VEH) [$F(2,42)=38.84$, $p<0.001$], as well as a significant pretreatment x drug x time interaction [$F(6,126)=2.46$, $p<0.01$].

Overall, MET-treated rats had BG levels 185% that of VEH-injected controls ($X=12.3 \pm 2.7$ vs 6.7 ± 1.7). Rats given E ($X=26.1 \pm 3.3$ mg/dl)

had BG levels significantly higher than rats given GLUC (X=2.1 ± 2.0 mg/dl) or VEH (X=0.2 ± 1.3 mg/dl) [as determined by the Newman-Keul's procedure].

Further analyses performed on the interaction effect, using the Newman-Keul's test, revealed that compared to VEH-pretreated rats MET-pretreated rats had higher BG levels following E at 20 and 40 minutes and had higher BG levels following GLUC at 20 minutes (See Figure 4a). Levels of BG were not changed by VEH injections in MET- or VEH-pretreated rats. However, pretreatment with MET did cause a significant increase in BG levels 90 minutes later (i.e. at the time of baseline determinations for E, GLUC or VEH injections). The increase in BG levels caused by Met alone was significantly higher than that caused be VEH pretreatment (X=13.1 ± 2.8 vs X=2.5 ± 1.9 mg/dl) [$t(23)=4.30$, $p<0.005$].

The results from this experiment demonstrate that MET itself can cause a small but reliable increase in BG levels. Furthermore, pretreatment of rats with this drug exacerbates the increase in BG caused by E or an injection of GLUC. Therefore, MET might be acting by slowing the clearance of the injected glucose from the blood and/or itself stimulating increases in BG levels. It has been reported that a high dose of MET (200 mg/kg) increases plasma glycerol levels (Ohno & Kuroshima, 1986). Since glycerol is a substrate for gluconeogenesis in the liver, this might account, in part, for the enhanced BG increase observed following footshock stress and E or glucose injections.

In conclusion, MET potentiates the increase in BG levels caused by footshock stress and injection of E, and this effect is independent of

alterations of the actions of E on specific glucoregulatory mechanisms. This effect may be related to the action of MET to increase plasma levels of glycerol, which is a substrate for gluconeogenesis in the liver.

Plasma Corticosterone. Analysis of Variance performed on the data indicated that there was no overall effect of pretreatment with Metopirone on plasma CORT levels [$F(1,24)=1.31$, n.s.]. However, there was a significant interaction effect, Pretreatment x Drug x Time of BG determination [$F(6,72)=2.45$, $p<0.05$]. Further analysis using the Newman-Keul's procedure revealed that E increased plasma CORT levels in VEH but not MET pretreated rats (See Figure 4b). A significant increase in CORT levels following E administration was observed at 60 minutes but not at 30 minutes. Folowing GLUC administration CORT levels were elevated above baseline at 30 minutes but not at 60 minutes, but this effect failed to reach significance (See Figure 4b).

The result with E (0.05 mg/kg ip) is consistent with the increase in CORT levels observed following administration of E (0.10 mg/kg ip) in Experiment 2 (See Figure 2b). The tendency for GLUC (500 mg/kg ip) to increase CORT levels was surprising given the abundent data indicating that CORT levels are usually high prior to the rats first meal at the beginning of the dark period and levels decline rapidly following the meal (and subsequent increase in BG levels; See Dallman, 1984 for a discussion of this issue as it relates to feeding behavior). The act of eating as well as the route of administration of the glucose are probably crucial factors influencing CORT levels following the exogenous

administration of glucose. Further studies should be carried out to determine what effect exogenous administration of glucose has on plasma CORT levels, as this is a critical issue for those interested in attributing the behavioral effects of glucose administration to a direct action in the CNS.

EXPERIMENT 5

The purpose of this experiment was to determine if CORT inhibited the BG increase caused by E. The experiment involved administering E alone or in combination with CORT to ADX rats. There are several points which should be discussed first, however.

Administration of E to ADX rats has been reported to cause only a slight rise in BG levels (Schaeffer, Chenoweth & Dunn, 1969). Administration of a glucocorticoid 15 or 30 minutes prior to injection did not affect the BG response of ADX rats to E. Injection of the glucocorticoid for three days prior to the administration of E did reinstate the normal hyperglycemic response to E in ADX rats. Thus, these authors concluded that CORT was necessary for E to activate hepatic glycogenolytic enzymes.

A major problem with the study of Schaeffer et al (1969) is that the authors reported that at 3 weeks postsurgery ADX rats had similar liver glycogen levels to controls. It is well established that glucocorticoid depletion causes a decrease in liver glycogen levels (Long et al, 1940; Hems & Whitton, 1980). Furthermore, one of the most

predominant effects of glucocorticoids is to promote glycogen synthesis (Long et al, 1940; Fleig et al, 1984). Therefore, the reduced BG increase in ADX rats following E administration may be due in part to the lower levels of liver glycogen rather than to an effect of glucocorticoids on hepatic enzymes involved in glycogen metabolism. [This effect of glucocorticoids is often referred to as a "permissive effect", which might be a catch-all term used to describe a variety of poorly understood effects attributed to glucocorticoids (Baxter, 1976).]

In the preceeding paragraph, data were cited indicating that longterm treatment with glucocorticoids leads to enhanced BG responses to E. Likewise, treatment of hepatocytes with dexamethasone for greater than 2 hours was required in order to demonstrate an enhancement of gluconeogenisis (Stumpo & Kletzien, 1981). On the other hand, several short-term effects of glucocorticoids have the opposite effect on BG levels. Glucocorticoids enhance insulin's effect of stimulating glycogen synthesis (Schudt, 1979, 1980) and acute treatment of hepatocytes with glucocorticoids was found to inhibit glucagon-stimulated glycogenolysis (Fleig et al, 1984). These short-term effects of glucocorticoids might translate into an initial effect of inhibiting a BG rise in response to a stimulus. Indeed, in our study, inhibition of CORT synthesis enhanced the initial hyperglycemic response to brief footshock stress (Bialik & Roberts, 1985). Furthermore, in the previous experiment it was found that inhibition of CORT synthesis also potentiated the increase in BG levels observed following E or GLUC treatment. Therefore, acute manipulation of glucocorticoid levels appears to have very different

effects on glucose metabolism than do more prolonged treatments.

The working hypothesis that was tested in the next series of experiments is that the predominant short-term effect of CORT on BG levels is to dampen the rise in BG levels caused by injection of E. The preparation that was used was the ADX rat. In this preparation, the rat would not be able to release adrenal hormones, therefore, circulating levels of E and CORT could be directly controlled by systemic administration. However, it should be kept in mind that these rats would have reduced glycogen levels in the liver and show attenuated increases in BG levels in response to adrenergic agonists, including E (See Experiment 2). Animals were treated with E, CORT or both E and CORT, and the BG response to these hormones was monitored. It was expected that E would raise BG levels more when it was administered alone than when it was administered with CORT.

We are ultimately interested in the behavioral consequences of CORT-E interactions. Behavioral data were discussed above (See pages 15-16) which led to the hypothesis that E enhances passive avoidance performance by increasing BG levels. Furthermore, CORT is hypothesized to reduce the potency of E on passive avoidance performance by attenuating the increase in blood glucose caused by E. In order to make direct comparisons with this behavioral work, the present study used the same doses and temporal parameters of drug administration that were used in the behavioral studies. That is, CORT (0.3 mg/kg sc) was administered 60 minutes prior to E (0.5 and 0.05 mg/kg sc).

Experiment 5a

Since this series of experiments involves subcutaneous (sc) administration of E and all our previous work involved ip injections, the first experiment in this series compared the BG response to E when it was adminstered ip or sc.

Method

Subjects. Subjects were 9 unoperated rats that had previously been used in Experiment 4 two weeks previously. The rats had received VEH pretreatment and GLUC or VEH injections, so that none of the rats had received E before the present experiment.

Procedure. At 1100 hr, rats were injected with E (0.5 mg/kg) by the ip (n=4) or sc route (n=5). Baseline BG determinations were made immediately prior to E administration and again 1/2, 1, 2, 3 and 4 hours later.

Results and Discussion

Analysis of Variance performed on the data revealed a significant Route of administration x Time of BG determination interaction [$F(4,28)=36.56$, $p<0.001$]. Further analyses using the Newman-Keul's procedure revealed that BG levels were significantly higher in the ip group compared to the sc group at 1/2 hr, but that the sc group had significantly higher BG levels at 2, 3 and 4 hr (See Figure 5a). These data clearly show that the route of administration is a major factor

determining the BG response to E.

Given the extent of the different BG responses observed following E by these two routes of administration, the route by which E is administered would be expected to be an important factor determining the behavioral effects of this hormone. If the rapid onset, short lasting increase in BG is the main determinant of the behavioral effect then ip injections would be more effective than sc injections, but if the duration of the increase in BG is the important factor then sc injections would be more effective.

Experiment 5b

The next experiment investigated the effect of CORT pretreatment on the hyperglycemic response following sc injections of E. The rationale for this experiment was outlined in detail above.

Method

Subjects. Subjects were 20 male Wistar rats, weighing 200-225 grams on delivery (Charles River Farms, St. Constant, Quebec). Rats were individually housed and maintained on a reversed 12-hr light/dark cycle. Groups were designated according to treatment as E injected or E and CORT injected (E+CORT).

Surgical Procedures. Adrenalectomies were performed as described in Experiment 1.

Hormone Administration. E (0.05 or 0.50 mg/kg sc) was administered

to induce an increase in BG in all rats. Half of the rats received vehicle injections 60 minutes before E was administered, while the other half received CORT (0.3 mg/kg sc).

Blood Glucose. Baseline BG determinations were made prior to the administration of CORT or VEH, prior to the administration of E, and again 40, 80 and 120 minutes later.

Plasma Corticosterone. Plasma CORT was determined as described previously. Three days or more following completion of testing in Experiment 5c (See below) all rats were decapitated and trunk blood collected in order to determine baseline CORT levels.

Results and Discussion

Plasma Corticosterone. One rat had high CORT levels and its data were discarded. The mean baseline plasma CORT levels for the 19 remaining ADX rats was 3.8 $\pm$ 0.5 ug/100 ml.

Blood Glucose. It was expected that BG levels would be increased by injection of E, and the magnitude of the E-induced increase in BG levels would be reduced in animals given CORT and E compared to rats given only E. Analysis of Variance performed on the data indicated main effects of Dose of E [$F(1,16)=31.43$, $p<0.001$] and Time of BG determination [$F(2,32)=23.03$, $p<0.001$] but no effect due to CORT pretreatment [$F(1,16)=1.08$, n.s.] and no significant interactions among the variables. It is clear from Figure 5b that the 0.50 mg/kg dose of E caused a greater increase in BG levels than the 0.05 mg/kg dose and furthermore, CORT pretreatment did not influence the effect of either

dose of E.

Since the expected effect of CORT pretreatment on BG levels following sc injections of E was not observed, the next experiment determined if CORT pretreatment would affect the BG response to ip injections of E. The BG response to ip injections of E has a much shorter latency and duration than the BG response to sc injections (See Figure 5a).

Experiment 5c

Method

Subjects. One week following the completion of the previous experiment 14 of the rats were randomly divided into two groups. Groups were designated according to treatment as CORT or CORT+E.

Procedure. CORT (300 ug/kg sc) was administered 60 minutes prior to the administration of E (0.05 mg/kg ip). Baseline BG determinations were made prior to E administration and again 30 and 60 minutes later.

Results and Discussion

Analysis of Variance performed on the data indicated only a main effect due to Time of BG determination [$F(1,12)=5.40$, $p<0.05$]. In Figure 5c, it can be seen that 0.05 mg/kg of E administered by the ip route caused an increase in BG levels that peaked at 30 minutes and was approaching baseline levels at 60 minutes. Again, ip injection of E had an effect on BG levels that occurred with a shorter latency and had a shorter duration than was observed following the same dose administered

subcutaneously (Compare with Figure 5b). Pretreatment with CORT did not affect the increase in BG levels caused by ip administration of E.

General Discusion for Experiment 5

It is clear from the present experiment that short-term pretreatment of ADX rats with a behaviorally-relevant dose of CORT (See Borrell et al, 1984a,b) does not influence the BG response to injections of E. Therefore, if the effect of CORT to reduce the efficacy of E in enhancing retention of a passive avoidance response (Borrell et al, 1984a,b) is due to an alteration of glucose supply to the brain, this effect must occur at a step beyond changes in BG levels. That is, since CORT did not alter the BG response to E, CORT must influence either the entry of glucose into the brain, the uptake of glucose into cells in the brain, and/or the utilization of glucose by cells in the brain. There is evidence to indicate that CORT can alter the blood-brain-barrier (Long & Holaday, 1985) and inhibit glucose uptake into brain cells (See Sapolsky, 1985; 1986). Further studies are warranted to investigate these issues.

From a behavioral perspective, the question of whether CORT attenuates the effect of E on behavior by blocking a metabolic effect of glucose on the brain may be addressed in the following manner. It is known that exogenous administration of glucose can facilitate retention performance in a passive avoidance task (Hall & Gold, 1985; Gold, 1986) as well as in a "Y-maze" task (See Experiments 10 and 11). The dose

response curve for glucose in the "Y-maze" task can be determined in ADX rats following injection of CORT (same dose as in Borrell et al, 1984a) or VEH. These results can be compared to the effects of CORT on the dose-response curve for manose and beta-hydroxybutyrate, two substances that can be used by the brain for fuel but whose uptake into brain cells is not influenced by CORT. If CORT blocks a metabolic effect of glucose on the brain to cause its behavioral effects, then CORT administration would attenuate the effect of glucose while not altering the effects of other metabolic fuels.

From a physiological perspective the question of whether CORT can, under some circumstances, attenuate the BG increase caused by stress or E administration is not straightforward. In the present experiment, there are a number of possible reasons for obtaining negative results. Several potentially important variables might be the use of ADX rats and the dose, route, and time of CORT administration. As discussed above, ADX rats have depleted glycogen stores in the liver and have impaired BG responses to adrenergic agonists. Perhaps, it would have been better to use ADX rats maintained on low dose glucocorticoid treatment. These rats would have higher levels of liver glycogen and should not be impaired in their BG responses to adrenergic agonists.

Furthermore, CORT was injected one hour prior to E administration in order to make direct comparisons to the behavioral work of Borrell et al (1984a,b). However, in terms of the physiological response of an animal to a stressor, the CORT and E would be released in much closer temporal proximity. Another point is that sc injection of CORT can be

expected to result in slower entry of the hormone into the general circulation than ip injection. Injection of 2.4 mg/kg ip (8 times the dose used here) can raise CORT levels by 20-25 ug/dl within 10 minutes (Newman, 1986). This is roughly equivalent to the levels of plasma CORT that we observe 20 minutes following the initiation of footshock stress (See Figure 2b).

To summarize, in order to more closely approximate the normal physiological responses to a stressor, both E and CORT should be injected by the ip route in close temporal proximity, and ADX rats that are maintained on low dose CORT treatment should be used as subjects. Using this procedure, the effect of CORT on the E-induced BG response can be determined, and these results would be comparable to the experiment in which blocking stress-induced CORT levels resulted in an enhanced BG response to footshock stress and E administration (Bialik & Roberts, 1985; Experiment 4).

In conclusion, it appears clear that the effect of CORT to inhibit the retention-enhancing effect of E on passive avoidance behavior (See Borrell et al, 1984a,b) is not mediated by changes in BG levels. However, the issues of whether this effect of CORT is due to an alteration in the accessibility of glucose to the brain, and whether CORT can inhibit the E-induced BG response under some circumstances are interesting and deserve further attention.

Adrenal Independent Hyperglycemia

To this point it has been established that adrenal E can account for the majority of the BG increase following footshock stress and 2-DG administration. On the other hand, adrenal CORT is necessary to observe the full effect(s) of E on BG levels, probably because of an influence on the activity of liver enzymes and by its role of increasing glycogen stores in the liver. The short-term effects of changes in CORT levels on E-induced changes in BG levels are unclear, but they too would be modulatory in nature if they were observed at all. Therefore, it is clear that release of adrenal E is by far the most important means by which the body causes rapid changes in BG levels in response to footshock or 2-DG stress. [We have also confirmed that adrenal E mediates the rise in BG levels observed following exposure of rats to a very different type of stressor, the odour of a predator (Roberts & Bialik, unpublished observations).]

As mentioned several times already, the conclusion that was summarized in the previous paragraph is in direct conflict with the views of several often- cited researchers (eg. Shimazu, 1979; Smythe et al, 1984). These authors claim that direct neural innervation of the liver is responsible for rapid changes in BG levels following exposure to stress. In the next experiment we make another effort to identify a hyperglycemic response that is independent of the adrenal medulla.

EXPERIMENT 6

As discussed previously, electrical stimulation of the hypothalamus is known to influence glucose metabolism by direct neural connections with the liver and by modulating hormone release from the pancreas and adrenal glands. Recently, it has been reported that there is a relative lack of anatomical specificity with respect to electrode sites in the ventral-lateral and ventral-medial hypothalamus which produce increases or decreases in BG (Atrens et al, 1984). These authors also reported that stimulation of both the VMH and LH increased several measures of energy utilization (Atrens et al, 1985), an effect which should lead to an increase in the rate of disappearance of glucose from the blood.

In our initial experiment, stimulation of the hypothalamus (through electrodes aimed at the lateral hypothalamus) caused only a slight decrease in BG levels two minutes following the initiation of one minute of stimulation. At 10 minutes following stimulation a significant increase in BG levels was observed (See below).

In the next experiment, it was determined to what extent BG levels change following hypothalamic stimulation when the release of E is prevented by removal of the adrenal medulla. It was expected that the drop in BG levels would be greater since the major counter-regulatory hormone had been removed. Following the expected initial drop in BG levels, an increase in levels should be observed which would be mediated by stimulation of hepatic glycogenolysis by direct neural connections with the hypothalamus.

Method

Subjects. Subjects were 8 male Wistar rats, weighing 200-225 grams on delivery (Charles River Farms, St. Constant, Quebec). Animals were housed two or three to a cage and maintained on a reversed 12-hr light/dark cycle (lights on at 2030). Water and Purina Lab Chow were provided ad libitum.

Electrodes. Electrodes were made from a 10 mm piece of monopolar stainless steel electrode wire, type 316. The wire was soldered to a RELI-A-TAC 220 P02 amphenol plug. The electrode wire was then insulated in epoxylite and then baked for 15 min at 100 C followed by 15 min at 350 C. The immersion-bake cycle was repeated six times to ensure proper insulation. The insulation was removed from the electrode tip with a fine sharpening stone (600-1200 grit) and finished with a ground glass slide. The tip was then checked under a microscope. Electrodes were then checked for current leakage.

Surgical Procedures: Electrode Implantation. Rats were anesthetized with Somnotol (65mg/kg ip) and stereotaxically implanted with electrodes aimed at the lateral hypothalamus. The coordinates were; A.P. + 6.5; D.V. + 0.9 and Midline $\pm$ 1.3 to 1.6. The indifferent electrode was made of uninsulated stainless steel wire wrapped around a stainless steel screw. The indifferent electrode was fastened to the skull caudal to the stimulating electrode. The entire assembly was fixed in place with dental acrylic cement bonded to three additional skull screws. Rats were allowed five days to recover from surgery before testing began.

Stimulation Parameters. A pilot study was run to determine

effective parameters. Non-fasted animals were placed in the stimulation chamber and given monophasic square wave pulses, 5-25 mv intensity, 2msec, 50 cycles/sec. The entire stimulation period lasted for 10 min per day. Rats received stimulation in bursts, 20-sec on/20 sec off. Current level and voltage were continuously monitored using an oscilloscope.

Blood Glucose. The baseline BG level was determined before the rat was placed in the stimulation chamber, and again at various intervals following stimulation. After several weeks of stimulation (5 or 6 tests per rat), rats were selected that demonstrated an increase in BG levels in response to "one minute" of continuous stimulation. In the final test prior to surgery, BG was measured immediately prior to stimulation and again 1, 10 and 20 minutes later.

Surgical Procedures: Adrenal Demedullation. On the same day that the final stimulation was given animals were anesthetized with Somnotol (65 mk/kg ip) and bilateral removal of the adrenal medulla was performed on all 8 rats as described in previous experiments. Animals were allowed 3 days to recover and then the BG reponse to hypothalamic stimulation was determined as described above. Another test was conducted 6 days later. Due to loss of headcaps during the experiment, complete data on only 5 rats were obtained and therefore only the data for these rats were analyzed and reported here.

Histology. At the end of the experiment, animals were given an overdose of Somnotol, perfused and their brains were fixed in 10% Formalin for histological verification of the electrode sites.

Results and Discussion

Blood Glucose. Analysis of variance performed on the BG data indicated a significant interaction between Test Day and Time After Stimulation [$F(4,16)=16.79$, $p<0.001$]. Post-hoc analyses with the Newman-Keul's procedure indicated that BG levels were higher at 10 and 20 minutes following stimulation when rats were tested prior to DEMED compared to tests performed 3 or 9 days following DEMED (See Figure 6). Furthermore, at 9 days following DEMED, stimulation produced a significant reduction in BG levels at 1 minute but this effect was not observed when testing occurred 3 days following DEMED or prior to DEMED. Inspection of Figure 6, clearly shows that hypothalamic stimulation produces a decrease in BG levels, an effect which is not observed in intact rats or 3 days following DEMED.

As in previous experiments utilizing various stressors, release of E from the adrenal medulla can account for the induced increase in BG levels following electrical stimulation of the hypothalamus.

EXPERIMENT 7

Hyperglycemia caused by Chronic Glucocorticoid Treatment

All of the experiments discussed to this point deal primarily with the effects of adrenal hormones on rapid changes in BG, effects probably mediated by changes in hepatic glycogenolysis (See the discussion for Experiment 3 though). Adrenal hormones also influence gluconeogenesis, which is the synthesis of glucose from the metabolic products of lipids

and proteins (See page 6). Both E and CORT enhance the process of gluconeogenesis. Chronic high levels of glucocorticoids have been associated with high BG levels (eg. Smythe et al, 1984) and impaired glucose tolerance in rats (Akerblom, Martin & Gary, 1973) and humans (eg. Karnieli, Cohen, Barzilai, Ish-Shalom, Armoni, Rafaelov & Barzilai, 1985). In the next study, it was investigated whether the increase in BG caused by the synthetic glucocorticoid, DEX (Bates & Garrison, 1971; 1973), was influenced by removal of endogenous CORT and/or E.

Experiment 7a

A preliminary study was required in order to select an appropriate concentration of DEX pellet to use. The hyperglycemic response to chronic implantation of different concentrations of DEX pellet was determined in the next experiment.

Method

Subjects. Subjects were 16 male Wistar rats, weighing 200-250 grams on delivery (Charles Rivers Farms, St. Constant, Quebec). All animals were housed individually (in plastic cages) and maintained on a reversed 12-hr light/dark cycle (lights on at 2030). Water and Purina Lab Chow were provided ad libitum throughout the experiment.

After at least 1 week acclimatization, rats were randomly assigned to one of four treatment groups. Groups were designated according to pellet concentration as 0% (i.e. cholesterol controls; CHOL), 5%, 15%,

and 25% DEX groups.

Dexamethasone Pellets. The pellets were made up of cholesterol and dexamethasone in one of the following concentrations. The control pellet was 240 mg CHOL, 5% DEX was 12 mg DEX + 228 mg CHOL, 15% DEX was 35 mg DEX + 200 mg CHOL, and the 25% DEX pellet was 60 mg DEX + 175 mg CHOL. The pellets were formed by melting powdered DEX into melted CHOL over high heat. The mixture was then dropped onto a pallet and allowed to cool, forming solid pellets of 200 $\pm$ 10 mg.

Surgical Procedures. The pellets were implanted one day prior to the start of BG testing. At this time, all rats were anesthetized with ether, a small incision was made in the back of the neck, and a pellet of 0, 5, 15, or 25% DEX was inserted and the incision closed with sutures and a wound clip.

Blood Glucose. Determinations of BG were made as described for previous experiments. Baseline BG values were determined immediately prior to implantation of the pellets. Subsequent determinations were made 1, 2 and 3 days later. The rats were weighed each time BG determinations were made.

Results and Discussion

Blood Glucose. Analysis of Variance performed on the data revealed a significant interaction between the concentration of DEX in the pellet and the time of the BG determination [$F(15,60)=11.72$, $p<0.001$]. Post-hoc comparisons, using the Newman-Keuls procedure, revealed that BG levels were significantly elevated for the 15% DEX group from day 3 and for the

25% DEX group from day 2 (See Figure 7a). Levels of BG for the 5% DEX pellet group did not differ from the CHOL pellet controls on any day tested.

Body Weight. Analysis of Variance performed on the body weight data indicated a significant interaction between DEX pellet concentration and time of body weight determination [$F(12,48)=9.48$, $p<0.001$]. Further analyses using the Newman-Keul's procedure indicated that, compared to the 0% DEX pellet group, the 5, 15 and 25% DEX pellet groups had significantly greater loss of body weight on days 2, 5 and 7 (See Figure 7b). Furthermore, compared to the 5% DEX group, the 15 and 25% groups had greater weight loss on days 5 and 7, and finally, the 25% DEX group had lost more weight than the 15% DEX group by day 7 (See Figure 7b).

Since the 5% DEX pellet group showed a significant loss of body weight but no increase in BG levels, it can be assumed that these two effects of the DEX pellet are, to some extent, controlled by different mechanisms.

Experiment 7b

The entire adrenal gland or just the adrenal medulla was removed and the increase in BG was observed for 4 days after implantation of a 25% DEX pellet. In the preceeding experiment, it was shown that this concentration of DEX pellet reliably produced hyperglycemia within several days.

Method

Subjects. Subjects were 28 male Wistar rats, weighing 200-250 grams on delivery (Charles Rivers Farms, St. Constant, Quebec). All animals were housed individually in plastic cages and maintained on a reversed 12-hr light/dark cycle (lights on at 2030). Water and Purina Lab Chow were provided ad libitum throughout the experiment. Adrenalectomized rats were given 0.9% saline instead of water. After at least 1 week acclimatization, rats were randomly assigned to one of six treatment groups. Groups were designated according to treatment as adrenalectomized and implanted with a DEX pellet (ADX+DEX) or a CHOL pellet (ADX+CHOL), adrenal demedullated and implanted with a DEX pellet (DEMED+DEX) or a CHOL pellet (DEMED+CHOL), and sham operated and implanted with a DEX pellet (SHAM+DEX) or a CHOL pellet (SHAM+CHOL).

Surgical Procedures. Adrenalectomies and adrenal demedullations were performed as described in Experiment 1. All rats were allowed at least 7 days to recover from surgery before the pellets were implanted. The 25% DEX and CHOL pellets were made and implanted as described in Experiment 7a.

Blood Glucose. Determinations of BG and body weight were made as described in previous experiments.

Plasma Corticosterone. Immediately following the final BG and body weight determination (day 4), rats were decapitated and trunk blood was collected into ice cooled, heparin rinsed glass centrifuge tubes. Plasma samples were stored and CORT determinations made as described in Experiment 1.

Results and Discussion

Plasma Corticosterone. Analysis of Variance performed on the data indicated a significant main effect of surgery [$F(2,24)=75.10$, $p<.001$] and a significant interaction between surgery and pellet implantation [$F(2,24)=59.44$, $p<.001$]. Post-hoc comparisons, using the Newman-Keuls procedure, showed that plasma CORT levels were reduced in the ADX+CHOL group (to 15%) and in the DEMED+CHOL group (to 51%) compared to the SHAM+CHOL group. Furthermore, in non-ADX rats, DEX pellet groups had plasma CORT levels only 16% of controls.

Blood Glucose. Analysis of variance performed on the BG data indicated a significant main effect due to pellet implantation [$F(1,23)=22.73$, $p<.001$] and a significant pellet by day interaction [$F(3,115)=31.05$, $p<.001$]. Post-hoc comparisons, using the Newman-Keuls procedure, showed that BG levels were higher for each of the DEX pellet implanted groups on days 2 and 3 (See Figure 7c). There were no differences in BG levels due to surgery in either the DEX or CHOL pellet implanted groups.

Body Weight. Analysis of Variance performed on the data indicated a significant main effect due to pellet implantation [$F(1,23)=24.07$, $p<.001$] and a significant interaction between pellet implantation and day of testing [$F(3,138)=17.69$, $p<.001$]. Post-hoc comparisons, using the Newman-Keuls procedure, showed that implantation of the DEX pellet caused a significant decrease in body weight on days 3 and 7 ($p<0.01$).

Interestingly, ADX rats implanted with CHOL pellets lost significantly more weight than the SHAM+CHOL and DEMED+CHOL groups,

while the ADX rats implanted with 25% DEX pellets lost significantly less weight than the SHAM+DEX and DEMED+DEX groups (See Figure 7d). [Similar effects were found when % weight loss was analysed instead of absolute weight loss - data not shown.]

General Discussion for Experiment 7

Neither adrenalectomy nor demedullation altered the rise in BG caused by implantation of a DEX pellet. The most important finding in this experiment was that the increase in BG caused by high levels of glucocorticoids (by subcutaneous implantation of a DEX pellet) was not mediated in any way by release of E from the adrenal medulla.

The implantation of DEX pellets probably causes weight loss, in part, by increasing the liberation of fatty acids and amino acids from peripheral stores (See Munck, 1971), and the hyperglycemia results, in part, from these substances being converted to glucose by the liver (Bates & Garrison, 1971; 1973). However, the effect of DEX must be influenced by other factors as well, since 5% DEX pellets caused a significant loss of weight but did not raise BG levels (See Figures 7a and 7b) and ADX rats showed significantly less weight loss but similar BG responses as SHAM and DEMED rats (See Figures 7c and 7d).

Furthermore, it is not known whether DEX-induced hyperglycemia is mediated entirely by peripheral mechanisms or whether the CNS influences the development of the hyperglycemia. In Experiment 8, it was determined if the disruption of brain NE systems influenced the BG response and loss of body weight caused by implantation of a DEX pellet.

that would affect BG levels are 1) the liberation of fatty acids and amino acids from peripheral tissues (which would cause weight loss), 2) the facilitation of gluconeogenesis by the liver (which would cause an increase in blood glucose levels) and 3) blockade of insulin release (BarsegRian & Levine, 1980). In order for brain NE lesions to have reduced DEX hyperglycemia, the lesions either reduced the effects of DEX on peripheral tissue catabolism or enhanced the utilization of glucose, maybe by interfering with the effect of DEX on the insulin receptor. In Figure 8b, it can be seen that NE depletion reduced weight loss caused by DEX, therefore it is assumed that peripheral tissue catabolism was inhibited by the disruption of the brain NE systems. This suggestion is tentative however, since it was found that the effects of DEX on body weight and BG levels are not necessarily inversely related (See Experiments 7a and b). It is not known whether brain NE depletions cause general metabolic alterations or whether effects are specific to the situation studied here.

In summary, the data indicate that brain NE might be involved in controlling some aspect(s) of peripheral glucose metabolism. We are currently investigating the effect of brain NE depletions on basal metabolic rate and metabolic rate following cold challenge. These studies are being carried out in collaboration with Dr. Peter Pawson at the National Research Council Laboratories. It has already been demonstrated that NE is involved in the control of energy metabolism in the brain (eg. Quach et al, 1978; Magistretti, Morrison, Shoemaker, Sapin & Bloom, 1981; Morrison & Magistretti , 1985; Harik, Busto &

EXPERIMENTS 8 and 9

Involvement of Brain Norepinephrine in Experimentally-induced Hyperglycemia

The effects of hypothalamic stimulation on liver enzymes (Shimazu & Matsushita, 1979) and BG (de Jong et al, 1977; Steffens et al, 1984) can be mimicked by application of NE. As well, Smythe et al (1984) have presented data demonstrating a correlation between medial basal hypothalamic NE activity and BG levels. Furthermore, it has been reported that rats with experimentally-induced diabetes have reduced NE activity in the hypothalamus during rest (Bitar, Koulu, Rapoport & Linnoila, 1986) and iontophoretic application of glucose decreases while application of 2-DG enhances the release of NE in the hypothalamus (McCaleb, Myers, Singer & Willis, 1979). Therefore, the involvement of brain NE systems in stress-, 2-DG- and DEX-induced hyperglycemia was determined by testing the ability of these agents to produce hyperglycemia in rats that had previously been depleted of brain NE (Experiment 8).

In Experiment 9, the hyperglycemic responses to the direct adrenergic agonists, E, clonidine and isoproterenol, and the indirect agonist, amphetamine, were determined in NE depleted rats. A potentiated increase in the BG response to either of the direct adrenergic agonists would indicate that the agonist was acting on supersensitive postsynaptic receptors to mediate the BG changes (See Zis & Fibiger, 1975; Spyraki & Fibiger, 1982). Since amphetamine acts on the NE system by facilitating release of this transmitter, destruction of the NE

neurons would eliminate any effect of amphetamine on BG that was mediated by an action on brain NE systems.

EXPERIMENT 8

Method

Subjects. Sixty-four male Wistar rats, weighing 200-250 grams upon receipt from the supplier (Charles River Farms, St. Constant, Quebec), served as subjects. Rats were housed in pairs in plastic cages, maintained on a 12-hr light/dark cycle (lights on at 0400) and allowed free access to water and Purina Lab Chow throughout the experiment (except that food was removed two hours prior to BG experiments and returned upon completion of that day's testing).

Surgical Procedures. Forebrain NE lesions were performed with the use of a Kopf stereotaxic apparatus while rats were anesthetized with Somnotol (65 mg/kg, ip). The head of the rat was held in the plane of Konig & Klippel (1963) [i.e. the skull was horizontal, which is achieved by placing the incisor bar at -4.2 mm for male rats in the 300 gram range]. Two burr holes were drilled in the skull 2.6 mm anterior to interaural zero and 1.1 mm each side of the midline suture (determined at bregma). A cannula (prepared from 30-gauge stainless steel tubing) was stereotaxically lowered to 3.7 mm above the interaural line, aimed at the dorsal NE bundle (DNB). Infusions of 2 ul, containing 4 ug of 6-OHDA (expressed as free base, Regis Chemical Co.) dissolved in 0.9% saline with 0.2 mg/ml ascorbic acid, were performed at a rate of 0.4

ul/min, and the cannula left in for an additional 30 seconds to permit diffusion of the drug.

Two more burr holes were drilled in the skull 1.4 mm anterior to interaural zero and 1.3 mm each side of the midline suture. The cannula was then be lowered to 1.0 mm above the interaural line and another 2 ul infused, aimed this time at the ventral NE bundle (VNB). Thus, rats received 4 ug of 6-OHDA in each ascending tract of both the dorsal and ventral NE fiber bundles. The incision was closed with wound clips.

At this time adrenal demedullations or sham operations were performed as described in Experiment 1. Rats were allowed three weeks to recover from surgery before the initiation of the BG experiments. The same groups of rats were used for the footshock stress and the 2-DG experiments. Different groups of rats were used for the DEX experiment, and these rats had their entire adrenal glands removed in order to remove endogenous sources of glucocorticoids in addition to sources of E.

Induction of Hyperglycemia. Application of electric foot-shocks was as described in Expegiment 3. Administration of 2-DG was as described in Experiment 1 and implantation of DEX pellets was carried out as described in Experiment 7.

Blood Glucose. Measurement of BG was carried out as described in previous experiments. Baseline determinations were made prior to the administration of footshock or 2-DG and again 20, 40, 60 and 120 minutes later. In the experiment with DEX, baseline determinations were made prior to pellet implantation and again each of the next 3 days.

Dissection and Assay Procedure. Within 1 to 2 weeks of completion of the BG tests, rats were decapitated, and their brains removed and dissected on saline-rinsed, ice-cooled plates according to the procedure previously used in this laboratory (Bialik et al, 1984a,b). The brainstem, hypothalamus and cortex were retained separately. Immediately after dissection, tissues were frozen in liquid nitrogen and then transferred to a freezer (-60 degrees C) until assayed for NE by a modification of the fluorescence method of Jacobowitz and Richardson (1978). Each brain part sampled was used as its own blank in the fluorescence calculations.

Results

Dexamethasone Pellets

Brain Norepinephrine. Analysis of Variance indicated that hypothalamic NE was significantly reduced to 31% of controls [$F(1,24)=240.17$, $p<.001$], hippocampal NE was significantly reduced to 7% of controls [$F(1,24)=291.56$, $p<.001$] and cortical NE was reduced to 15% of controls [$F(1,24)=348.60$, $p<.001$]. On the other hand, brainstem NE was significantly increased to 109% of controls [$F(1,24)=5.63$, $p<.05$]. These data are presented in Table 8.

Blood Glucose. The increase in BG was approximately 50% less in lesioned rats than in non-lesioned controls. Adrenalectomized rats showed little difference in BG levels compared to sham operated controls. When the data is collapsed across surgery the effect of NE

depletions is more clearly observed (See Figure 8a).

Body Weight. As in previous experiments, implantation of the 25% DEX pellet caused a profound loss of weight over days and ADX rats showed less % weight loss than SHAM-operated rats [$F(1,25)=4.98$, $p<0.05$]. Furthermore, NE depleted rats showed less weight loss in response to the DEX pellet than SHAM-lesioned controls (See Figure 8b). This effect was also found when % weight loss was analysed (data not shown).

Footshock Stress

Brain Norepinephrine. A sample of the animals used in this study showed that lesioned animals had NE levels only 38% of controls in the hypothalamus and 24% in the hippocampus.

Blood Glucose. Analysis of Variance performed on the data indicated main effects due to Lesion [$F(1,22)=5.60$, $p<0.05$], Surgery [$F(1,22)=47.20$, $p<0.001$] and Time of BG determination [$F(4,88)=133.85$, $p<0.001$], as well as an interaction between Surgery and Time [$F(4,88)=17.14$, $p<0.001$]. Animals depleted of brain NE showed a significantly higher BG increase to footshock than controls, and this was most evident in rats with intact adrenals (See Figure 8c). Animals which had their adrenal glands intact, showed an increase in BG levels while DEMED rats had levels that were below baseline (See Figure 8d).

Administration of 2-DG

Brain Norepinephrine. The same rats that were used in the footshock

experiment were used in this study.

Blood Glucose. Analysis of Variance performed on the data indicated main effects due to Lesion [$F(1,27)=5.46$, $p<0.05$], Surgery [$F(1,27)=69.32$, $p<0.001$] and Time of BG determination [$F(3,81)=40.38$, $p<0.001$], as well as, a significant interaction among these three variables [$F(3,81)=3.04$, $p<0.05$]. Figure 8d clearly shows that 2-DG caused an increase in BG levels and that depletion of brain NE enhanced this effect. Furthermore, adrenal demedullation greatly attenuated the increase in BG levels caused by 2-DG (See Figure 8f). Post-hoc analyses of the three-way interaction, using the Newman-Keul's procedure, indicated that NE-depleted rats had significantly higher BG levels than non-depleted rats at 40 ($p<0.01$), 60 ($p<0.01$) and 120 minutes ($p<0.05$), while the NE-depleted+DEMED rats and the non-depleted+DEMED rats did not differ at any time point.

Additionally, it should be noted that the early BG response (i.e. at 20 minutes) to 2-DG in adrenal demedullated rats is thought to be due to direct neural stimulation of the liver by the brain (See Experiment 1). In Figure 8g, it can be seen that the BG response at 20 minutes is very small in non-depleted+DEMED rats, while this response is much larger in NE-depleted+DEMED rats. In fact, if an a priori t-test is used to analyse this comparison, the difference is significant [$t(5)=2.24$, $p<0.05$]. Given this difference, it may be that NE-depleted rats show enhanced BG responses to stressors largely as a result of potentiating the direct neural stimulation of the liver rather than potentiating the effect of E released from the adrenal medulla. So far, in the rat, the

only BG response that might be mediated by direct neural stimulation of the liver is the early response to a large dose of 2-DG (See Figure 1a and 3g). [It should be noted that these responses are only known to be independent of adrenal hormones, other hormonal factors such as glucagon have not been ruled out.] Information relating to this question is obtained in the next experiment, where the BG response to the administration of several adrenergic agonists, including E, is determined.

General Discussion for Experiment 8

Depleting hypothalamic and forebrain NE with 6-OHDA potentiated the rise in BG caused by two different stressors, footshock and injection of 2-DG. The increase in blood glucose caused by these stressors is largely due to the release of E from the adrenal medulla and in the next experiment it is determined if brain NE depletions potentiate the BG response to ip injections of E.

It was also found that depletion of brain NE attenuated both the rise in BG and the weight loss caused by DEX. These data indicate that brain NE is involved in the metabolic response to chronic glucocorticoid treatment. This is an important finding since it demonstrates that brain NE systems can influence peripheral metabolic actions of glucocorticoids.

It has been assumed that chronic glucocorticoid treatment causes hyperglycemia by effects entirely in the periphery. The effects of DEX

that would affect BG levels are 1) the liberation of fatty acids and amino acids from peripheral tissues (which would cause weight loss), 2) the facilitation of gluconeogenesis by the liver (which would cause an increase in blood glucose levels) and 3) blockade of insulin release (BarsegRian & Levine, 1980). In order for brain NE lesions to have reduced DEX hyperglycemia, the lesions either reduced the effects of DEX on peripheral tissue catabolism or enhanced the utilization of glucose, maybe by interfering with the effect of DEX on the insulin receptor. In Figure 8b, it can be seen that NE depletion reduced weight loss caused by DEX, therefore it is assumed that peripheral tissue catabolism was inhibited by the disruption of the brain NE systems. This suggestion is tentative however, since it was found that the effects of DEX on body weight and BG levels are not necessarily inversely related (See Experiments 7a and b). It is not known whether brain NE depletions cause general metabolic alterations or whether effects are specific to the situation studied here.

In summary, the data indicate that brain NE might be involved in controlling some aspect(s) of peripheral glucose metabolism. We are currently investigating the effect of brain NE depletions on basal metabolic rate and metabolic rate following cold challenge. These studies are being carried out in collaboration with Dr. Peter Pawson at the National Research Council Laboratories. It has already been demonstrated that NE is involved in the control of energy metabolism in the brain (eg. Quach et al, 1978; Magistretti, Morrison, Shoemaker, Sapin & Bloom, 1981; Morrison & Magistretti , 1985; Harik, Busto &

Martinez, 1982), and it may be that central NE systems are also involved in controlling peripheral metabolic responses to various challenges.

EXPERIMENT 9

In the previous experiment, it was found that lesioning the ascending NE fiber bundles altered the BG response to various stressors. The responses to footshock and 2-DG stress were potentiated, while the response to implantation of a DEX pellet was somewhat attenuated. In the next experiment, it was determined if similar lesions to the ascending NE fiber bundles affect BG responses to adrenergic agonists, which are presumed to be non-stressful. The hyperglycemic responses to the direct adrenergic agonists E, CLON and ISO, and the indirect agonist, amphetamine (AMPHET), were determined in NE depleted rats.

Method

Subjects. Twenty male Wistar rats, weighing 200-250 grams upon receipt from the supplier (Charles River Farms, St. Constant, Quebec), served as subjects. Rats were housed in pairs in plastic cages. Rats were maintained on a reversed 12-hr light/dark cycle (lights on at 2030) and allowed ad libitum access to water and Purina Lab Chow throughout the experiment (except for two hours preceeding BG experiments). Groups were designated according to treatment as dorsal plus ventral NE bundle lesions (DVNB) and sham lesions (SHAM). All rats received a single injection of each of the drugs. At least 3 days were allowed between

test days.

Surgical Procedures. Rats received DVNB lesions as outlined in the previous experiment.

Drug Administration. All drugs were injected intraperitoneally and the doses were as follows; clonidine - 0.05; isoproterenol - 0.05; epinephrine - 0.05; and amphetamine - 0.5 mg/kg.

Determinations of BG and brain NE were carried out as previously described.

Results and Discussion

Inspection of Figures 9a, 9b, 9c and 9d clearly indicate that depletion of brain NE had no effect on the increase in BG caused by CLON [$F(1,17)=0.00$,N.S.], ISO [$F(1,17)=2.42$,N.S.], E [$F(1,17)=0.02$,N.S.] or AMPHET [$F(1,16)=0.60$,N.S.].

Of special importance is the finding that amphetamine did not cause much of a change in BG levels. This drug is thought to enhance the release of NE from terminals in the brain, an effect that would be hypothesized by Smythe et al (1984) to cause increases in BG levels. Since amphetamine is thought to affect brain NE systems by facilitating release of this transmitter, any effects of amphetamine on BG that were mediated by central NE systems should be altered in NE depleted rats. However, there was no difference in the BG response to AMPHET in intact compared to NE depleted rats. In conclusion, there is no evidence to indicate that brain NE systems are involved in the BG responses to E, ISO, CLON or AMPHET.

The results from experiments 8 and 9 indicate that brain NE systems are not responsible for initiating the BG response to several challenges. However, these systems appear to be involved in modulating the BG response to several challenges when these challenges include a stressful component (footshock, 2-DG and possibly chronic DEX administration) but these systems are not involved in any way in modulating the hyperglycemic responses to various adrenergic agonists.

EXPERIMENTS 10 and 11

Blood Glucose and Conditioned Behavior

The entire paper to this point has been concerned with determining the physiological mechanisms that control BG levels in behaving rats. It is clear that adrenal E is the major mediator of the hyperglycemic response in each case that the response occurs within several hours of treatment. It is also clear that the easiest way to block the BG response during aversively-motivated conditioning is to remove the adrenal medullae. Furthermore, the BG response to the conditioning procedure can be potentiated by administering exogenous glucose. Therefore, the next two experiments investigate the effects of altering BG levels on acquisition and retention of an aversively-motivated "Y-maze" task.

EXPERIMENT 10

It has been reported that posttraining administration of glucose can enhance the retention of a passive avoidance response (Hall & Gold, 1985; Gold, 1986). The present experiment determines if posttraining injections of glucose can alter retention performance in an aversively-motivated discrimination task.

Method

Subjects. Subjects were 48 male Wistar rats weighing 200-225 grams on delivery (Charles River Farms, St. Constant, Quebec). Rats were maintained as described for previous experiments. After at least one week of acclimatization to the animal quarters, rats were randomly assigned to one of four groups. Groups were designated according to amount of glucose that they were injected with. Rats received either 2000 mg/kg ip of D-glucose (2000), 1000 mg/kg (1000), 500 mg/kg (500) or 0 mg/kg (VEH).

Avoidance Training Apparatus and Procedures. A "Y-maze", adapted from Flexner, Flexner & Roberts (1967) was used for behavioral testing. The maze had two black arms and one white arm, each arm measuring 51 cm long, 14 cm wide and 23 cm high. The floor was made of 0.3 mm diameter stainless steel rods, inserted in the walls 3 cm from the ground. The rods were connected by neon bulbs to permit scrambling of the shock (A.C. electrical current, 0.5 mA for 0.5 seconds). A microprocessor, fabricated by Carleton University Science Workshop, controlled the

presentation of the shock.

Training was initiated by placing the rat in one of the black arms. After 15 seconds, the microprocessor delivered a 0.5 mA shock every 2 seconds. The rat had to leave the start box (black arm) and enter the white arm of the maze in order to escape/avoid shock. The grids in the white arm were never electrified, making this arm the "safe area" for the rat. The rat had to stay in the white arm or "safe area" for 30 seconds before the trial was considered completed. If the rat left the "safe area" it received a footshock every 2 seconds until it returned to the "safe area". The trial continued until the rat stayed in the "safe area" for 30 seconds.

The sequence of training trials was two active avoidance trials (startbox was black arm #1 and then black arm #2) followed by one passive avoidance trial (startbox was the white arm or "safe area"). For the passive avoidance trials, the rat was placed in the white arm and it received shocks every 2 seconds only if it left the safe white arm. The trial continued until the rat had stayed in the white arm for 60 seconds consecutively. Training continued until the rat had performed 2 active and 2 passive avoidance trials without receiving a shock (i.e. 4 consecutive errorless trials).

A retention test was carried out 48 hours later. The procedure was identical to that of training, except no shock was delivered. The rat received three trials, one that started from each of the three arms of the "Y-maze". The sequence was always active (black #1) - passive (white) - active (black #2). There was a 30 second intertrial interval.

Drug Administration. Immediately following the completion of training animals were weighed, injected with one of the drug treatments described above, and returned to their home cage.

Results and Discussion

There were no differences among groups in acquisiton of the avoidance response. When either the active avoidance or the passive avoidance latencies were analyzed separately there were no differences in retention performance among groups. However, if the criterion for correct performance on the retention trials was that the latency in the passive avoidance trial was 30 seconds or more longer than the mean of the active avoidance trials, it is clear that the 500 and 1000 mg/kg doses of glucose enhanced performance while the 2000 mg/kg dose had no effect or may even have impaired performance (See Figure 10).

The rationale for using a retention performance criterion that reflects a relationship between performance on the active and passive avoidance components of the task actually reflects the purpose for designing this "Y-maze" task. That is, during training the rat is trained to associate the white arm with "safety". On alternating trials, a correct response required running from a black arm to the white arm (active avoidance) or not running from the white arm (passive avoidance). Therefore, if the rat performed perfectly on the retention test, it would leave the black arm very quickly (short latency) and would stay in the white arm for the entire trial (60 seconds). The score obtained by subtracting the passive avoidance latency from the active

avoidance latency should be large if the rat performs both tasks optimally. This score should get progressively smaller as the rat performs more poorly on the active avoidance trial (longer latency) and/or on the passive avoidance trial (shorter latency). The criterion of 30 seconds was an arbitrary figure, but it was felt that the rat had considerable recall of the training if it took relatively longer to leave the white arm than it did to leave the black arm.

One aspect of this task is that good performance on the retention trial cannot be due to either hyper- or hypo-activity as it can in active and passive avoidance tasks, respectively. Therefore, the data from this last experiment support the view proposed by others that posttraining administration of glucose can alter memory processes. Furthermore, this view was extended to include effects of posttraining glucose on a discrimination task.

EXPERIMENT 11

The converse of the last experiment would be to prevent the increase in BG caused by the avoidance training and test retention performance. In the next experiment, the BG response to the avoidance training was prevented by removing the adrenal medulla (See Bialik & Roberts, 1985). Both DEMED and SHAM rats were trained in the "Y-maze" task and given post-training injections of either glucose or vehicle.

Method

Subjects. Subjects were 24 male Wistar rats, weighing 200-250 grams on delivery (Charles River Farms, St. Constant, Quebec). Animals were maintained as described previously. Groups were designated according to surgery and posttraining drug administraiton as DEMED+GLUC, DEMED+VEH, SHAM+GLUC and SHAM+VEH.

Avoidance Training and Retention. Training and retention trials were carried out as described in Experiment 10. The only difference was that the acquisition criterion was increased from 4 to 6 consecutive errorless trials. It was hoped that this change would increase the retention performance of control rats so that a deficit in performance could be observed in the operated rats if it were to occur.

Drug Administration. Immediately following training animals were administered one of the drug treatments described above.

Results and Discussion

Analysis of Variance performed on the data indicated that DEMED rats made significantly more incorrect trials in reaching the acquisition criterion than sham-operated rats [$F(1,42)=5.21$, $p<0.05$]. Since there was a significant difference in acquisition performance between DEMED and SHAM rats, it was impossible to compare the retention performance between these two groups. However, it is clear from the data that posttraining injection of GLUC (500 mg/kg ip) facilitated retention performance in DEMED rats compared to DEMED rats given posttraining vehicle injections (See Figure 11). A similar effect was not observed in

SHAM rats. It is clear that SHAM rats show an increase in BG levels following footshock similar to that experienced in the present task and that DEMED rats do not show this increase in BG levels (See Figure A). the exogenous GLUC would combine with the exogenous glucose to give the observed BG levels (if measured). This summated level of BG can vary depending on numerous factors including handling, housing, feeding etc. If the BG levels were too high, poorer performance might be observed (Hall & Gold, 1985; Gold, 1986; See also Figure 10). However, in DEMED rats the level of BG would increase very little in response to these variables (and may even decrease; See Figure 6). Therefore, the effect of exogenous administration of GLUC to DEMED rats would more likely bring the BG levels within the "optimal range" for this conditioned task, whereas BG levels for SHAM rats may be increased too much and levels may be outside this "optimal range".

The present data do not allow a comparison of retention performance of DEMED rats to that of SHAM rats because DEMED rats demonstrated an acquistion deficit. However, the discovery of the acquisiton deficit in the DEMED rats is an important finding, since previous work assumes that DEMED rats have altered retention performance (See Borrell et al, 1984a,b). These investigators used a one trial passive avoidance task in which acquisition behavior cannot be measured. Furthermore, it should be determined whether pretraining injections of glucose can alleviate this deficit. Finally, with respect to the original hypothesis described in the introduction (See page 3), when DEMED is combined with brain NE depletion (which impairs the local release of glucose from glycogen in

brain tissue) an even greater deficit in avoidance performance would be expected.

Future Experiments

The hypothesis has been suggested that a deficit in the energy supply to the brain might be an important factor in the behavioral impairments observed in DEMED rats. A direct method of addressing this hypothesis would be to compare a number of physiologically different fuels (eg. beta-hydroxybutyrate, manose, fructose), to the facilitative effects of glucose on learning and memory in DEMED and normal rats.

An additional line of research which is worth pursuing (and will be pursued), is to attempt to determine exactly what role might BG levels play in the normal and abnormal functioning of memory? In Appendix A, I suggest that BG levels are an important part of the physiological state of an organism at the time that certain experiences occur (eg. aversive conditioning), and this physiological state acts as a cue that is associated with the avoidance response in the organism's memory.

SUMMARY

Original Goals

The original goals of this paper were: 1) to determine the physiological mechanisms that mediated BG increases in response to

various stressors, 2) to determine if alterations in BG levels altered conditioned behavior.

With respect to the first goal, it was clearly demonstrated that release of adrenal E was responsible for the BG increase following several stressors, including behaviorally-relevant footshock. Furthermore, this effect of E was found to be mediated mainly by an interaction with alpha-2 adrenergic receptors. Despite numerous claims to the contrary, and an extended effort on our part, we failed to identify a rapid increase in BG levels that was independent of the adrenal medulla, especially when behaviorally-relevant footshock was used as the stressor.

With respect to the second goal of this paper, it was demonstrated that posttraining administration of glucose could enhance the retention of an aversively-motivated discrimination response. It was also demonstrated that DEMED rats, who do not show the normal increase in blood glucose levels caused by the conditioning paradigm, are impaired in acquiring this discrimination response. Furthermore, exogenous administration of glucose enhanced retention performance in DEMED rats. An hypothesis was developed in which to investigate the psychological nature of the role that changes in BG levels might play in memory processing of aversively-motivated conditioned responses.

Other Findings

In the process of carrying out the numerous studies in this paper, several additional findings were made. First, adrenergic agonists

stimulated the release of CORT and this effect may be partially due to the increase in BG levels caused by these agents. Second, DEMED can prevent the increase in BG levels caused by electrical stimulation of the hypothalamus. Third, brain NE systems can modulate stress-induced BG responses and possibly alter peripheral metabolic actions of glucocorticoids.

CONCLUSION

The purpose of the proposed experiments was to relate adrenal hormone effects on the supply of glucose to the brain (glucose is the major substrate for cerebral energy metabolism) to the effects of these hormones on the storage of information. Ultimately, the goal is to determine if alterations in the control of the BG response to stress are involved in (1) alterations in cognitive function following application of acute or chronic stressors, or administration of stress-related hormones; (2) alterations in cognitive function during normal ageing, which may be precipitated or exacerbated by stress and/or stress hormones (eg. Sapolsky, Krey & McEwen, 1984; Sapolsky, Krey, McEwen & Rainbow, 1984); (3) alterations in emotional behavior and/or cognitive function in animal models of depression or anxiety states (which might be precipitated or exacerbated by stress).

The group of experiments that has been proposed here is a starting point in an attempt to relate a very global response "a change in glucose levels in the blood" to a very basic and important function of the brain "a change in the ability to store and/or process information."

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Table 1. Mean (± S.E.M.) regional brain norepinephrine (NE) levels (ug/g wet weight) for rats receiving 6-OHDA or VEH infusions into both the dorsal and ventral ascending NE bundles (DVNB and SHAM, respectively). Numbers in brackets indicate the amount of NE remaining in that brain part compared to the respective controls. DVNB and adrenalectomized (ADX) groups were compared to the SHAM group, while the DVNB+ADX group was compared to the ADX group.

Group	n	Cortex	Hippocampus	Hypothalamus	Brainstem
SHAM	7	0.0883±0.005	0.1585±0.009	0.9745±0.061	0.2609±0.011
DVNB	6	0.0081±0.001 (9%)	0.0112±0.003 (7%)	0.2740±0.033 (28%)	0.2937±0.003 (113%)
ADX	8	0.0807±0.002 (91%)	0.1534±0.012 (97%)	1.0212±0.032 (105%)	0.2775±0.009 (106%)
DVNB +ADX	7	0.0167±0.005 (21%)	0.0096±0.003 (6%)	0.3970±0.061 (39%)	0.2905±0.011 (105%)

Figure 1a. Mean change in blood glucose levels at various times following injection of 2-DG (500 mg/kg ip) in adrenalectomized (ADX), demedullated (DEMED) and sham-operated (SHAM) rats. * indicates a significant difference from SHAM-operated controls ($p<0.01$). † indicates a significant difference from baseline for the ADX and DEMED groups ($p<0.01$). Comparisons were made using the Newman-Keul's procedure.

Figure 1a

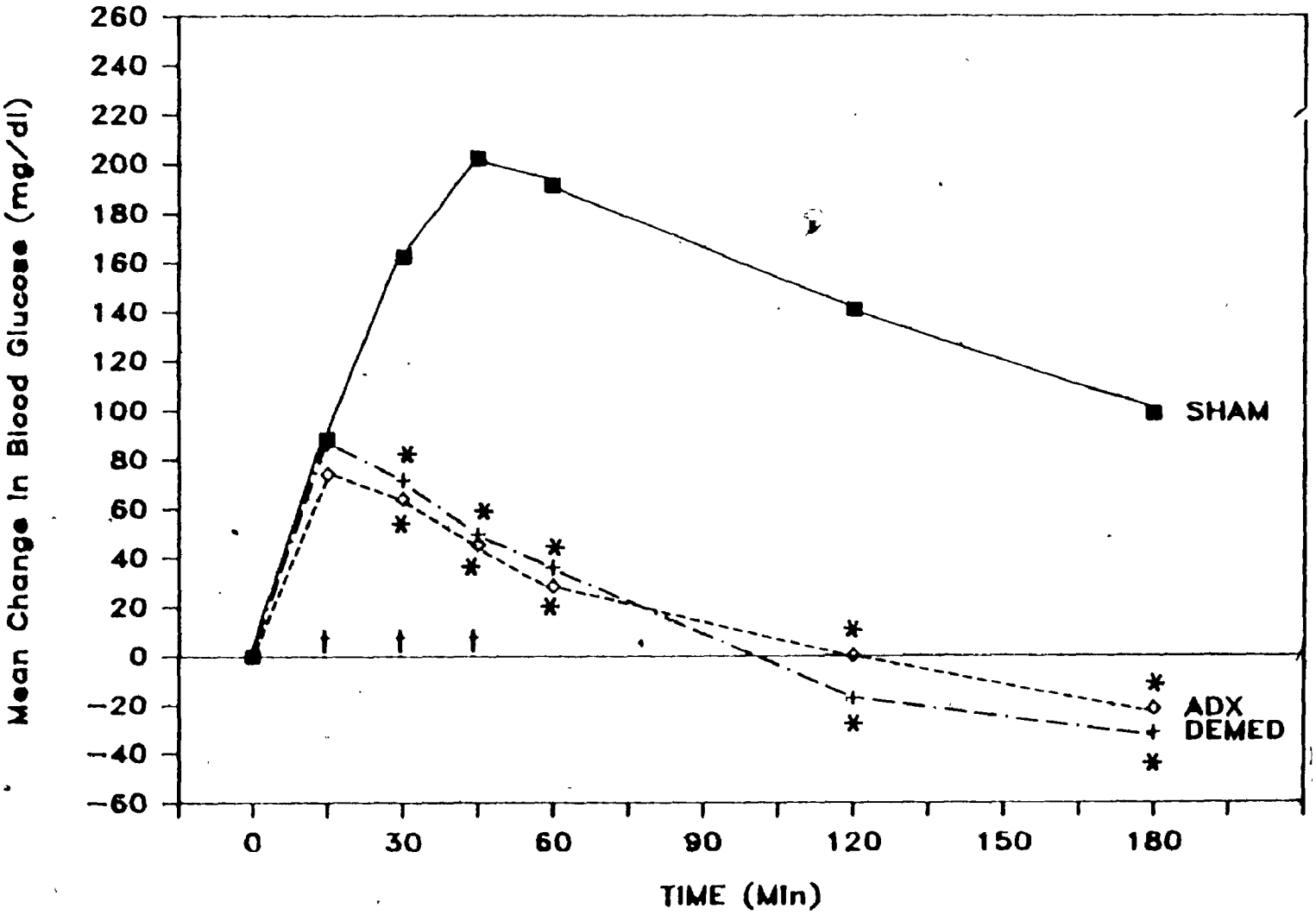

Mean Change In Blood Glucose (mg/dl)
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
0
30
60
90
120
150
180
TIME (Min)
SHAM
ADX
DEMED

Figure 1b. Mean change in blood glucose levels at various times following injection of 2-DG (125 mg/kg ip) in demedullated (DEMED), adrenal denervated (DEN) and sham-operated (SHAM) rats. * indicates a significant difference from SHAM-operated controls ($p<0.01$ as determined by the Newman-Keul's test).

Figure 1b

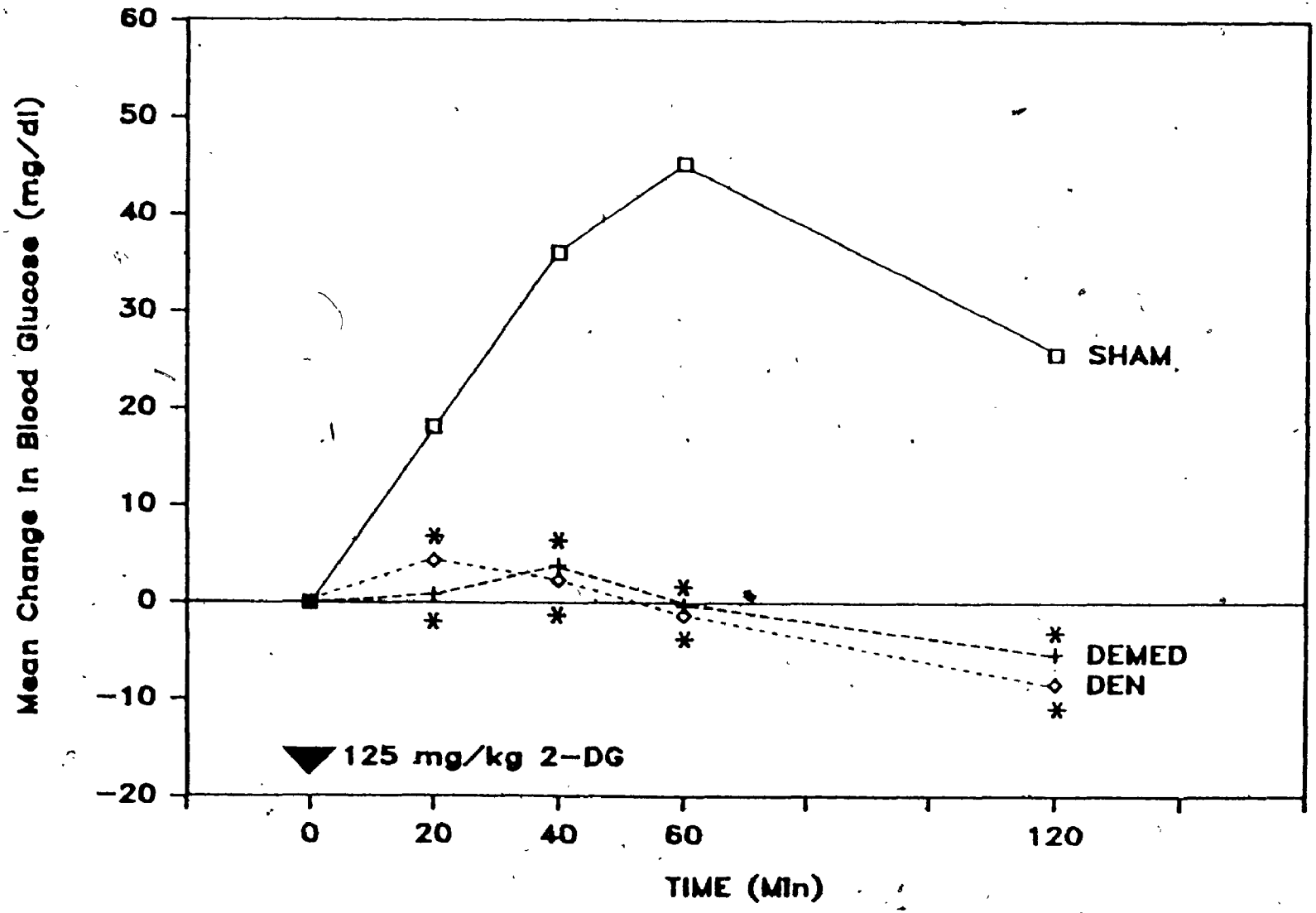

Mean Change In Blood Glucose (mg/dl)
60
50
40
30
20
10
0
−10
−20
0
20
40
60
120
TIME (Min)
SHAM
DEMED
DEN
125 mg/kg 2-DG

Figure 2a. Mean plasma corticosterone levels 20 minutes following injection (0.10 mg/kg ip) of clonidine (CLQN), isoproterenol (ISO) or epinephrine (E).

Figure 2a

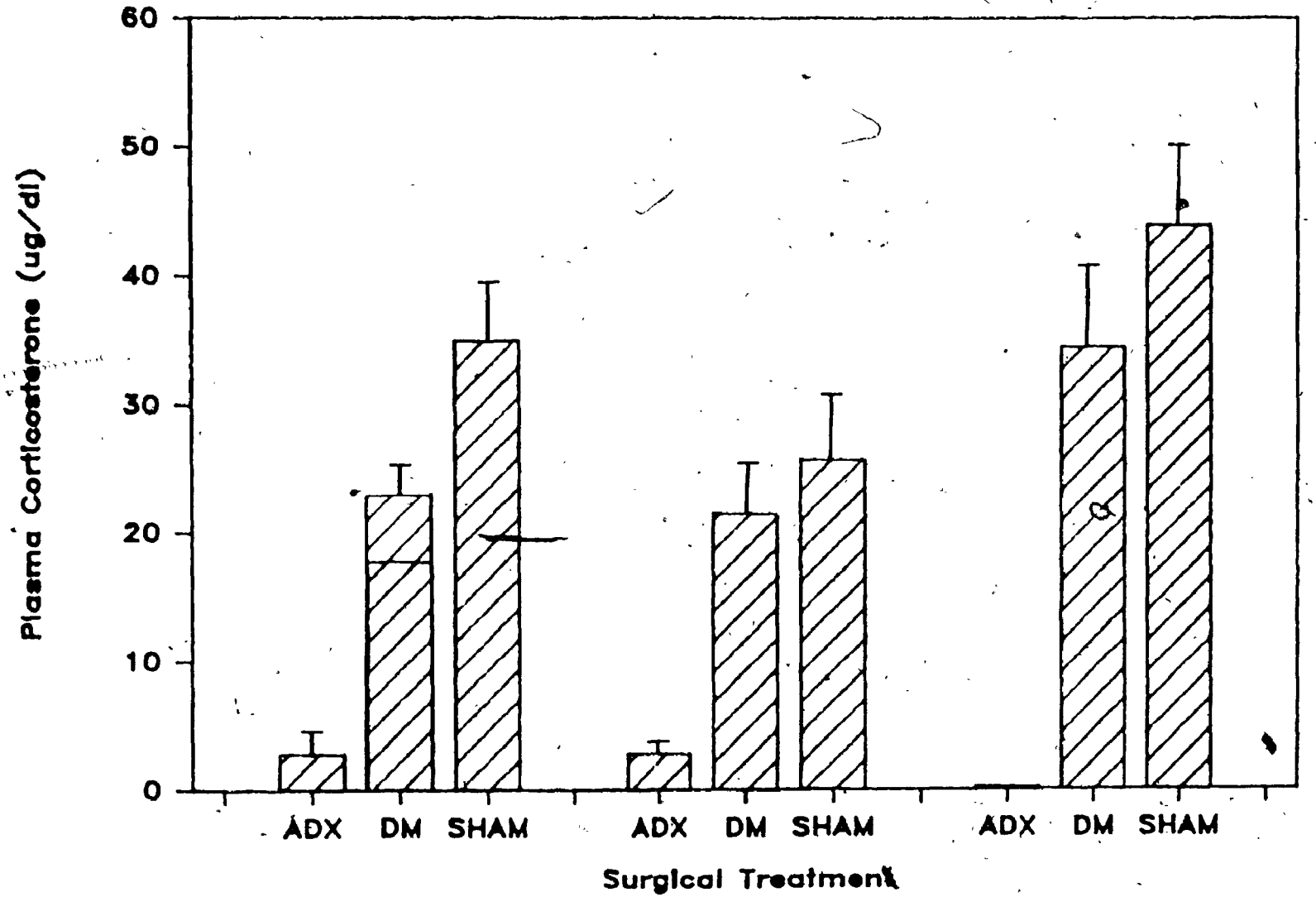

60
50
40
30
20
10
0
Plasma Corticosterone (ug/dl)
ADX
DM
SHAM
ADX
DM
SHAM
ADX
DM
SHAM
Surgical Treatment
CLON
ISO
E

Figure 2b. Mean plasma corticosterone levels 20 minutes following injection (ip) of several adrenergic agonists or 30 minutes following the administration of footshock stress. The doses of the drugs were 0.50 mg/kg for phenylephrine (PHENYL) and 0.10 mg/kg for clonidine (CLON), isoproterenol (ISO) and epinephrine (E). The footshock data were taken from Experiment 1b. Rats had received 15 footshocks following the procedure described in the text (See page 40).

Figure 2b

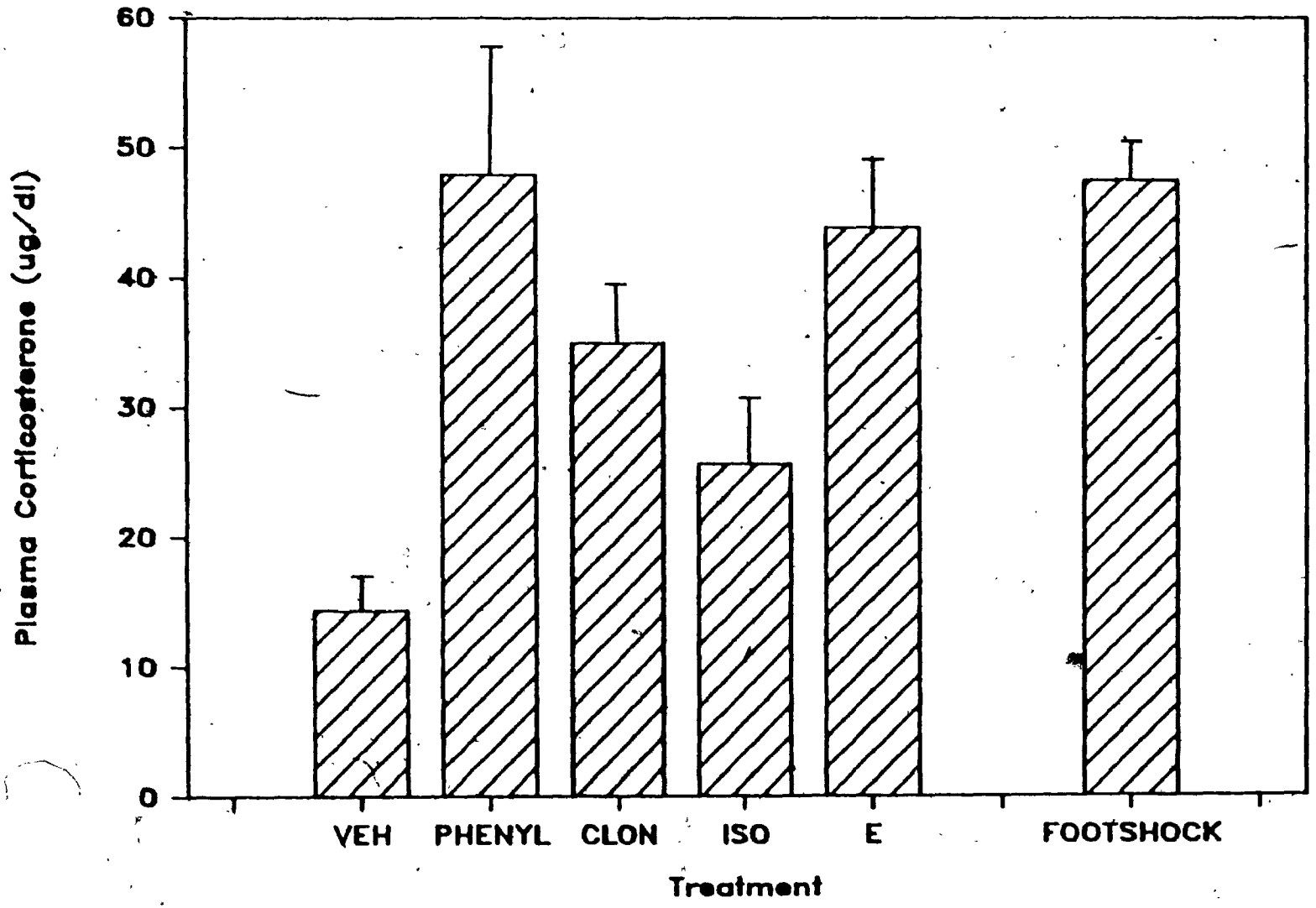

60
50
40
30
20
10
0
Plasma Corticosterone (ug/dl)
VEH
PHENYL
CLON
ISO
E
FOOTSHOCK
Treatment

Figure 2C. Mean change in blood glucose levels for the three Surgical Treatments and the four Doses of Clonidine. Data are collapsed across Sampling Time. * indicates a significant difference from the ADX group at the same dose level ($p<0.01$). † indicates a significant difference from the DEMED group at the same dose level ($p<0.01$). Comparisons were made using the Newman-Keul's test.

Figure 2c

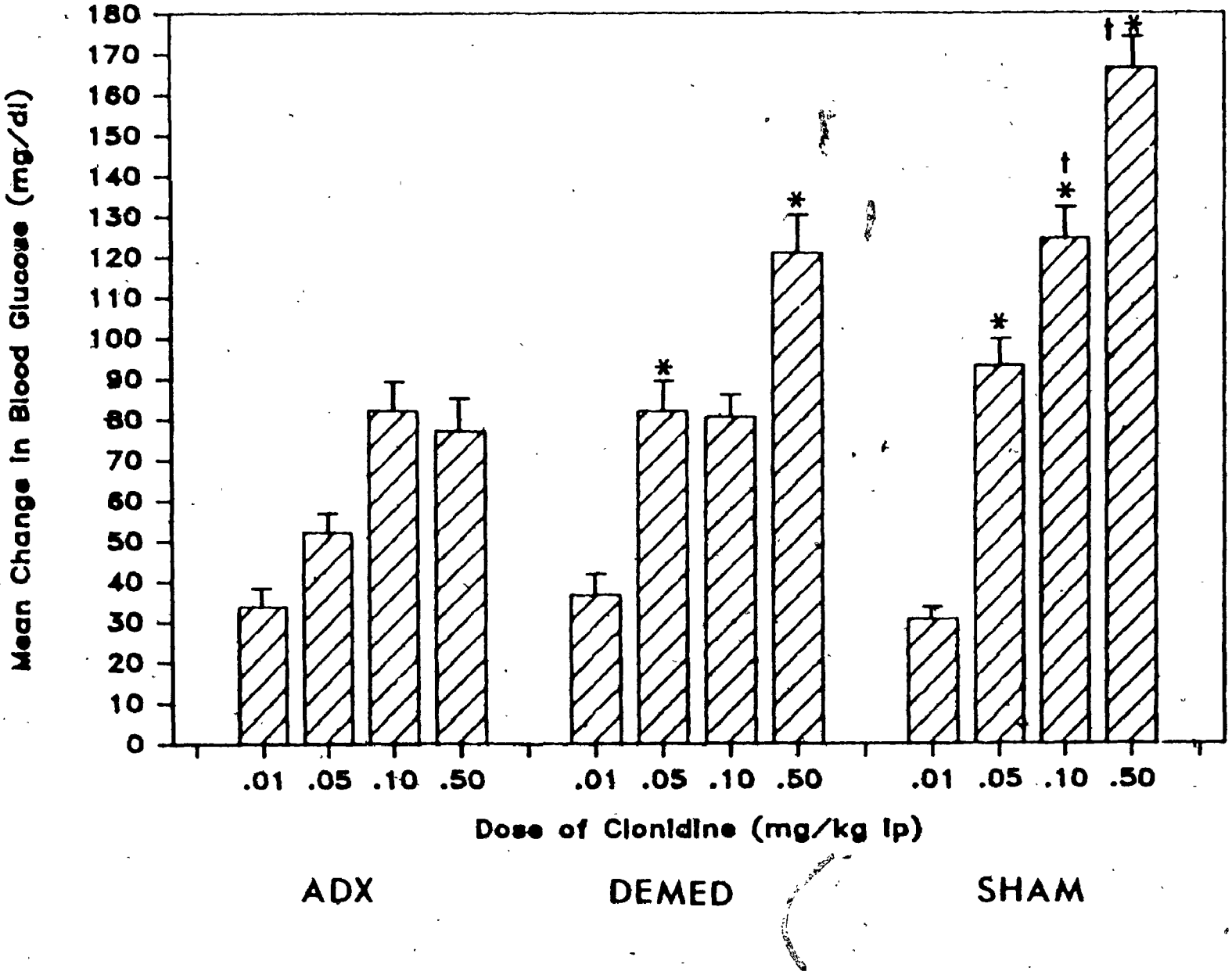
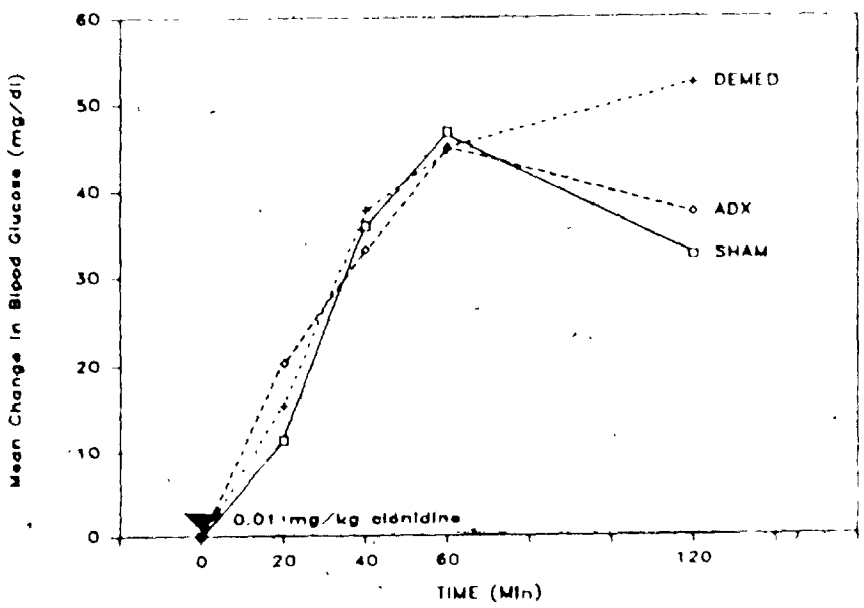
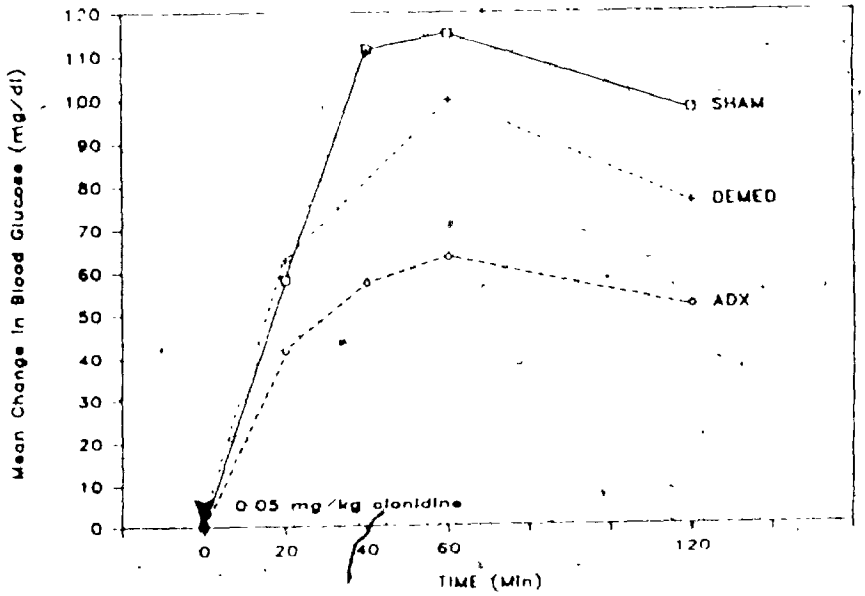
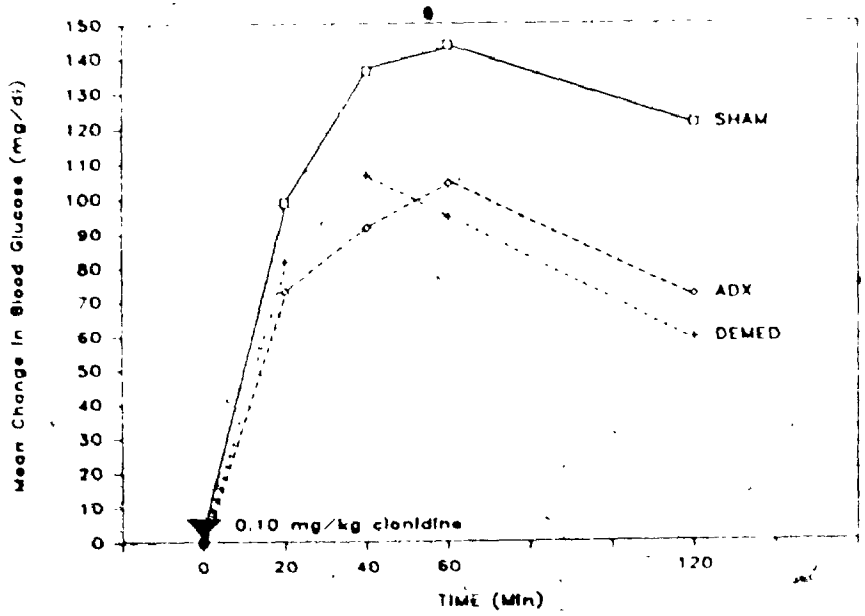

Mean Change in Blood Glucose (mg/dl)
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
.01 .05 .10 .50 .01 .05 .10 .50 .01 .05 .10 .50
Dose of Clonidine (mg/kg ip)
ADX
DEMED
SHAM

Figure 2d. Mean change in blood glucose levels at various times following the ip injection of clonidine in adrenalectomized (ADX), demedullated (DEMED) and sham-operated rats (SHAM). Data are shown for 0.01 (panel a), 0.05 (panel b), 0.10 (panel c) and 0.50 (panel d) mg/kg doses of clonidine.

Figure 2d


Mean Change In Blood Glucose (mg/dl)
60
50
40
30
20
10
0
DEMED
ADX
SHAM
0.01 mg/kg clonidine
0
20
40
60
120
TIME (Min)


Mean Change In Blood Glucose (mg/dl)
120
110
100
90
80
70
60
50
40
30
20
10
0
SHAM
DEMED
ADX
0.05 mg/kg clonidine
0
20
40
60
120
TIME (Min)


Mean Change In Blood Glucose (mg/dl)
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
SHAM
ADX
DEMED
0.10 mg/kg clonidine
0
20
40
60
120
TIME (Min)

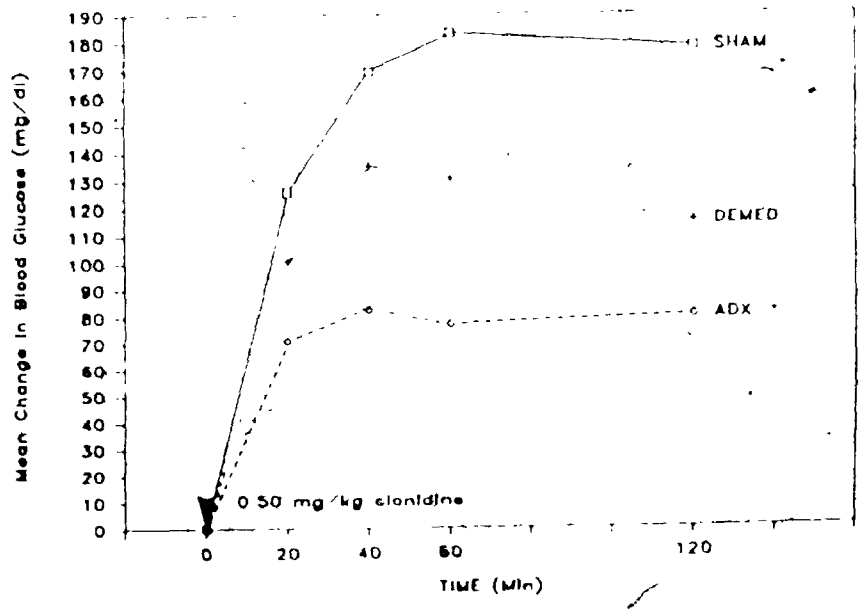
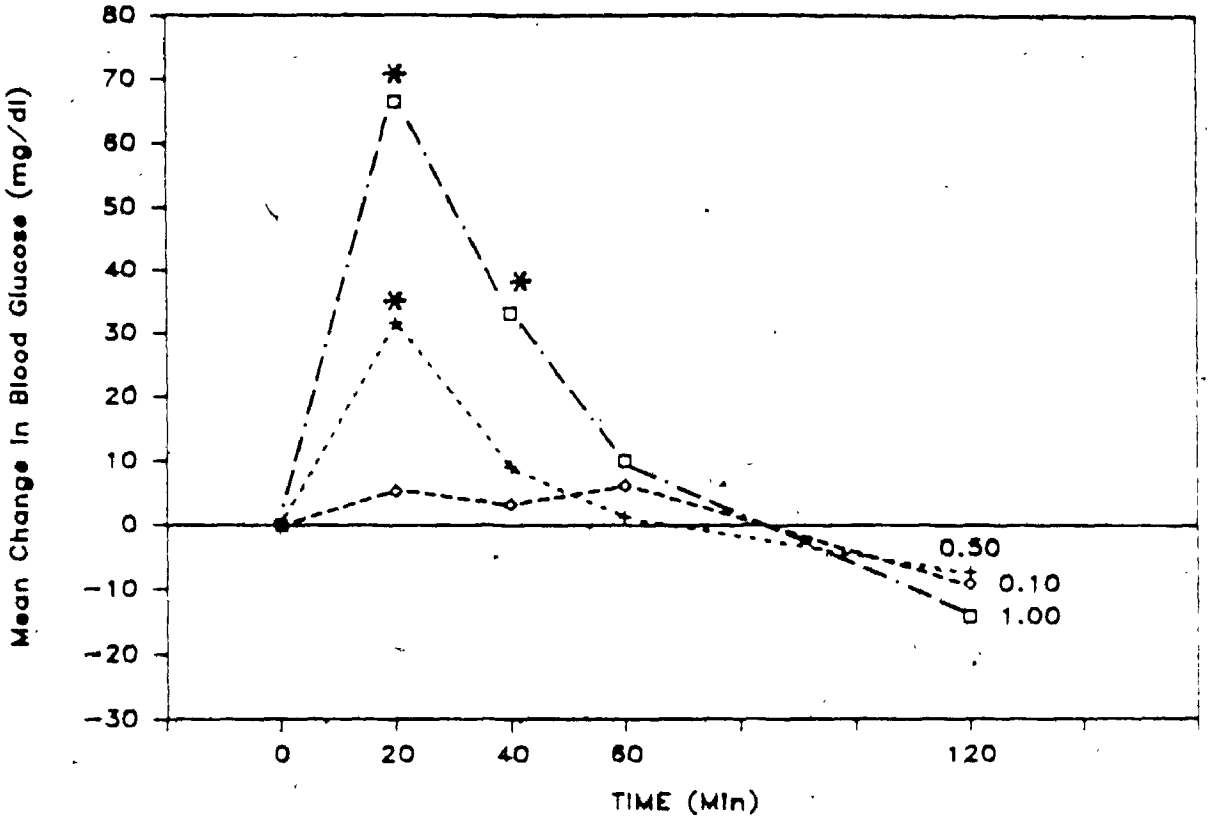

Mean Change In Blood Glucose (mg/dl)
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
SHAM
DEMED
ADX
0.50 mg/kg clonidine
0
20
40
60
120
TIME (Min)

Figure 2e. Mean change in blood glucose levels at various times following three doses of phenylephrine. Data are shown for sham-operated rats (SHAM) in the top panel and for adrenalectomized rats (ADX) in the bottom panel. * indicates a significant difference from baseline ($p<0.01$). Comparisons were made using the Newman-Keul's procedure.

Figure 2

SHAM



ADX

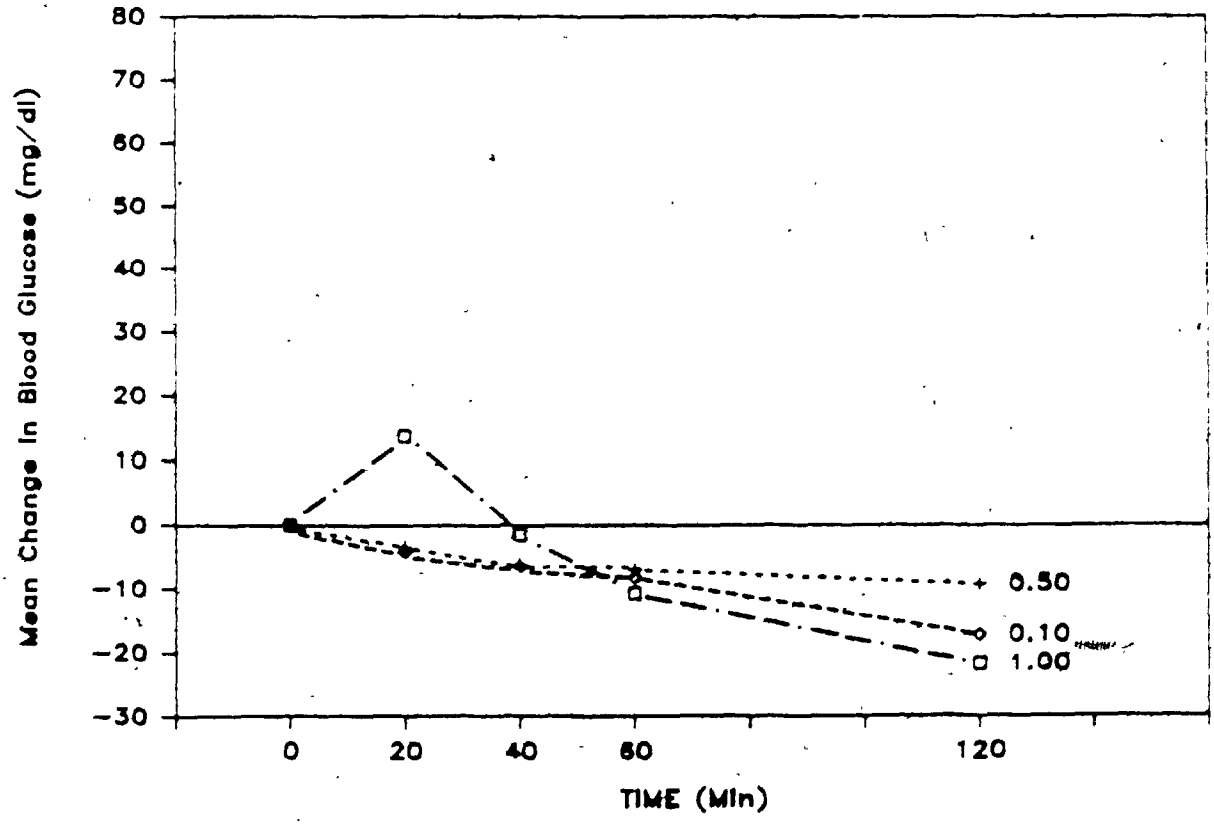


Figure 2g Mean change in blood glucose levels at various times following the injection of isoproterenol in adrenalectomized (ADX), demedullated (DEMED) and sham-operated rats (SHAM). Data are collapsed across Dose of Isoproterenol. * indicates a significant difference from the SHAM group ($p<0.05$ as determined by the Newman-Keul's test).

Figure 2g

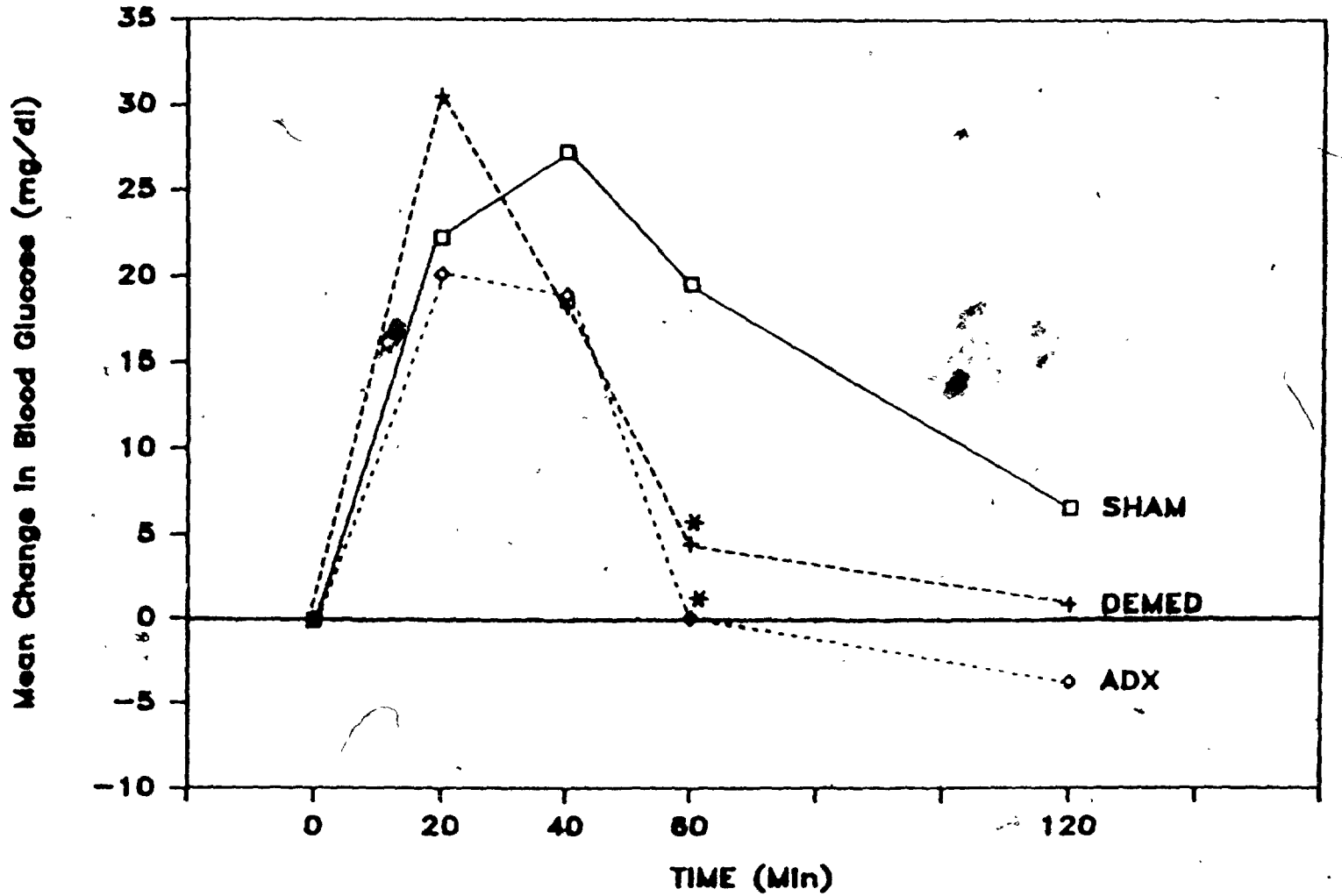
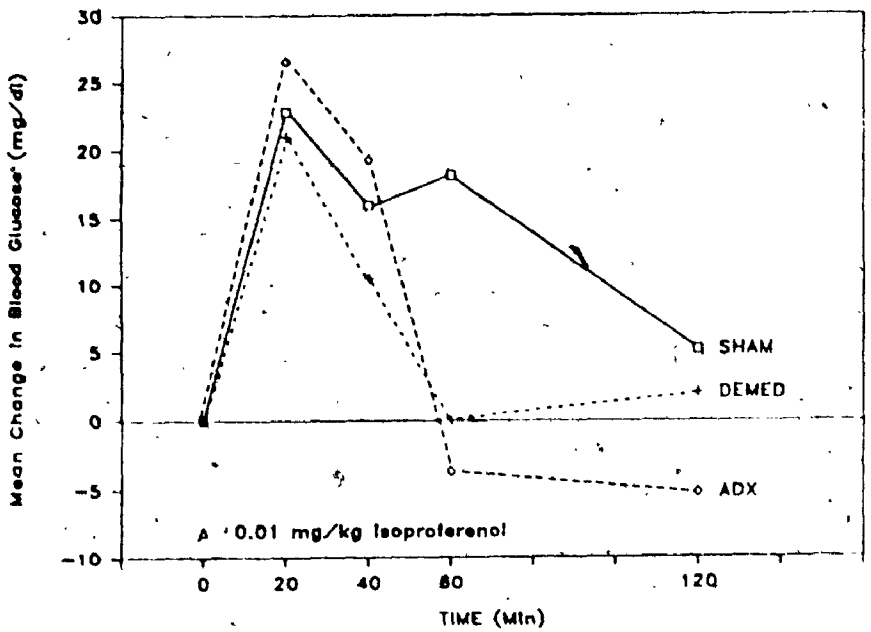
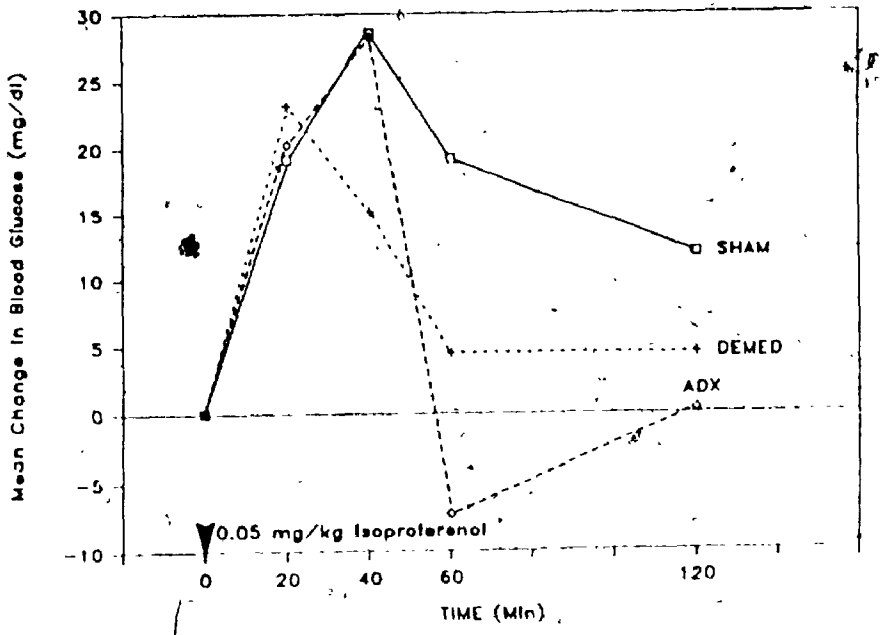
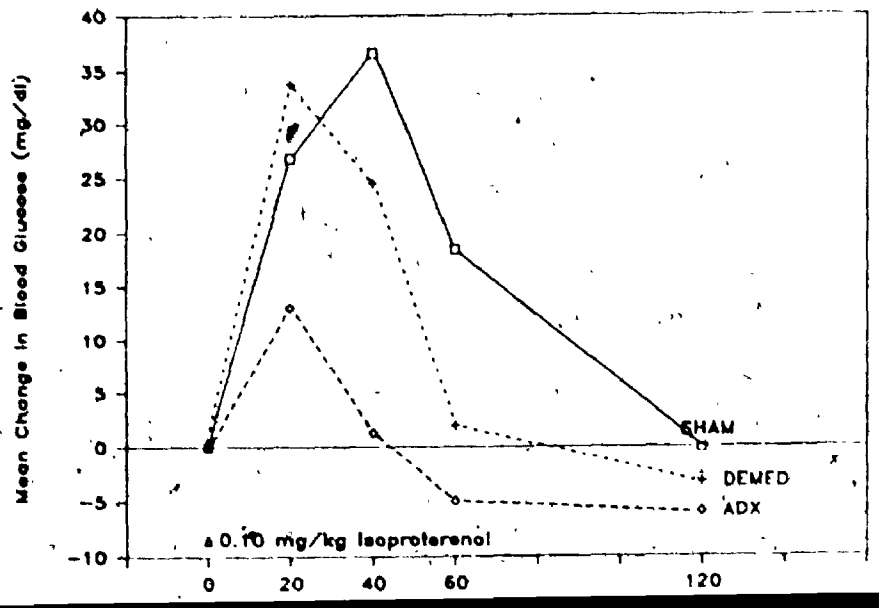

Mean Change In Blood Glucose (mg/dl)
35
30
25
20
15
10
5
0
-5
-10
0
20
40
60
120
TIME (Min)
SHAM
DEMED
ADX

Figure 2f. Mean change in blood glucose levels at various times following the ip injection of isoproterenol in adrenalectomized (ADX), demedullated (DEMED) and sham-operated rats (SHAM). Data are shown for 0.01 (panel a), 0.05 (panel b), 0.10 (panel c) and 0.50 (panel d) mg/kg doses of isoproterenol.

Figure 2f


Mean Change in Blood Glucose (mg/dl)
30
25
20
15
10
5
0
-5
-10
0
20
40
80
120
TIME (Min)
SHAM
DEMED
ADX
0.01 mg/kg Isoproterenol


Mean Change in Blood Glucose (mg/dl)
30
25
20
15
10
5
0
-5
-10
0
20
40
60
120
TIME (Min)
SHAM
DEMED
ADX
0.05 mg/kg Isoproterenol


Mean Change in Blood Glucose (mg/dl)
40
35
30
25
20
15
10
5
0
-5
-10
0
20
40
60
120
SHAM
DEMED
ADX
0.10 mg/kg Isoproterenol

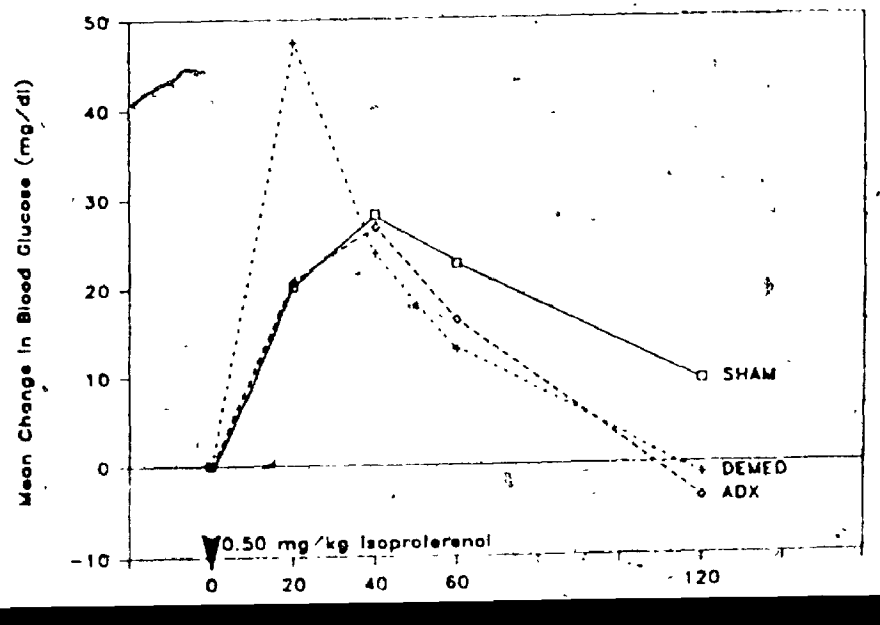

Mean Change in Blood Glucose (mg/dl)
50
40
30
20
10
0
-10
0
20
40
60
120
SHAM
DEMED
ADX
0.50 mg/kg Isoproterenol

Figure 2h. Mean change in blood glucose levels at various times following the administration of clonidine plus isoproterenol (administered as a cocktail, 0.01 mg/kg ip of each drug) for adrenalectomized (ADX), demedullated (DEMED) and sham-operated rats (SHAM). In each group, a significant increase in blood glucose levels that lasted throughout the testing period was observed.

Figure 2h

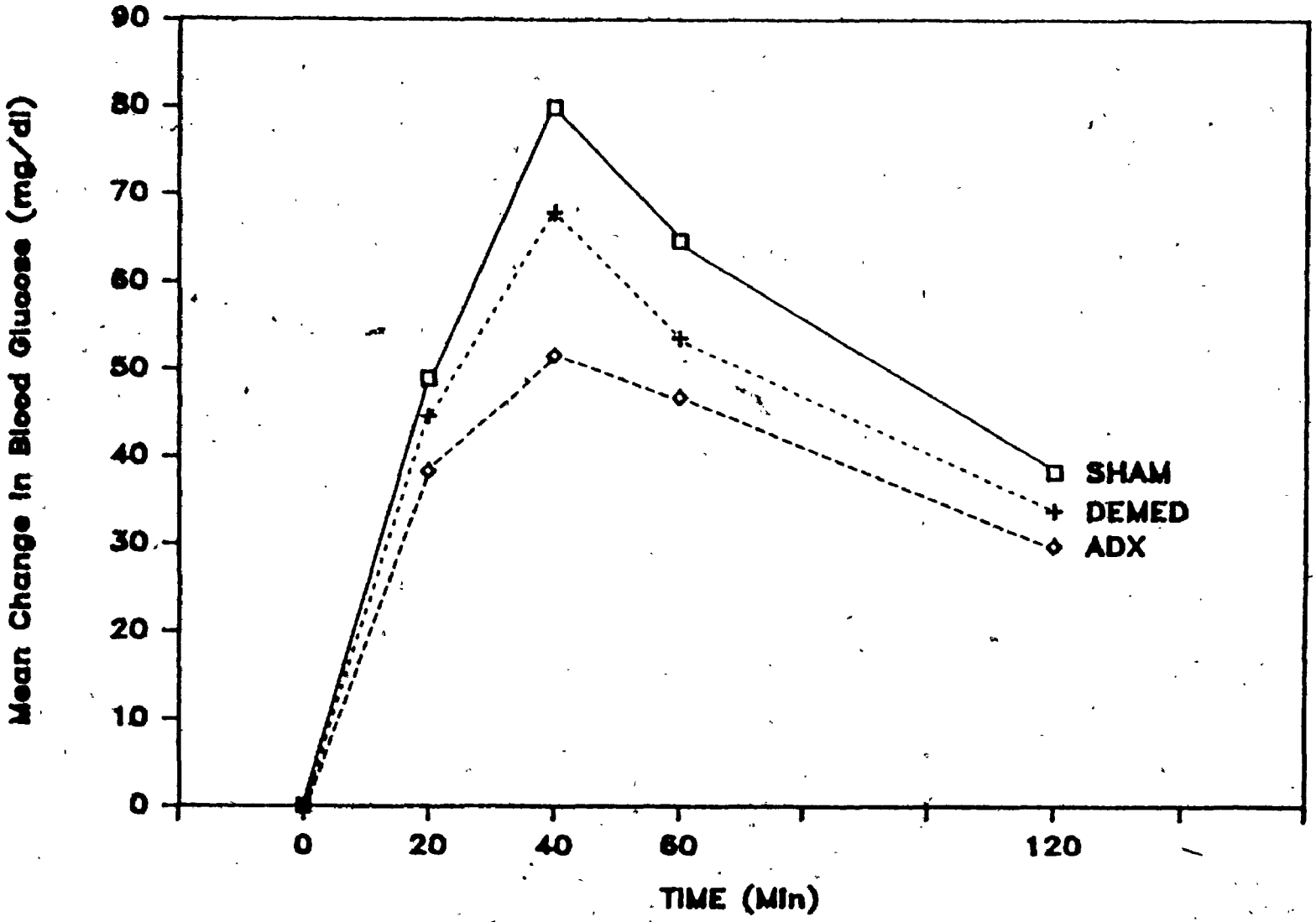
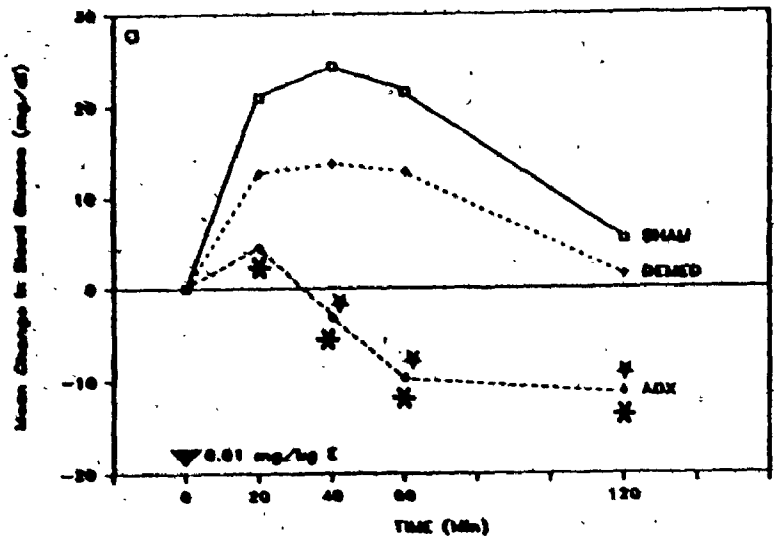
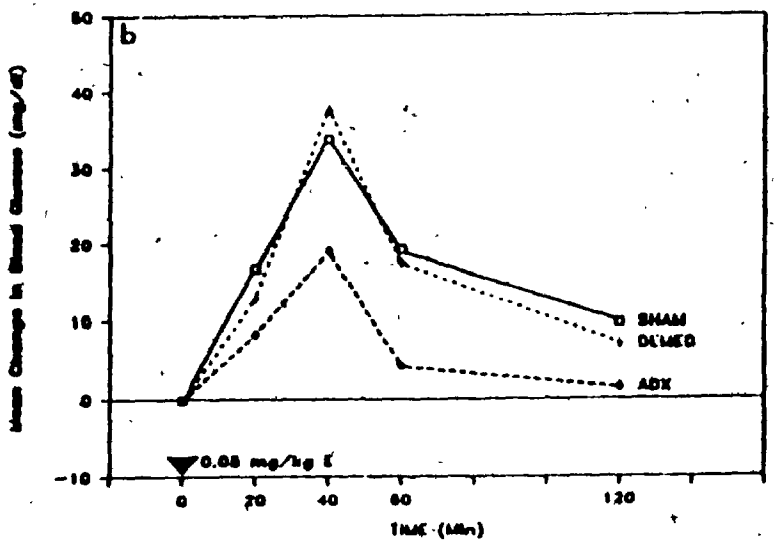
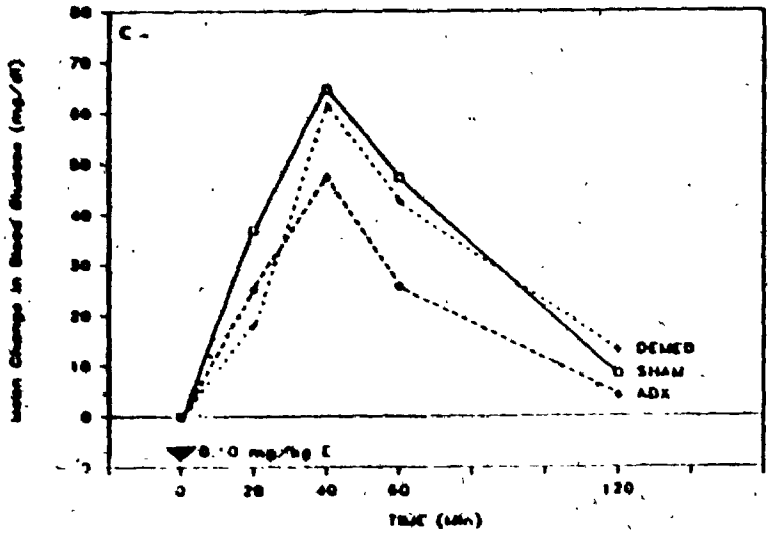

Mean Change In Blood Glucose (mg/dl)
90
80
70
60
50
40
30
20
10
0
0
20
40
60
120
TIME (Min)
SHAM
DEMED
ADX

Figure 21. Mean change in blood glucose levels at various times following the ip injection of epinephrine in adrenalectomized (ADX), demedullated (DEMED) and sham-operated rats (SHAM). Data are shown for 0.01 (panel a), 0.05 (panel b), 0.10 (panel c) and 0.50 (panel d) mg/kg doses of epinephrine. * indicates a significant difference from the SHAM group ($p<0.01$). + indicates a significant difference from the DEMED group ($p<0.01$). Comparisons were made using the Newman-Keul's test).

Figure 2i


a
SHAM
DEMED
ADX
0.01 mg/kg E
TIME (Min)


b
SHAM
DEMED
ADX
0.05 mg/kg E
TIME (Min)


c
DEMED
SHAM
ADX
TIME (Min)

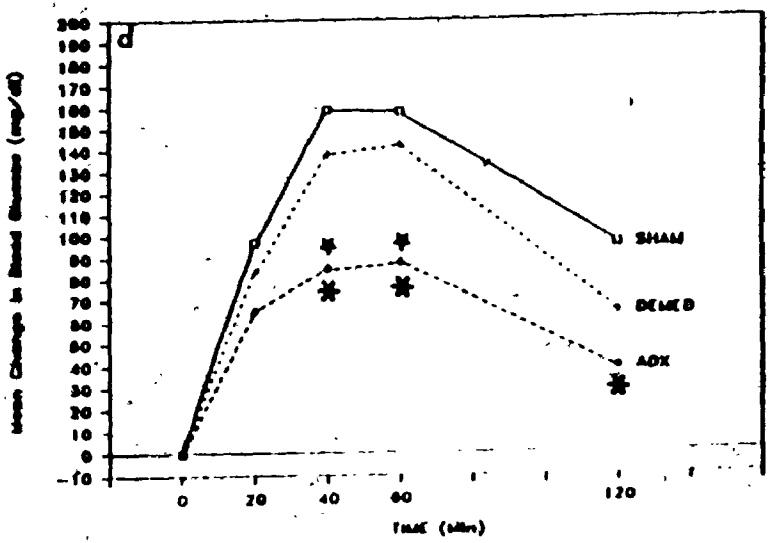

d
SHAM
DEMED
ADX
TIME (Min)

Figure 3a. Mean change in blood glucose levels following the injection of epinephrine for groups administered adrenergic blocking agents. The receptor antagonists were propranolol (PROP), phentolamine (PHENT), both drugs (BOTH), or vehicle (VEH). Data are collapsed across Sampling Time. * indicates a significant difference from VEH-injected controls ($p<0.01$ as determined by the Newman-Keul's test).

Figure 3a

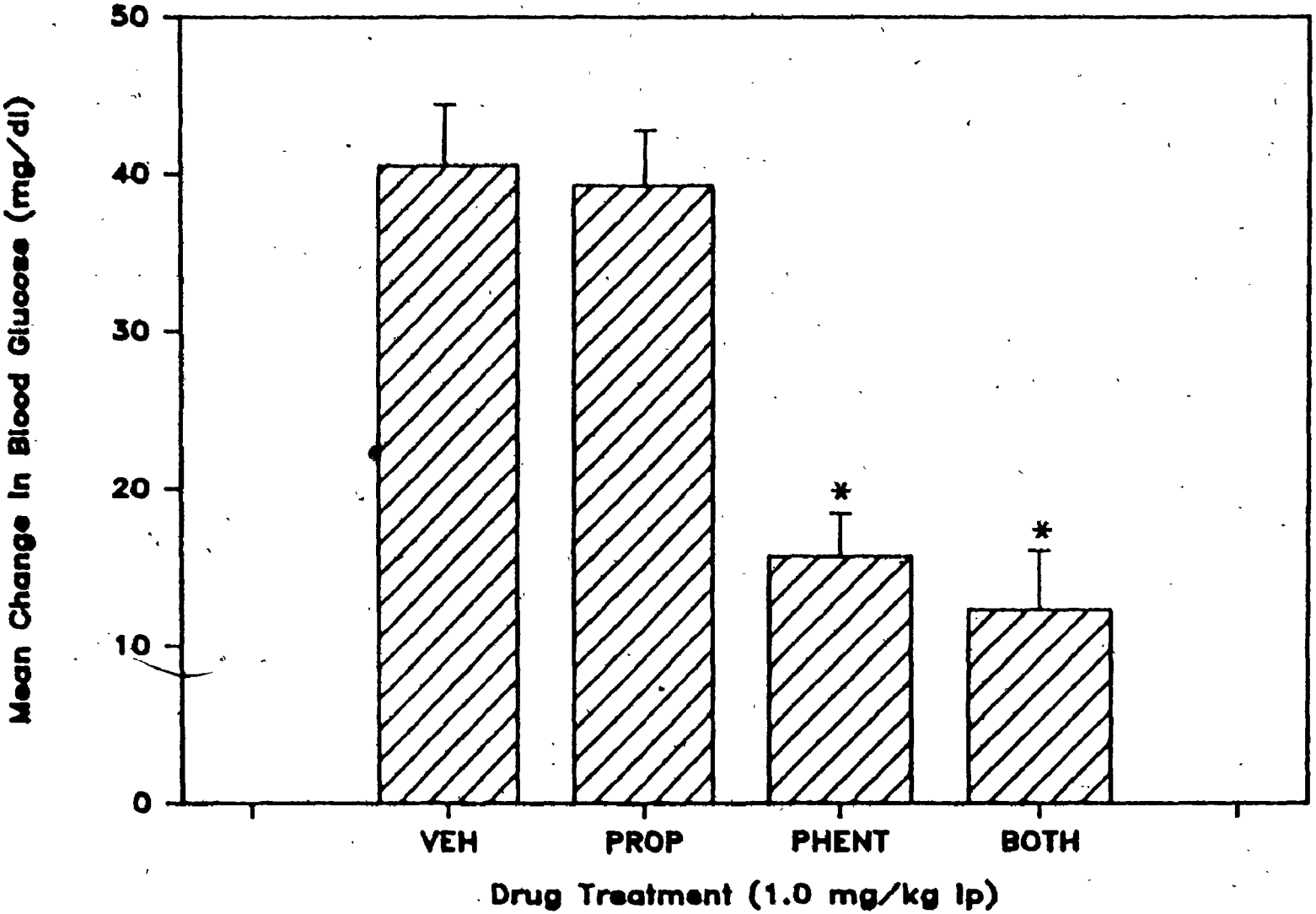

50
40
30
20
10
0
Mean Change In Blood Glucose (mg/dl)
*
*
VEH
PROP
PHENT
BOTH
Drug Treatment (1.0 mg/kg ip)

Figure 3b. Mean change in blood glucose levels at various times following the injection of epinephrine (E) for groups administered adrenergic blocking agents (1.0 mg/kg ip). The receptor antagonists were propranolol (PROP), phentolamine (PHENT), both drugs (BOTH), and vehicle (VEH). * indicates a significant difference from VEH-injected controls for the groups administered PHENT (i.e. PHENT and BOTH groups) [$p<0.01$ as determined by the Newman-Keul's test].

Figure 3b

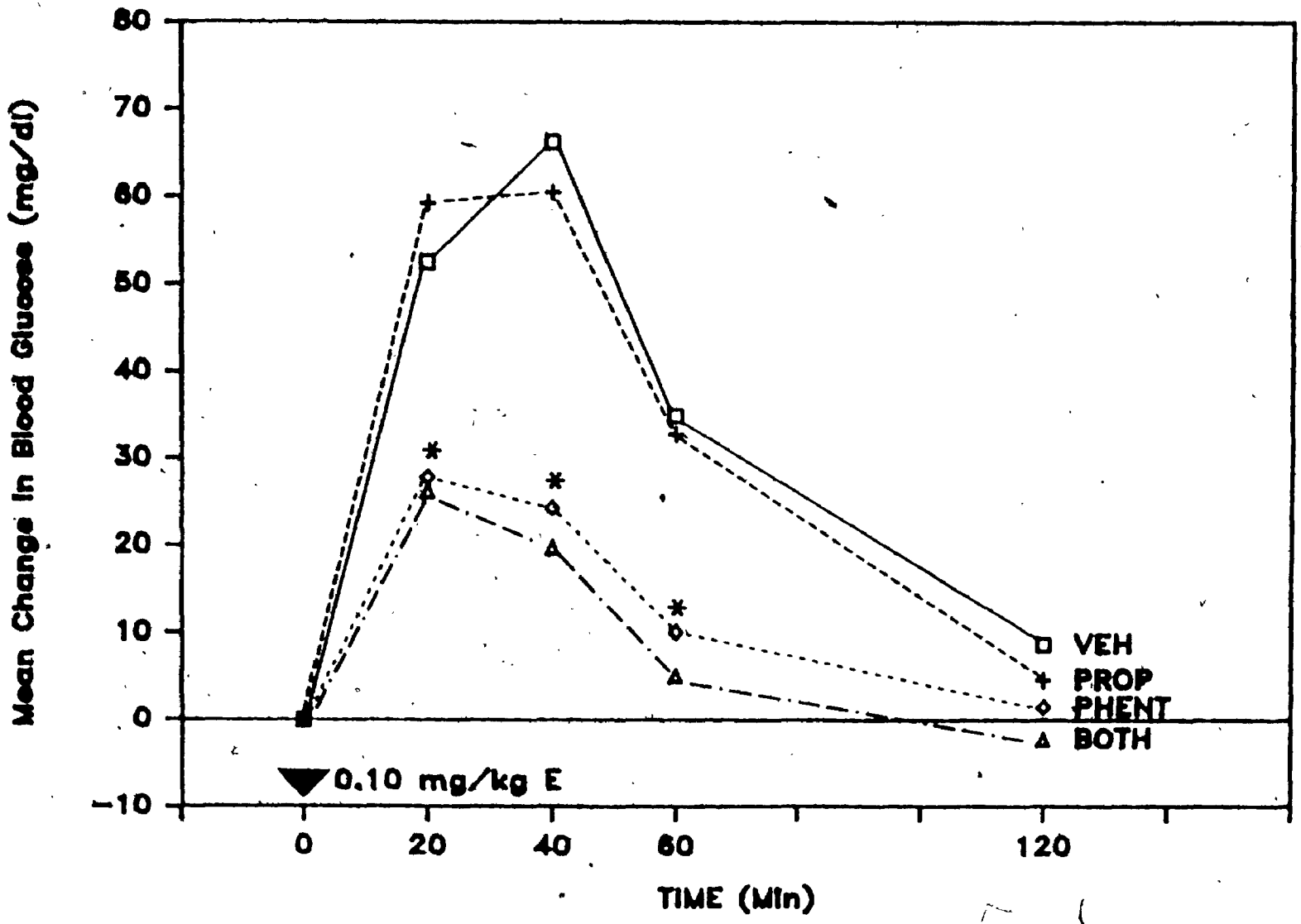

Mean Change In Blood Glucose (mg/dl)
80
70
60
50
40
30
20
10
0
-10
0.10 mg/kg E
0
20
40
60
120
TIME (Min)
VEH
PROP
PHENT
BOTH

Figure 3c. Blood glucose levels following the presentation of brief footshock stress in groups administered adrenergic blocking agents. The receptor antagonists were propranolol (PROP), phentolamine (PHENT), both drugs (BOTH), and vehicle (VEH). Data are collapsed across Sampling Time. * indicates a significant difference from VEH-injected controls ($p<0.01$ as determined by a priori t-tests).

Figure 3c

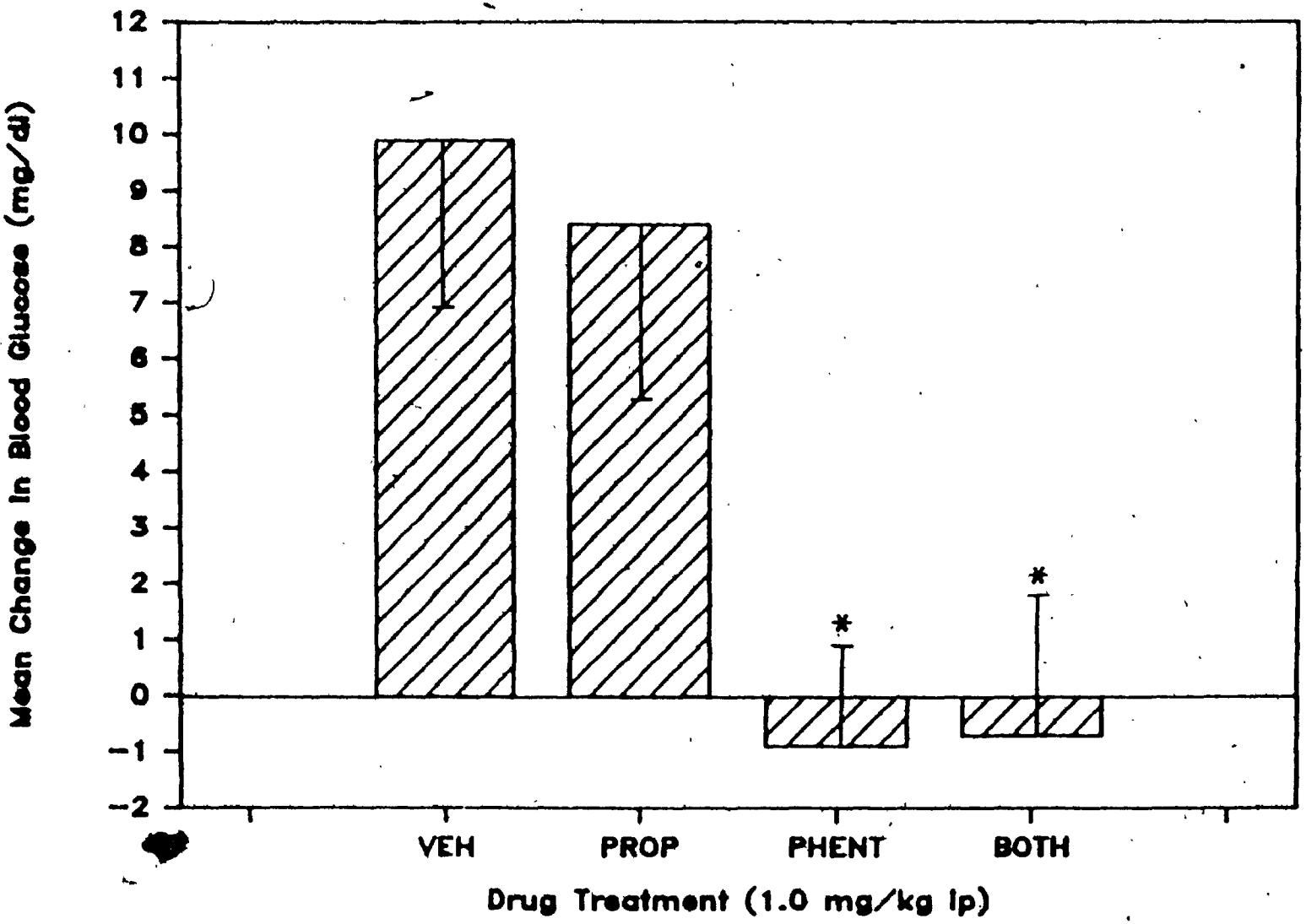

Mean Change In Blood Glucose (mg/dl)
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
−2
*
*
VEH
PROP
PHENT
BOTH
Drug Treatment (1.0 mg/kg ip)

Figure 3d. Mean change in blood glucose levels at various times following brief footshock stress for groups administered adrenergic blocking agents (1.0 mg/kg ip). The receptor antagonists were propranolol (PROP), phentolamine (PHENT), both drugs (BOTH), and vehicle (VEH).

Figure 3d

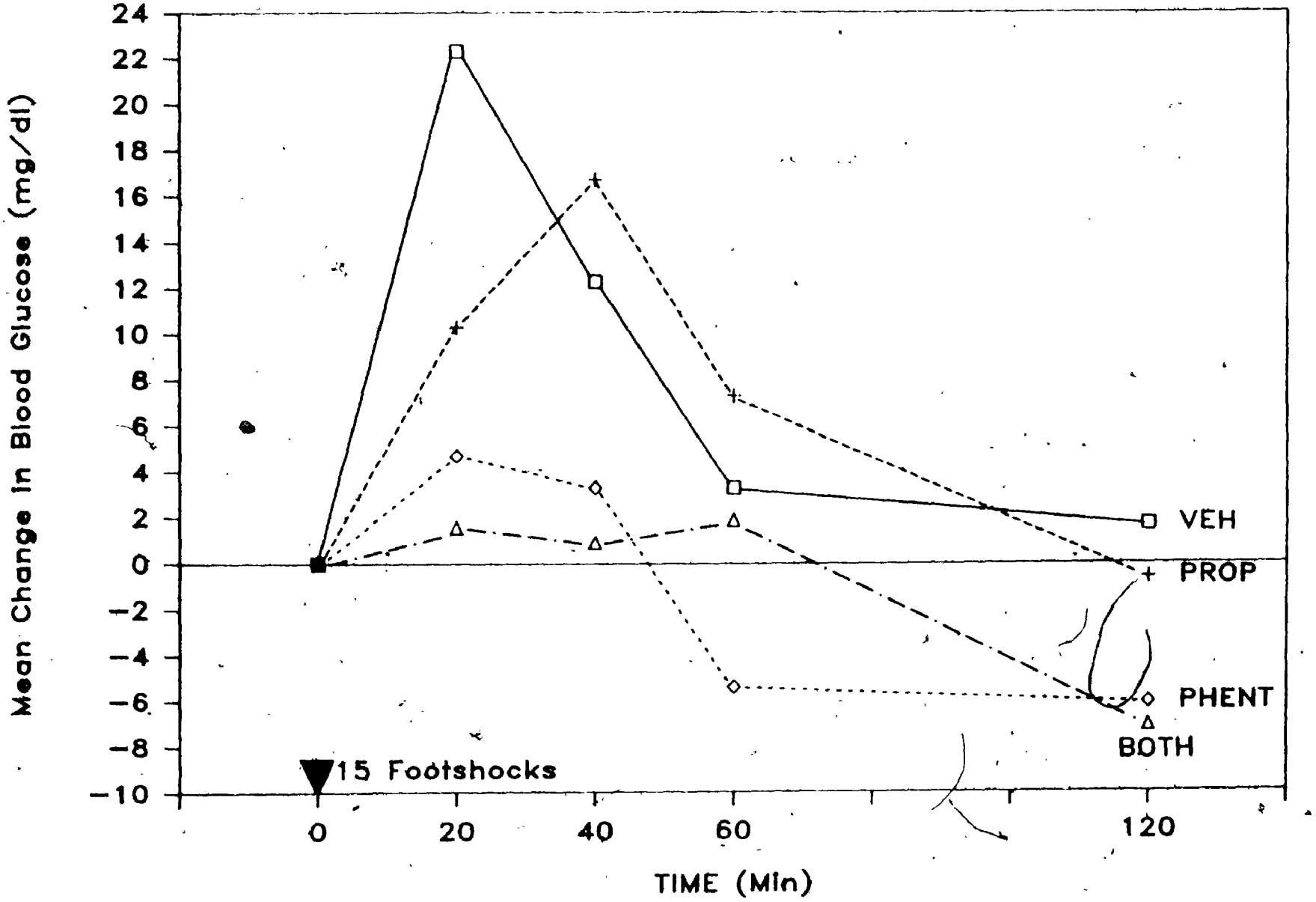

Mean Change in Blood Glucose (mg/dl)
24
22
20
18
16
14
12
10
8
6
4
2
0
−2
−4
−6
−8
−10
0
20
40
60
120
TIME (Min)
15 Footshocks
VEH
PROP
PHENT
BOTH

Figure 3e. Mean change in blood glucose levels at various times following the injection of epinephrine (E) for groups adminstered phentolamine (PHENT), propranolol (PROP), yohimbine (YOH), phenoxybenzamine (PHENOXY), and vehicle (VEH). The receptor blockers were administered at a dose of 5 mg/kg ip. * indicates a significant difference from VEH-injected controls ($p<0.05$). Comparisons were made using the Newman-Keul's procedure.

Figure 3e

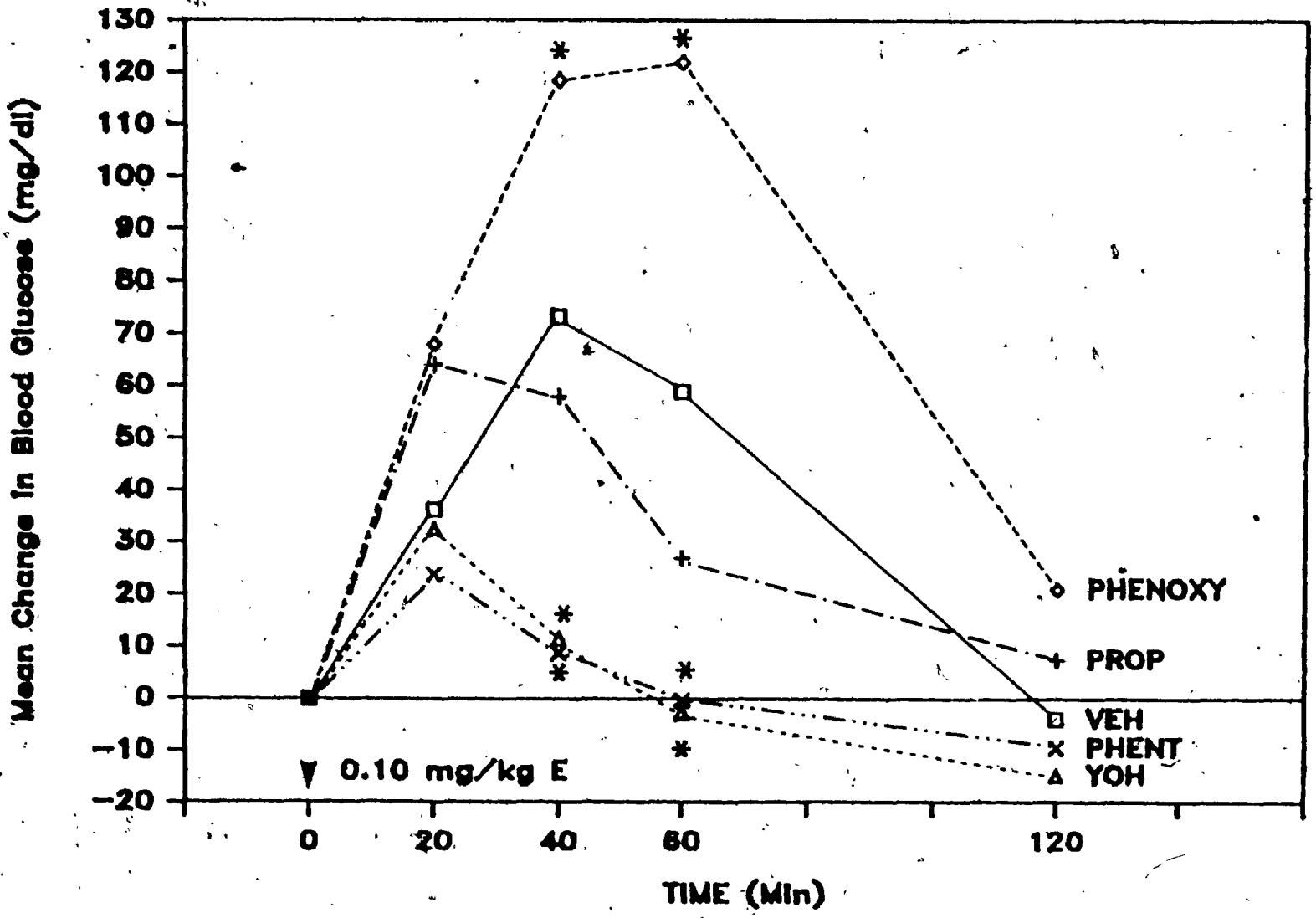

Mean Change In Blood Glucose (mg/dl)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
0
20
40
60
120
TIME (Min)
0.10 mg/kg E
PHENOXY
PROP
VEH
PHENT
YOH

Figure A. Mean change in blood glucose levels at various times following the presentation of brief footshock stress for adrenalectomized (ADX), demedullated (DEMED) and sham-operated rats (SHAM) [rats were 2-hr food deprived]. Data are taken from Bialik & Roberts (1985). * indicates a significant difference from ADX and DEMED groups ($p<0.01$ as determined by the Newman-Keul's test).

Figure A

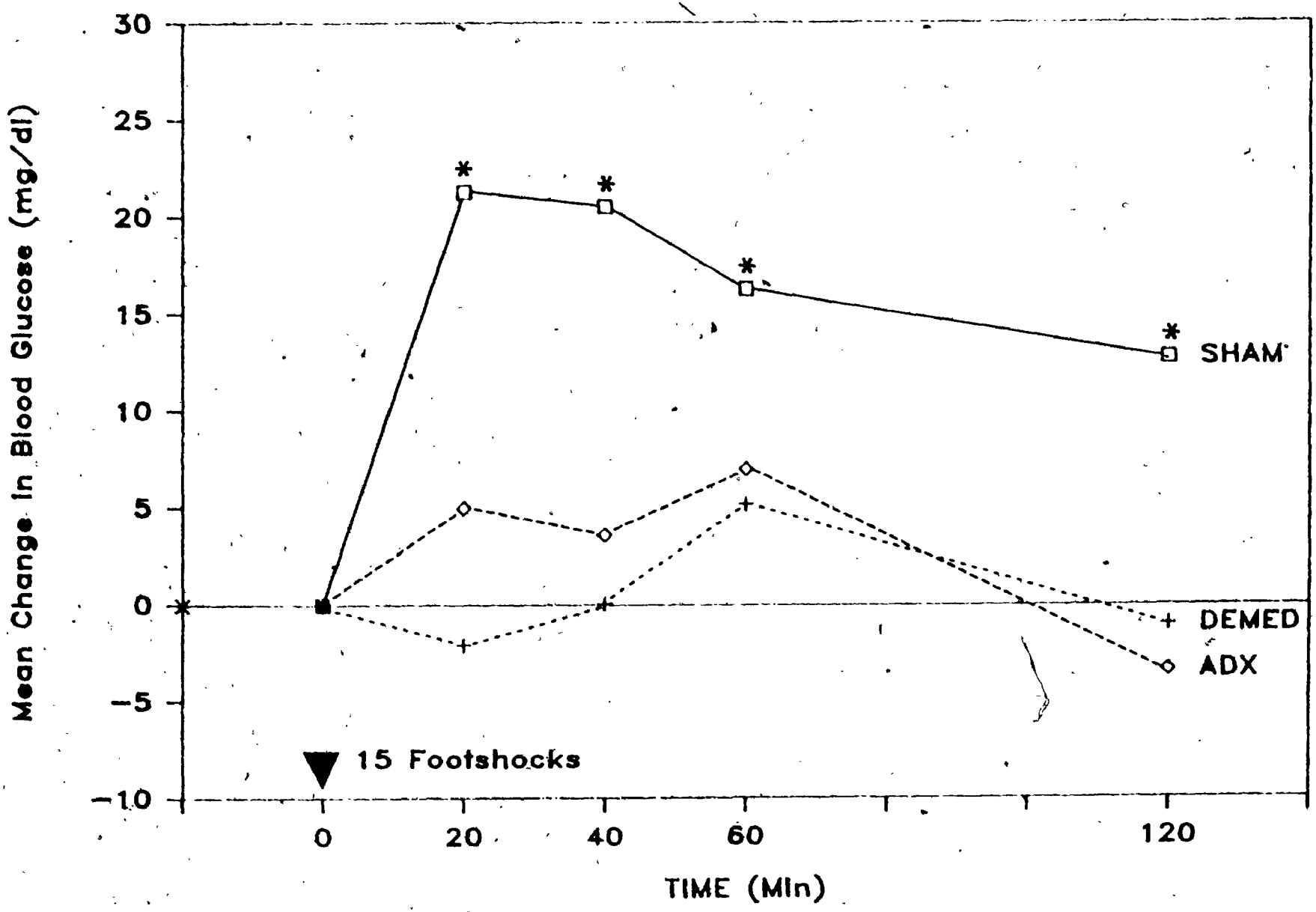

30
25
20
15
10
5
0
−5
−10
Mean Change In Blood Glucose (mg/dl)
SHAM
DEMED
ADX
15 Footshocks
0
20
40
60
120
TIME (Min)

Figure 3f. Mean change in blood glucose levels at various times following the presentation of footshock stress for demedullated (DEMED), adrenal denervated (DEN), and sham-operated rats (SHAM) [rats were 24-hr food deprived]. * and ** indicate significant differences from baseline ($p<0.05$ and $p<0.01$, respectively). Comparisons were made using the Newman-Keul's test.

Figure 3f

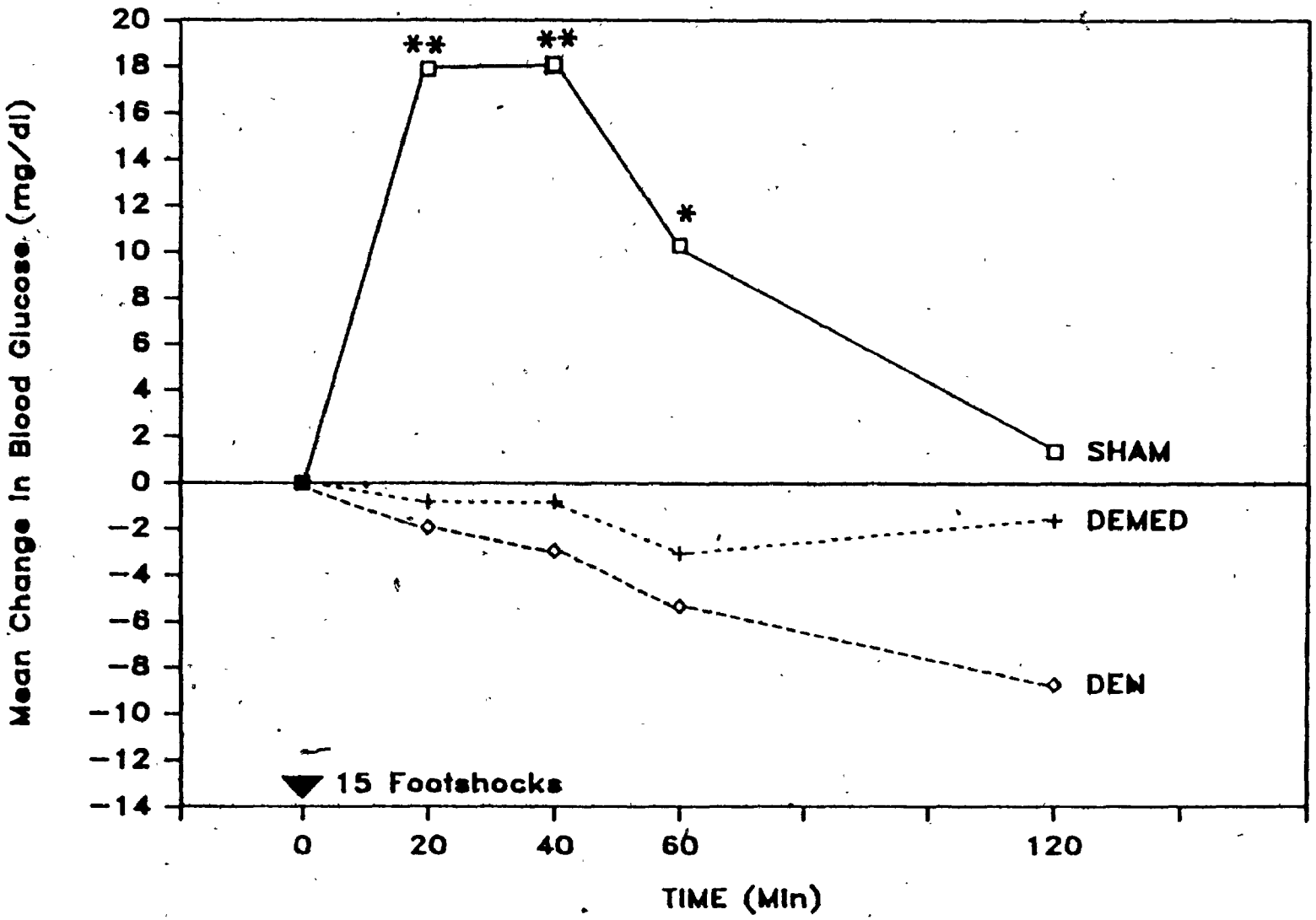

Mean Change In Blood Glucose (mg/dl)
20
18
16
14
12
10
8
6
4
2
0
−2
−4
−6
−8
−10
−12
−14
**
**
*
SHAM
DEMED
DEN
15 Footshocks
0
20
40
60
120
TIME (Min)

Figure 3g. Mean change in blood glucose levels at various times following the administration of 2-DG (125 mg/kg ip) in demedullated (DEMED) and sham-operated rats (SHAM) [rats were 24-hr food deprived]. * indicates a significant difference from the DEMED group ($p<0.01$). ✱ indicates a significant difference from baseline ($p<0.01$). Comparisons were made using the Newman-Keul's test).

Figure 3g

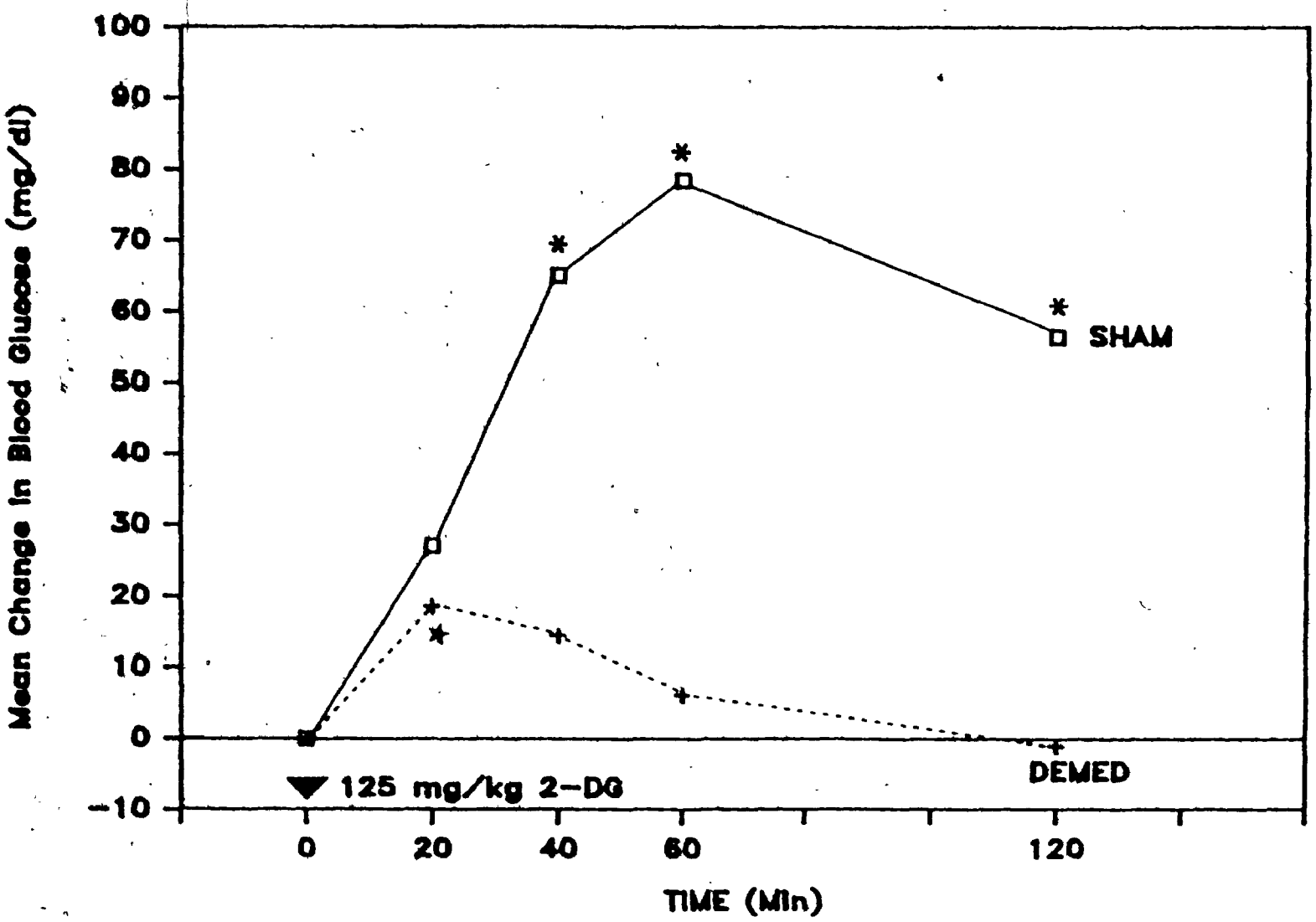
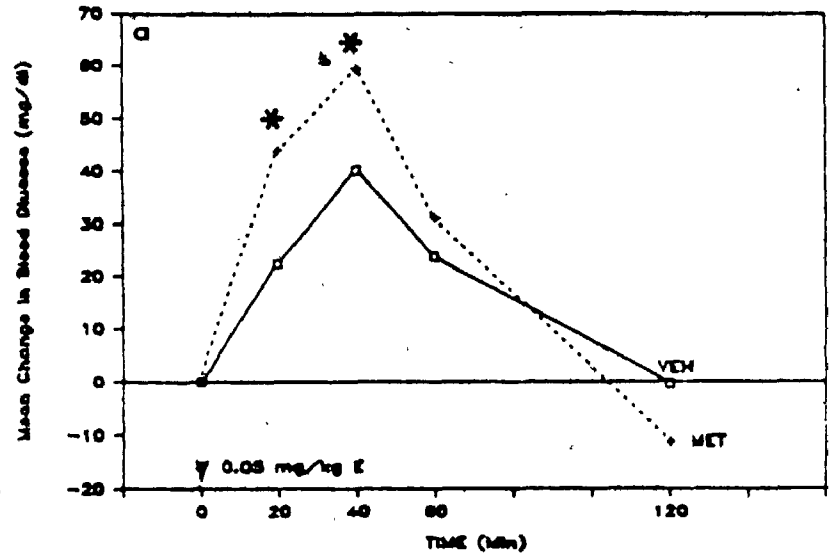
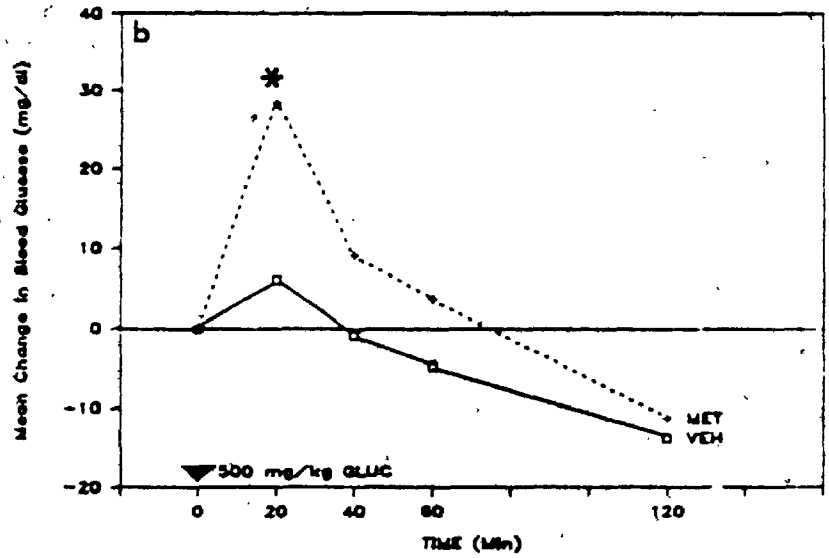

Mean Change in Blood Glucose (mg/dl)
100
90
80
70
60
50
40
30
20
10
0
-10
SHAM
DEMED
125 mg/kg 2-DG
0
20
40
60
120
TIME (Min)

Figure 4a. Mean change in blood glucose levels at various times following the ip injection of epinephrine (E) [panel a], glucose (GLUC) [panel b], and vehicle (VEH) [panel c] in metopirone (MET; 50 mg/kg ip 2-hr previously) or vehicle (VEH) pretreated rats. * indicates a significant difference from VEH-pretreated controls ($p<0.01$ as determined by the Newman-Keul's test).

Figure 4a


a
Mean Change in Blood Glucose (mg/dl)
70
60
50
40
30
20
10
0
-10
-20
*
*
VEH
MET
0.05 mg/kg E
0
20
40
60
120
TIME (Min)


b
Mean Change in Blood Glucose (mg/dl)
40
30
20
10
0
-10
-20
*
MET
VEH
500 mg/kg GLUC
0
20
40
60
120
TIME (Min)

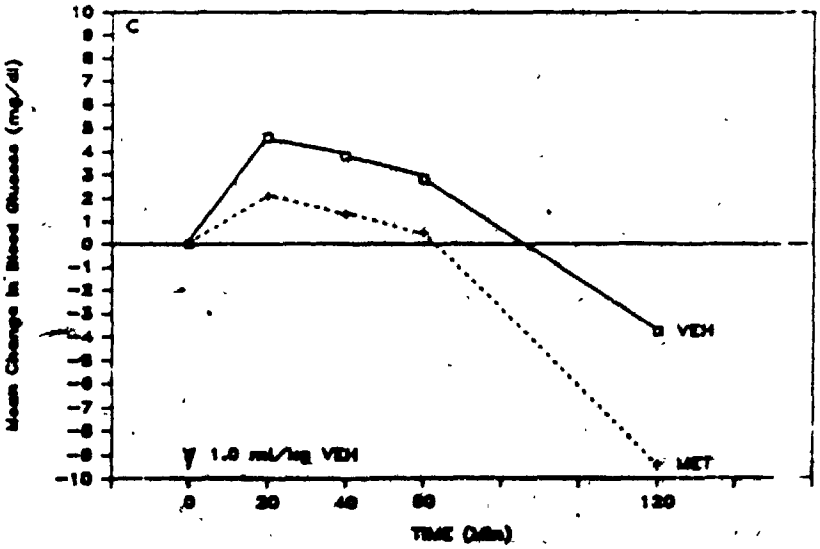

c
Mean Change in Blood Glucose (mg/dl)
10
0
-10
VEH
MET
1.0 ml/kg VEH
0
20
40
60
120
TIME (Min)

Figure 4b. Mean change in plasma corticosterone levels at various times following administration of epinephrine (E), glucose (GLUC), and vehicle (VEH) for rats pretreated with metopirone (MET) or the metopirone vehicle (VEH). * indicates a significant difference from baseline ($p<0.05$ as determined by the Newman-Keul's procedure).

Figure 4b

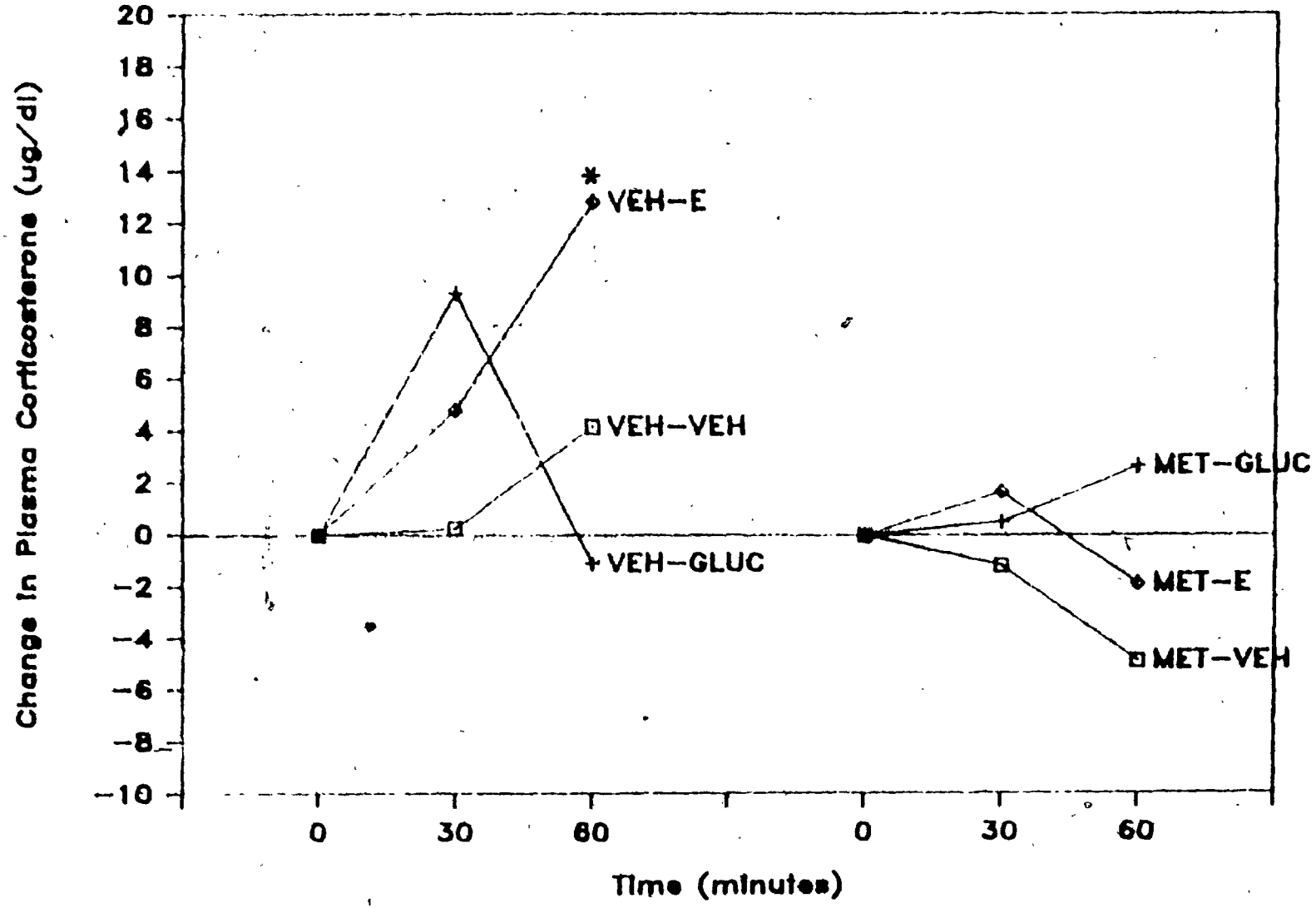

Change In Plasma Corticosterone (ug/dl)
20
18
16
14
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
VEH-E
VEH-VEH
VEH-GLUC
MET-GLUC
MET-E
MET-VEH
0
30
60
0
30
60
Time (minutes)

Figure 5a. Mean change in blood glucose levels at various times following the adminstration of epinephrine by subcutaneous (sc) or intraperitoneal (ip) injections. * indicates a significant difference from the ip group ($p<0.01$ as determined by the Newman-Keul's test).

Figure 5a

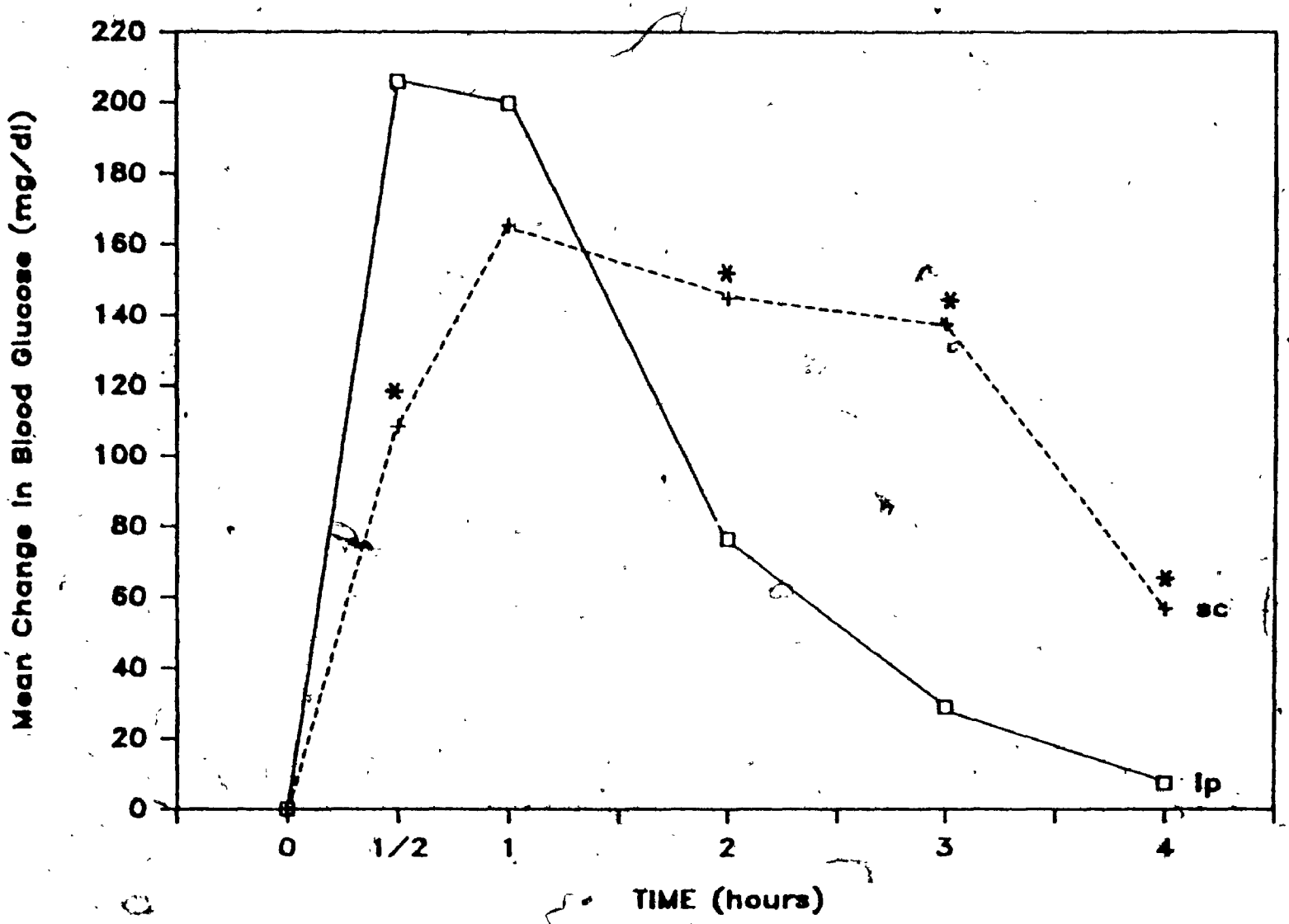

Mean Change in Blood Glucose (mg/dl)
220
200
180
160
140
120
100
80
60
40
20
0
0
1/2
1
2
3
4
TIME (hours)
sc
ip

Figure 5b. Mean change in blood glucose levels following the administration of 0.05 and 0.50 mg/kg sc epinephrine (E) with and without pretreatment with corticosterone (CORT) [0.300 mg/kg sc, injected 1 hour previously].

Figure 5b

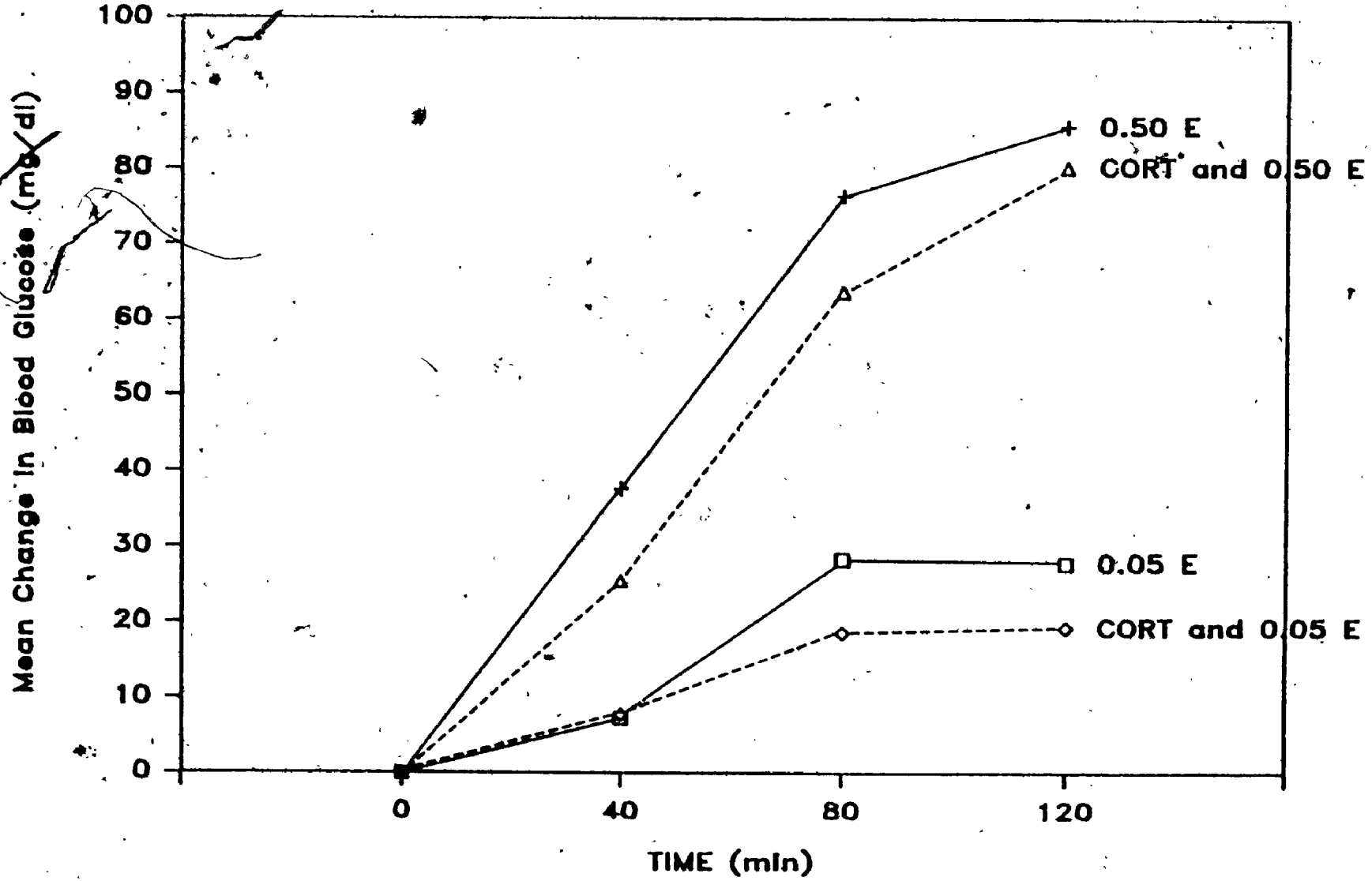

Mean Change In Blood Glucose (mg/dl)
100
90
80
70
60
50
40
30
20
10
0
0
40
80
120
TIME (min)
0.50 E
CORT and 0.50 E
0.05 E
CORT and 0.05 E

Figure 5c. Mean change in blood glucose levels following the administration of epinephrine (0.05 mg/kg ip) with and without pretreatment with corticosterone (CORT) [0.300 mg/kg sc, injected 1 hour previously].

Figure 5c

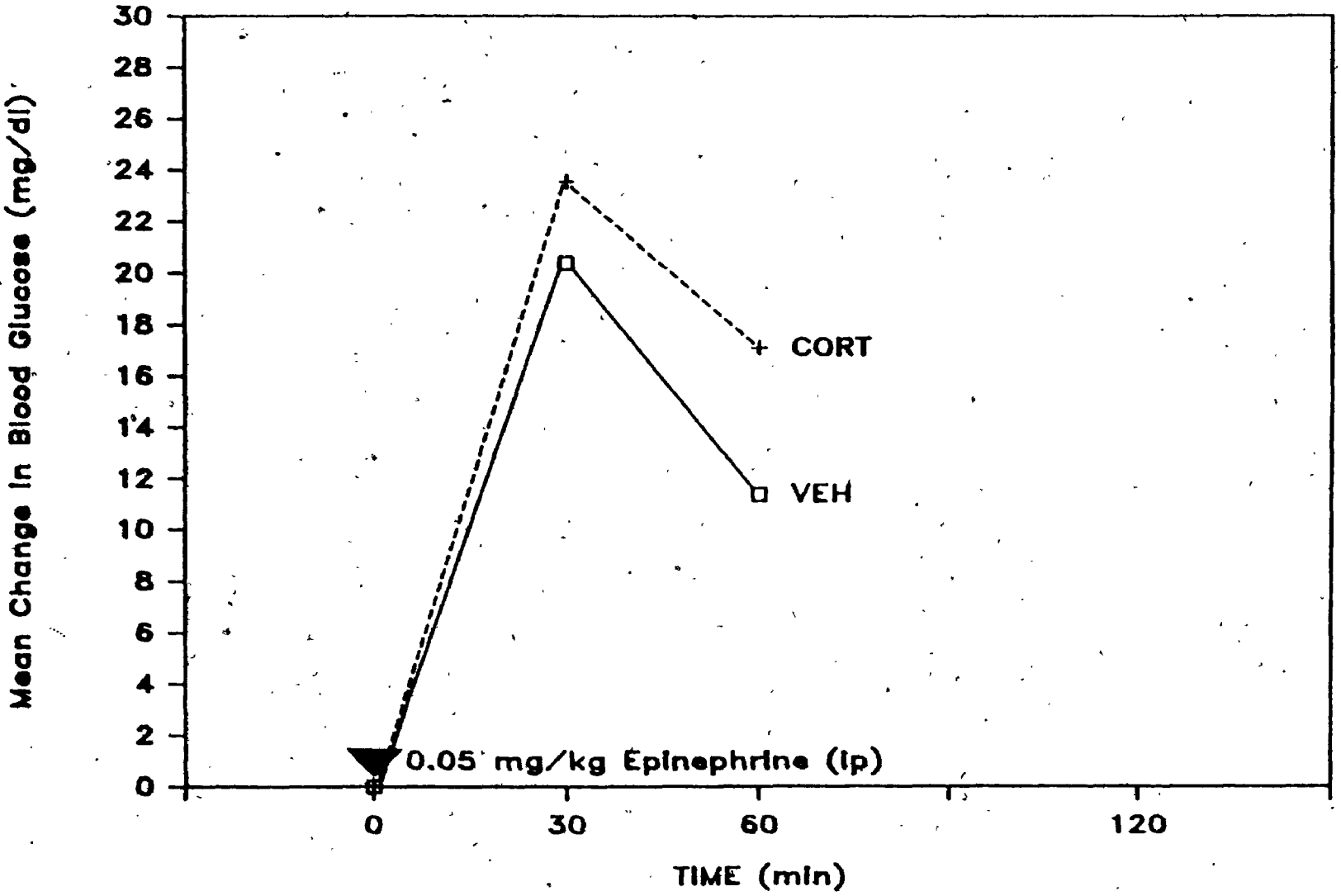

Mean Change In Blood Glucose (mg/dl)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
CORT
VEH
0.05 mg/kg Epinephrine (ip)
0
30
60
120
TIME (min)

Figure 6. Mean change in blood glucose levels at various times following electrical stimulation of the lateral hypothalamus in intact rats, in the same rats 3 days following DEMED, and in the same rats 9 days following surgery. A significant difference from baseline is indicated by * ($p<0.01$ as determined by the Newman-Keul's test).

Figure 6

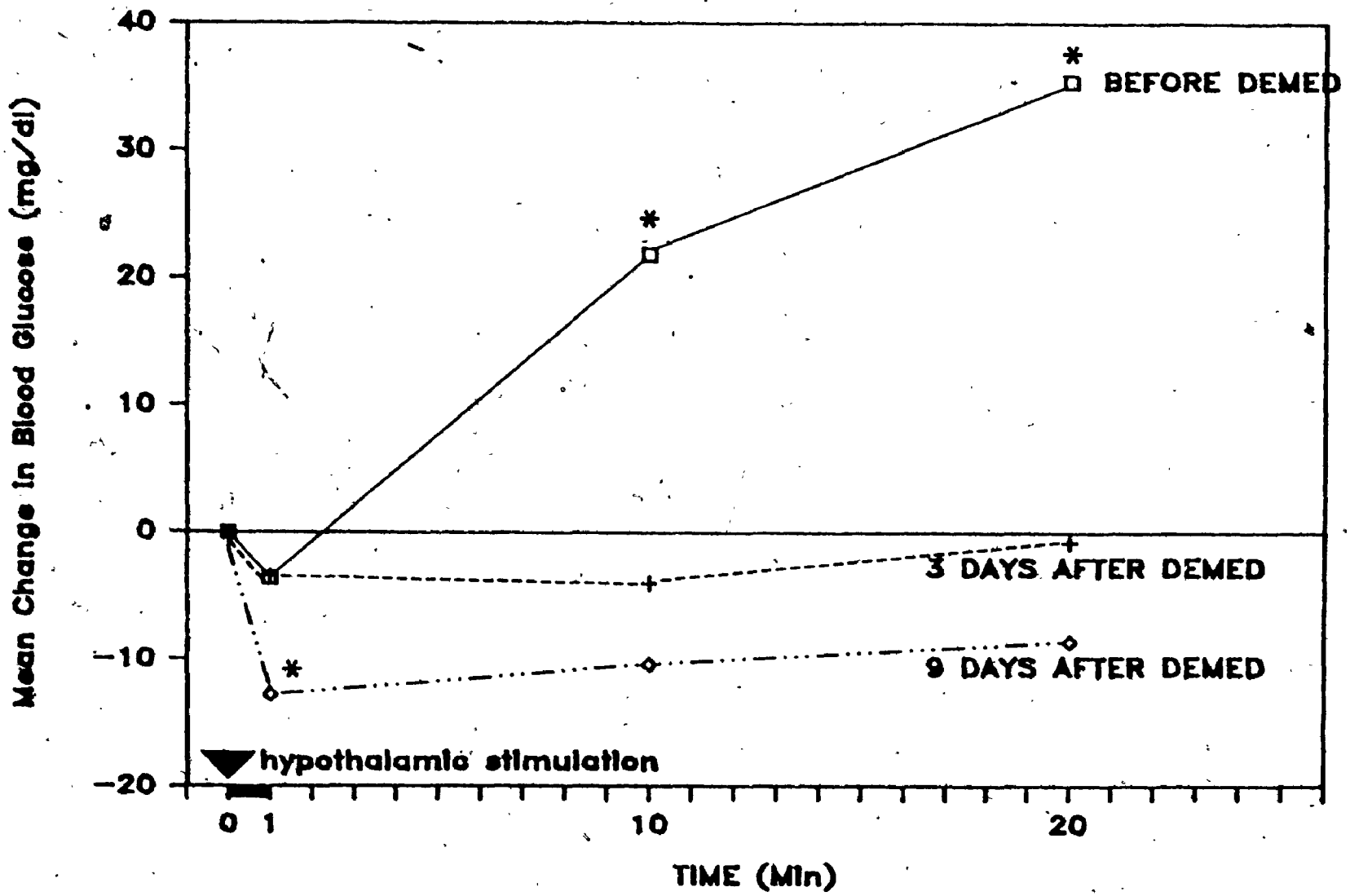

40
30
20
10
0
−10
−20
Mean Change in Blood Glucose (mg/dl)
BEFORE DEMED
3 DAYS AFTER DEMED
9 DAYS AFTER DEMED
hypothalamic stimulation
0 1
10
20
TIME (Min)

Figure 7a. Mean change in blood glucose levels at various times following implantation of different concentrations of dexamethasone (DEX) pellets (0, 5, 15 or 25%). * indicates a significant difference from the 0% DEX pellet control group ($p<0.01$). † indicates a significant difference from the 15% DEX group ($p<0.01$). Comparisons were made using the Newman-Keul's test.

Figure 7a

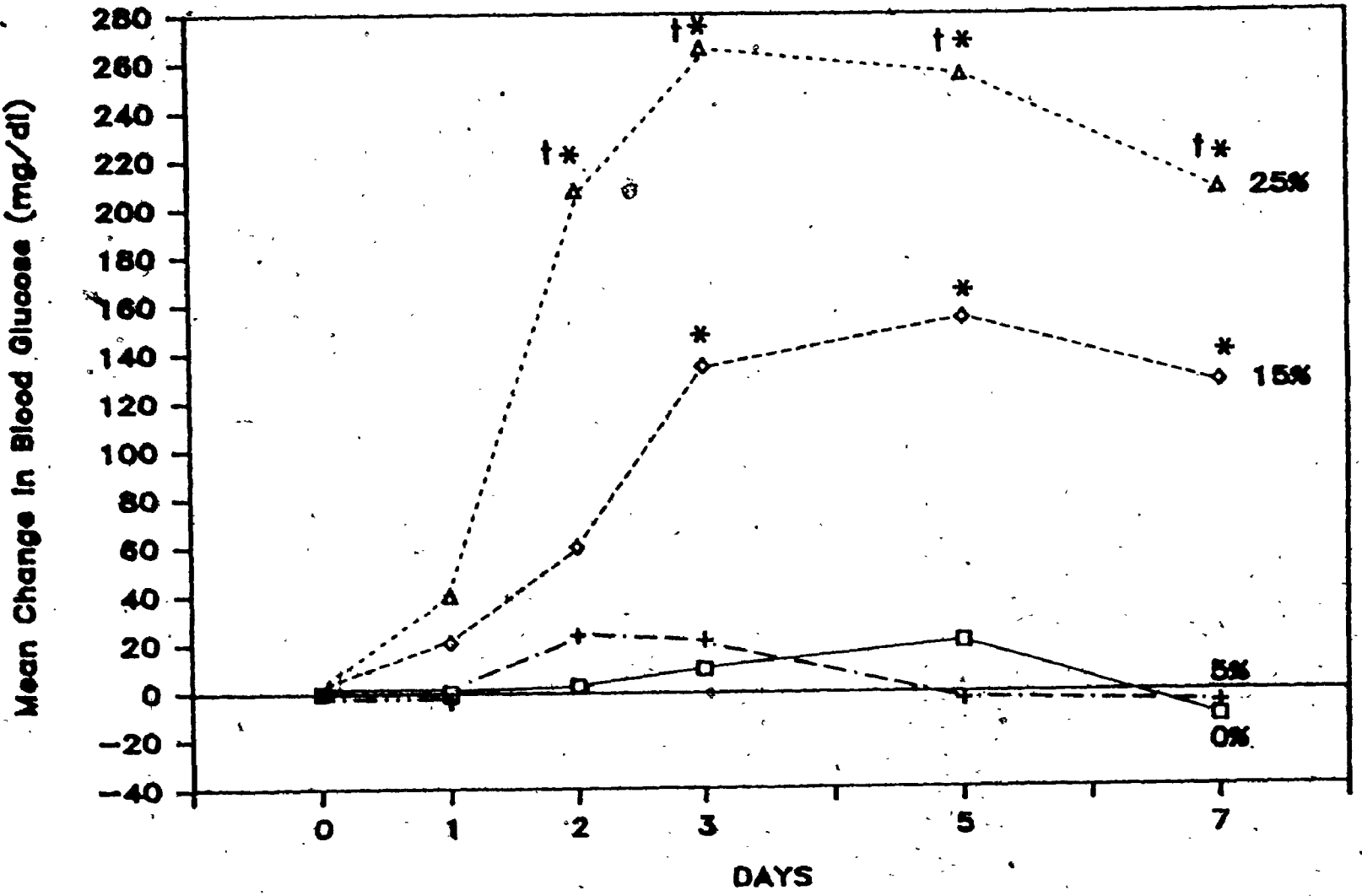

Mean Change In Blood Glucose (mg/dl)
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
0
1
2
3
5
7
DAYS
†*
†*
†*
25%
*
*
15%
5%
0%

Figure 7b. Mean change in body weight at various times following implantation of different concentrations of dexamethasone (DEX) pellet (0, 5, 15 or 25%). * indicates a significant difference for the 0% DEX pellet control group from the other groups ($p<0.01$). † indicates a significant difference for the 5% DEX group from the 15% and 25% DEX groups ($p<0.01$). X indicates a significant difference for the 15% DEX group from the 25% DEX group ($p<0.05$). Comparisons were made using the Newman-Keul's test.

Figure 7b

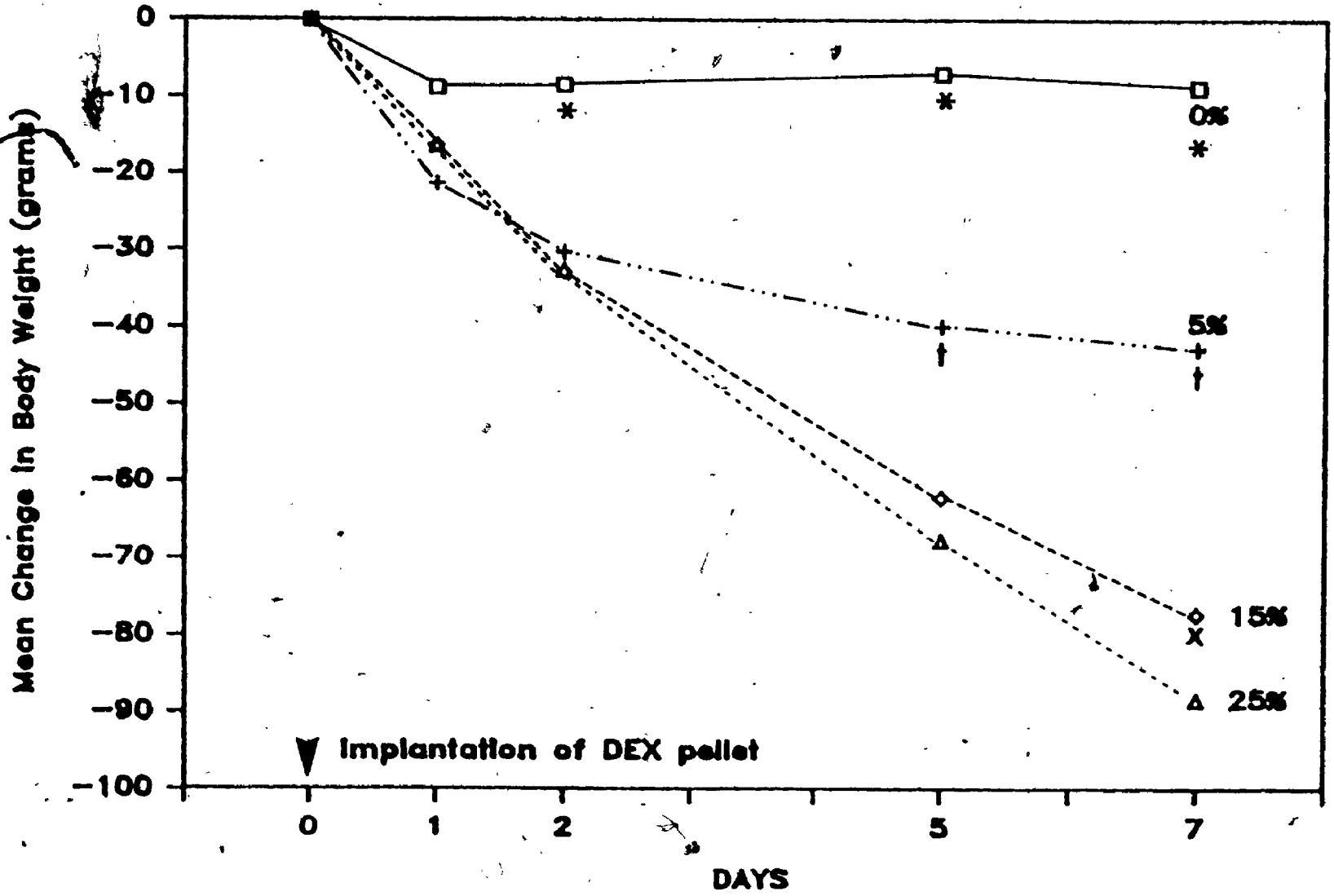

Mean Change in Body Weight (grams)
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0%
5%
15%
25%
Implantation of DEX pellet
0
1
2
5
7
DAYS

Figure 7c. Mean change in blood glucose levels at various times following implantation of a 0% (i.e. cholesterol only) or a 25% dexamethasone (DEX) pellet in adrenalectomized (ADX), demedullated (DEMED), and sham-operated (SHAM) rats. On days 2 and 5, there was a significant difference between each 25% DEX-implanted group and their respective 0% DEX pellet control group ($p<0.01$ as determined by the Newman-Keul's test).

Figure 7c

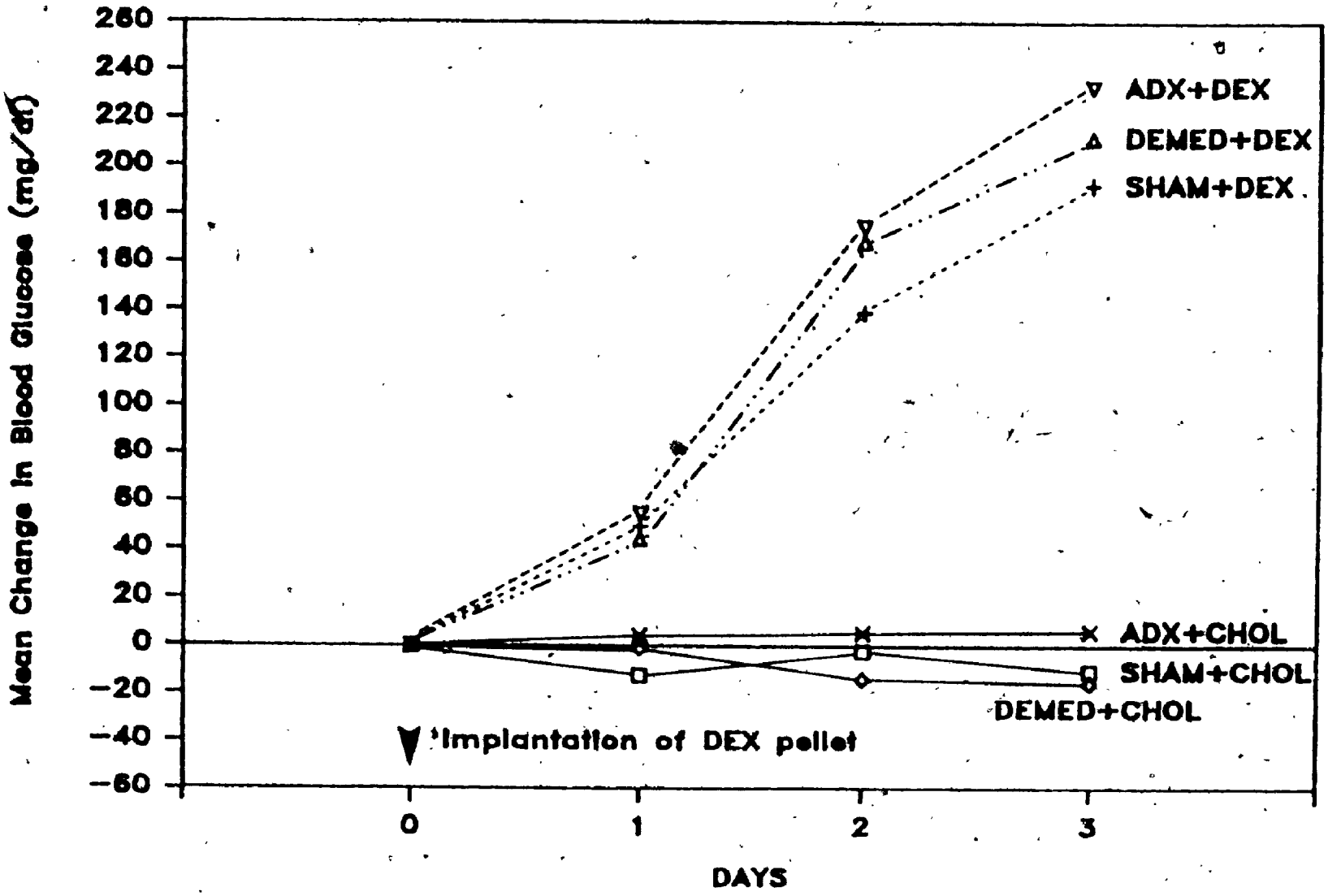
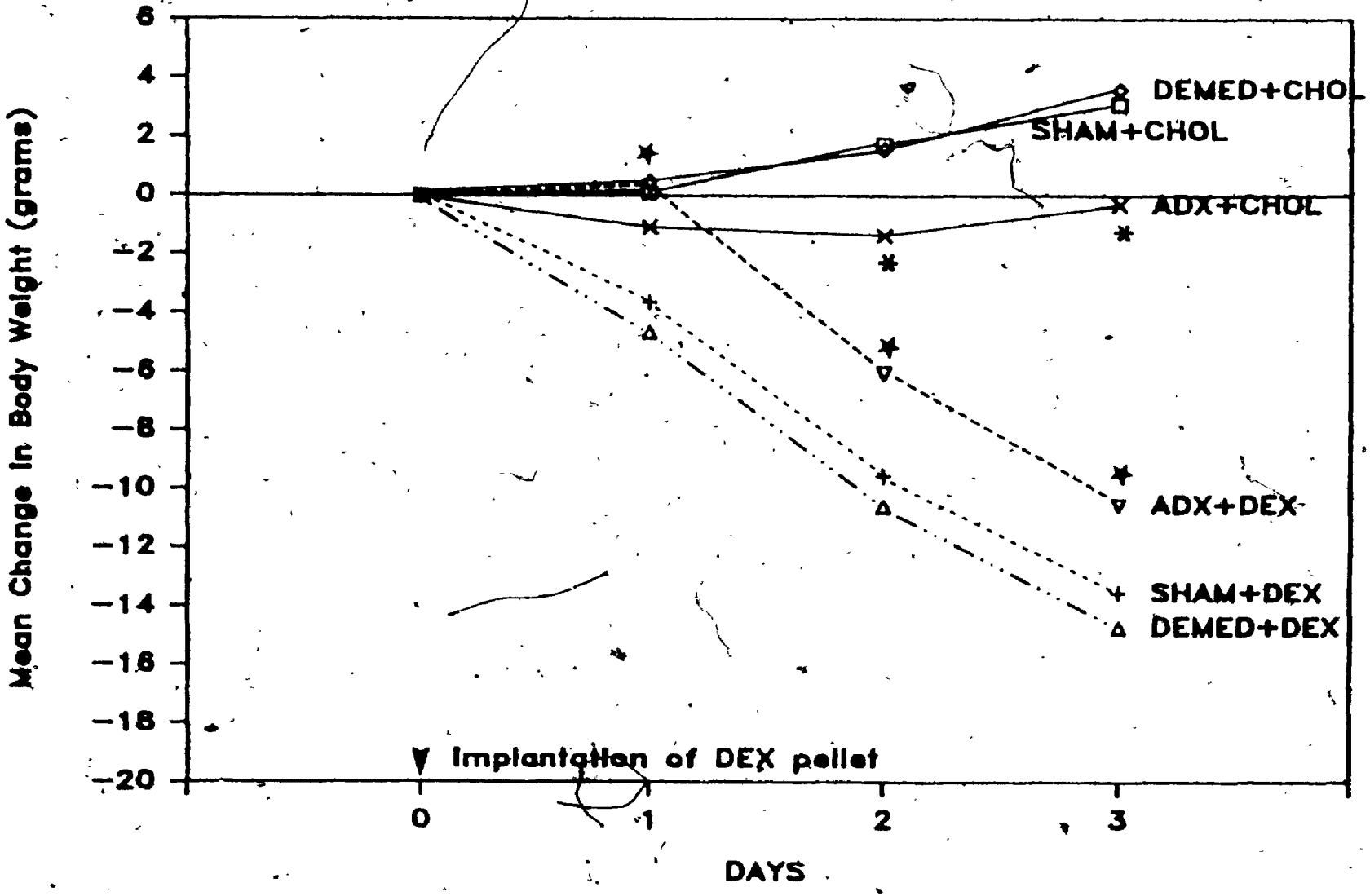

Mean Change in Blood Glucose (mg/dl)
260
240
220
200
180
160
140
120
100
80
60
40
20
0
−20
−40
−60
0
1
2
3
DAYS
ADX+DEX
DEMED+DEX
SHAM+DEX
ADX+CHOL
SHAM+CHOL
DEMED+CHOL
Implantation of DEX pellet

Figure 7d. Mean change in body weight at various times following implantation of a 0% (i.e. cholesterol only, CHOL) or a 25% dexamethasone (DEX) pellet in adrenalectomized (ADX), demedullated (DEMED), and sham-operated rats (SHAM). On days 2 and 3, each of the 25% DEX groups had lost significantly more weight than their respective CHOL control groups ($p<0.01$). In addition, * indicates a significant difference for the ADX+CHOL group compared to the DEMED+CHOL and SHAM+CHOL groups ($p<0.01$) and ✱ indicates a significant difference for the ADX+DEX group compared to the DEMED+DEX and SHAM+DEX groups ($p<0.01$). Comparisons were made with the Newman-Keul's test.

Figure 7d


Mean Change In Body Weight (grams)
6
4
2
0
−2
−4
−6
−8
−10
−12
−14
−16
−18
−20
0
1
2
3
DAYS
DEMED+CHOL
SHAM+CHOL
ADX+CHOL
ADX+DEX
SHAM+DEX
DEMED+DEX
Implantation of DEX pellet

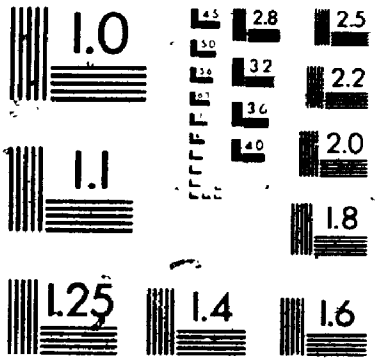


Figure 8a. Mean change in blood glucose levels at various times following the implantation of a 25% DEX pellet for norepinephrine depleted (DVNB) and control rats (SHAM). Data are collapsed across Surgical Treatment. * indicates a significant difference from the SHAM-operated control group ($p<0.05$). The comparison was made using an a priori t-test [$t(13)=2.50$, $p<0.05$].

Figure 8a

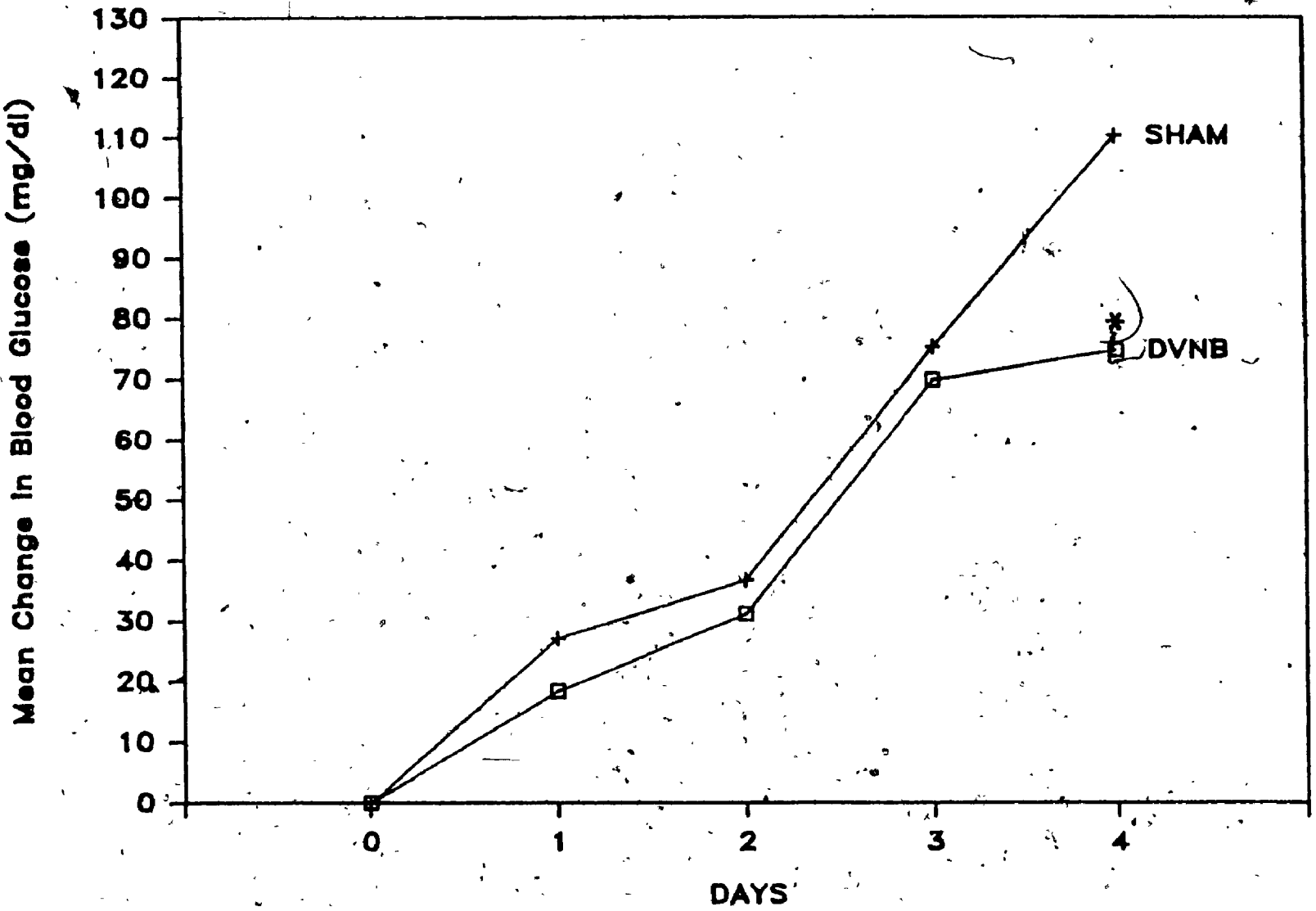

Mean Change in Blood Glucose (mg/dl)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
DAYS
SHAM
DVNB

Figure 8b. Mean change in body weight at various times following the implantation of a 25% dexamethasone (DEX) pellet for norepinephrine depleted (DVNB), norepinephrine depleted and adrenalectomized (DVNB+ADX), non-lesioned adrenalectomized rats (ADX), and non-lesioned sham-operated rats (SHAM). Both the ADX group and the DVNB group had lost significantly less weight than the SHAM group on day 2 ($p<0.05$), day 3 ($p<0.01$), and day 4 ($p<0.01$). Comparisons were made using the Newman-Keul's test.

Figure 8b

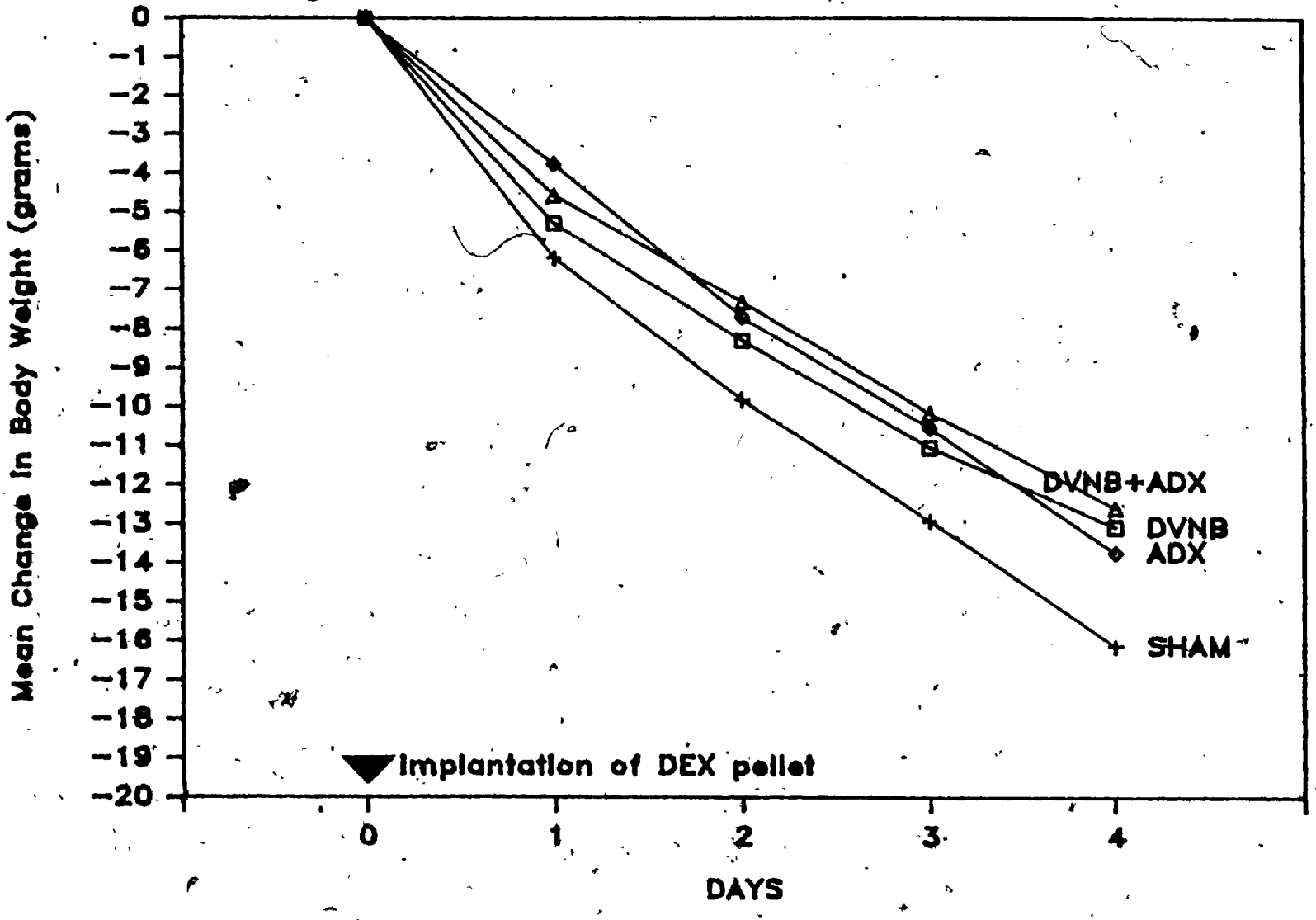

Mean Change in Body Weight (grams)
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
-19
-20
Implantation of DEX pellet
0
1
2
3
4
DAYS
DVNB+ADX
DVNB
ADX
SHAM

Figure 8c. Mean change in blood glucose levels at various times following administration of brief footshock-stress to rats which were depleted of brain NE (DVNB) or received sham-operations (SHAM). Overall, the DVNB group had significantly higher blood glucose levels than the SHAM group (See text for details).

Figure 8c

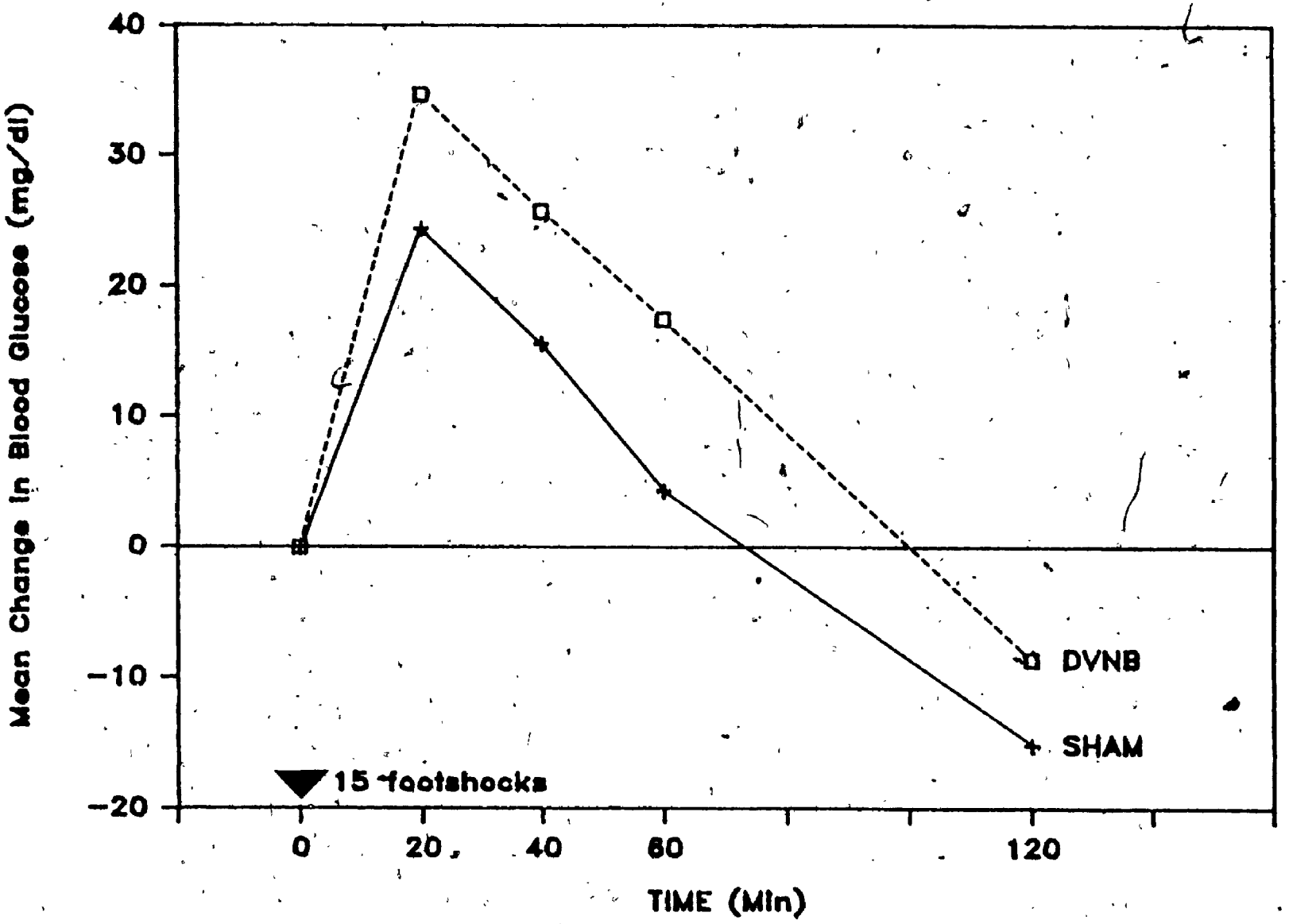

40
30
20
10
0
−10
−20
Mean Change In Blood Glucose (mg/dl)
DVNB
SHAM
15 footshocks
0
20
40
60
120
TIME (Min)

Figure 8d. Mean change in blood glucose levels at various times following administration of footshock stress in demedullated (DEMED) and sham-operated rats (SHAM). * indicates a significant difference from baseline ($p<0.01$ as determined by the Newman-Keul's procedure).

Figure 8d

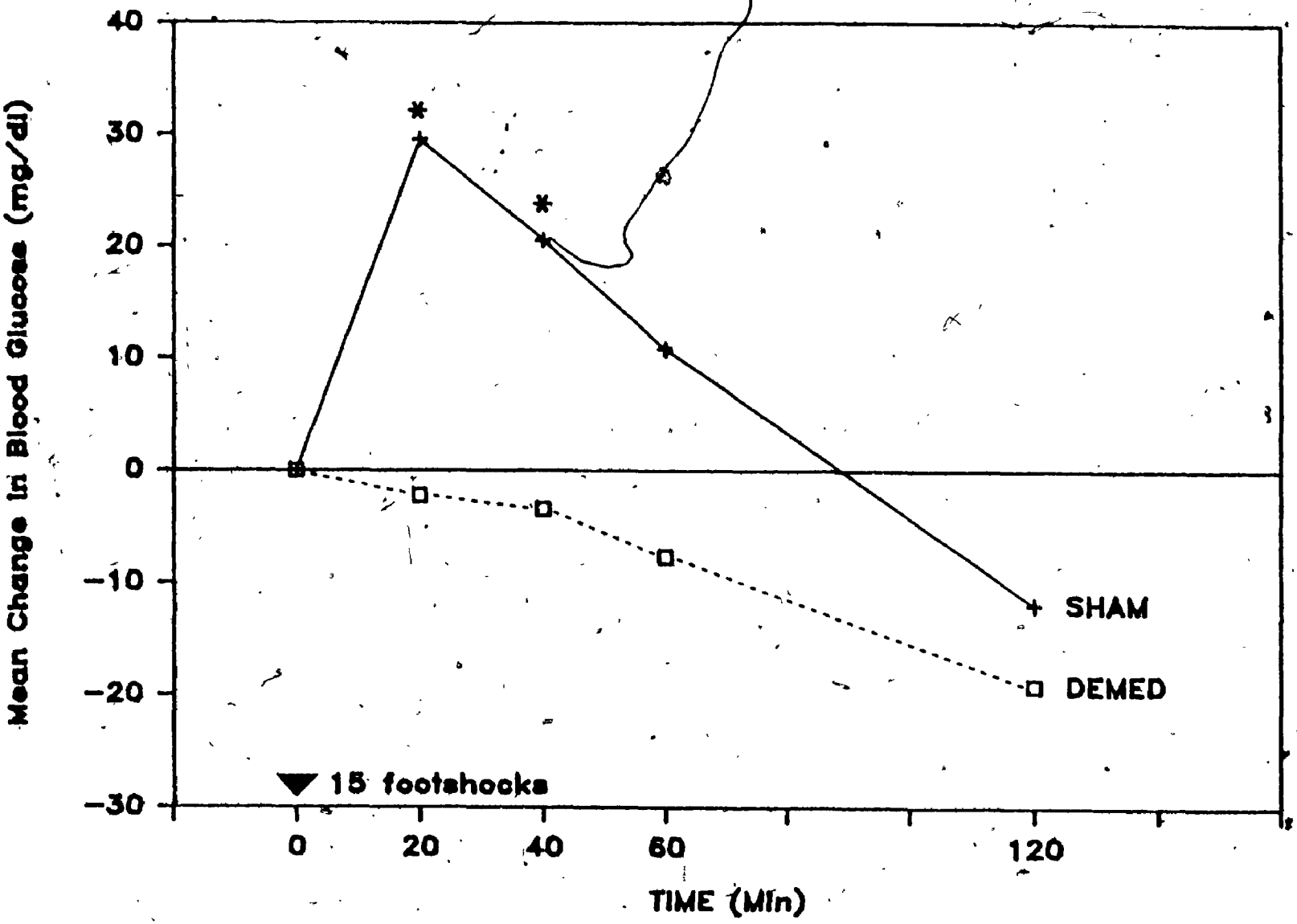

Mean Change in Blood Glucose (mg/dl)
40
30
20
10
0
−10
−20
−30
0
20
40
60
120
TIME (Min)
SHAM
DEMED
15 footshocks
*
*

Figure 8e. Blood glucose levels at various times following administration of 2-DG (250 mg/kg ip) to rats which were depleted of brain NE (DVNB) or received sham operations. Overall, the DVNB group had significantly higher blood glucose levels than the SHAM group (See text for details).

Figure 8e

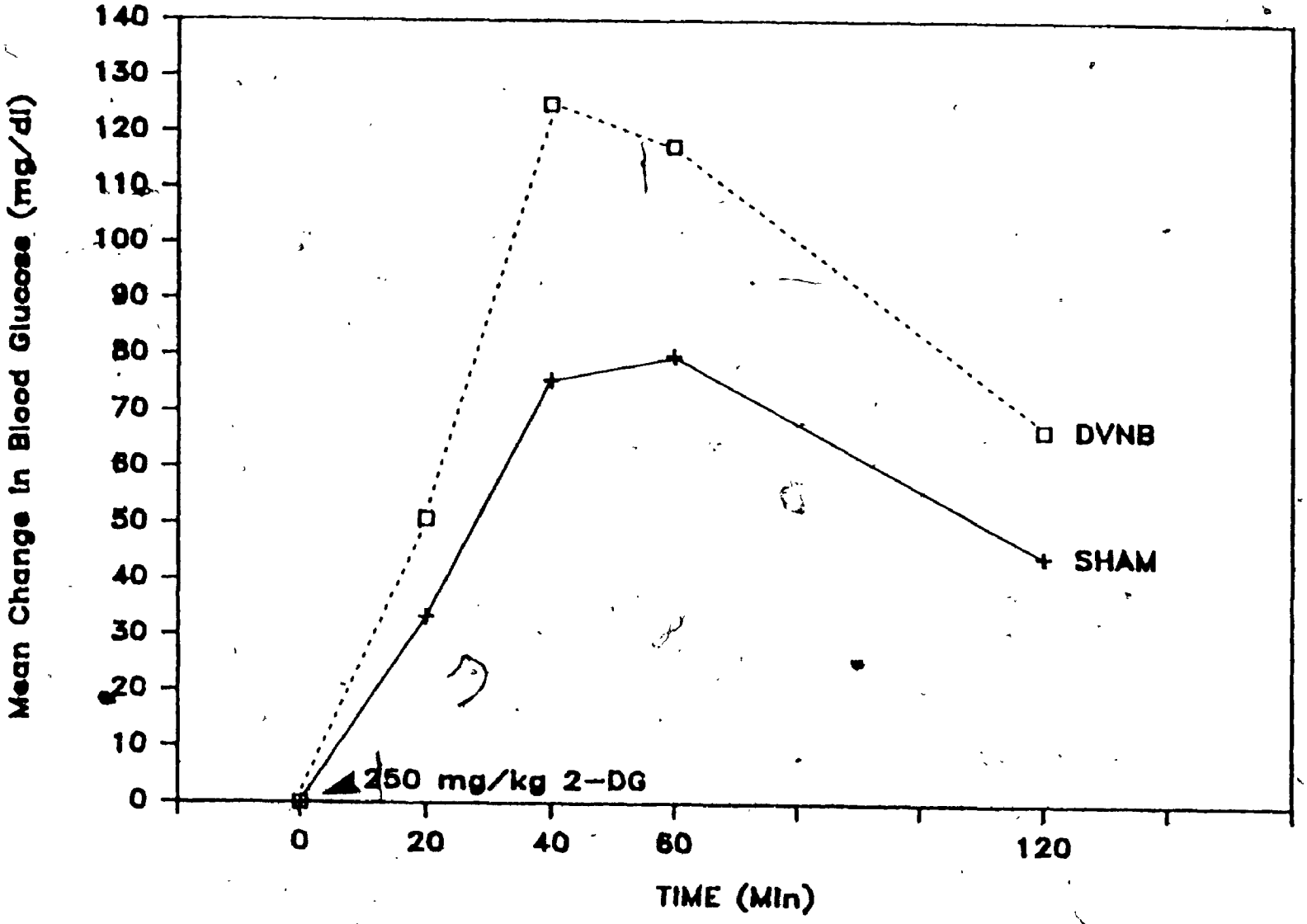

Mean Change In Blood Glucose (mg/dl)
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
250 mg/kg 2-DG
DVNB
SHAM
0
20
40
60
120
TIME (Min)

Figure 8f. Mean change in blood glucose levels at various times following the administration of 2-DG (250 mg/kg ip) in demedullated (DEMED) and sham-operated rats (SHAM). Data are collapsed across the Brain Lesion Factor. See text for details.

Figure 8f

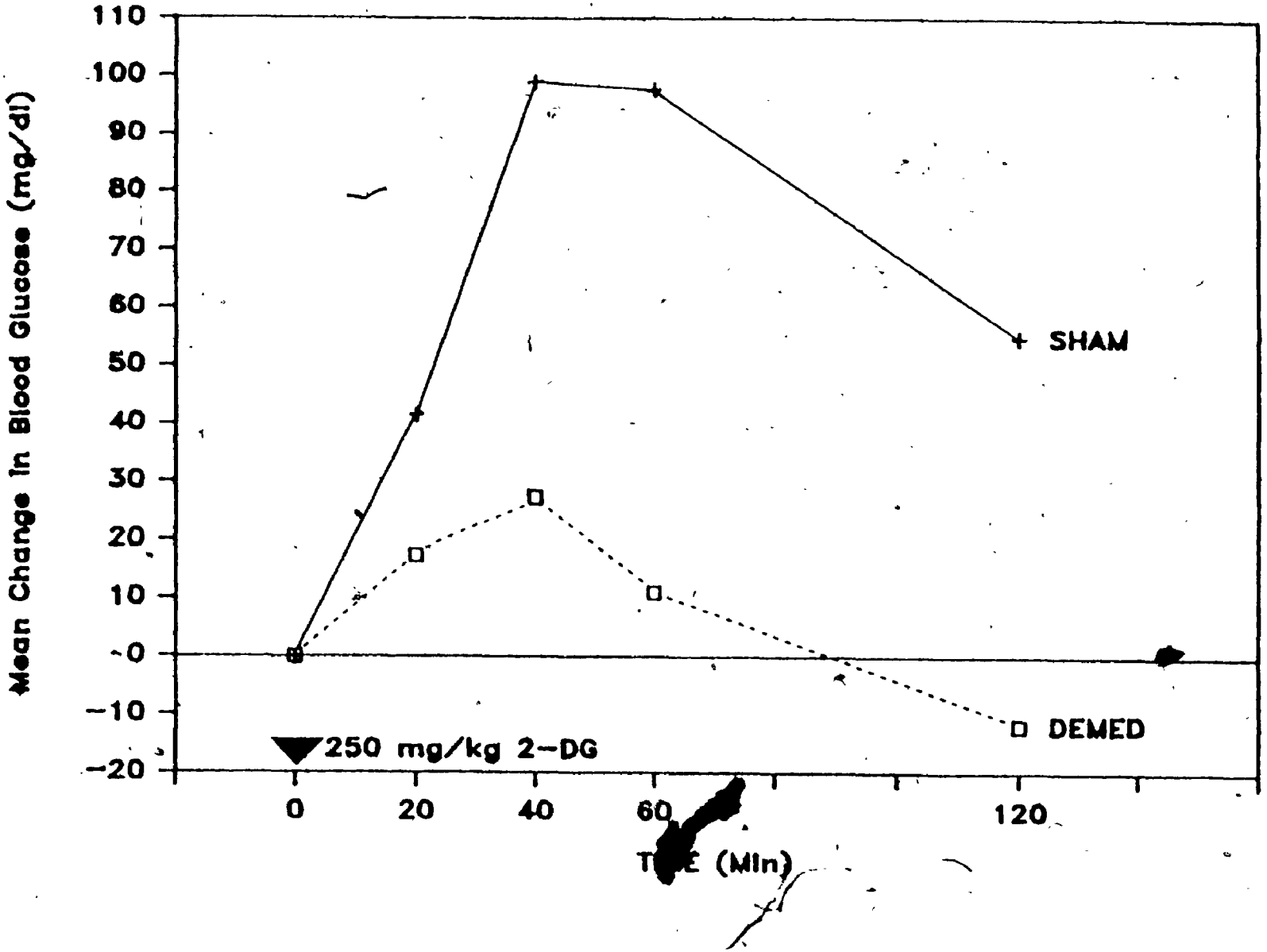

Mean Change in Blood Glucose (mg/dl)
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
SHAM
DEMED
250 mg/kg 2-DG
0
20
40
60
120
TIME (Min)

Figure 8g. Mean change in blood glucose levels at various times following the administration of 2-DG (250 mg/kg ip) in demedullated rats (DEMED) that had also been depleted of brain norepinephrine (DVNB+DEMED) or had received sham operations (DEMED). * indicates a significant difference from the DEMED group ($p<0.05$ as determined by an a priori t-test).

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Figure 8g

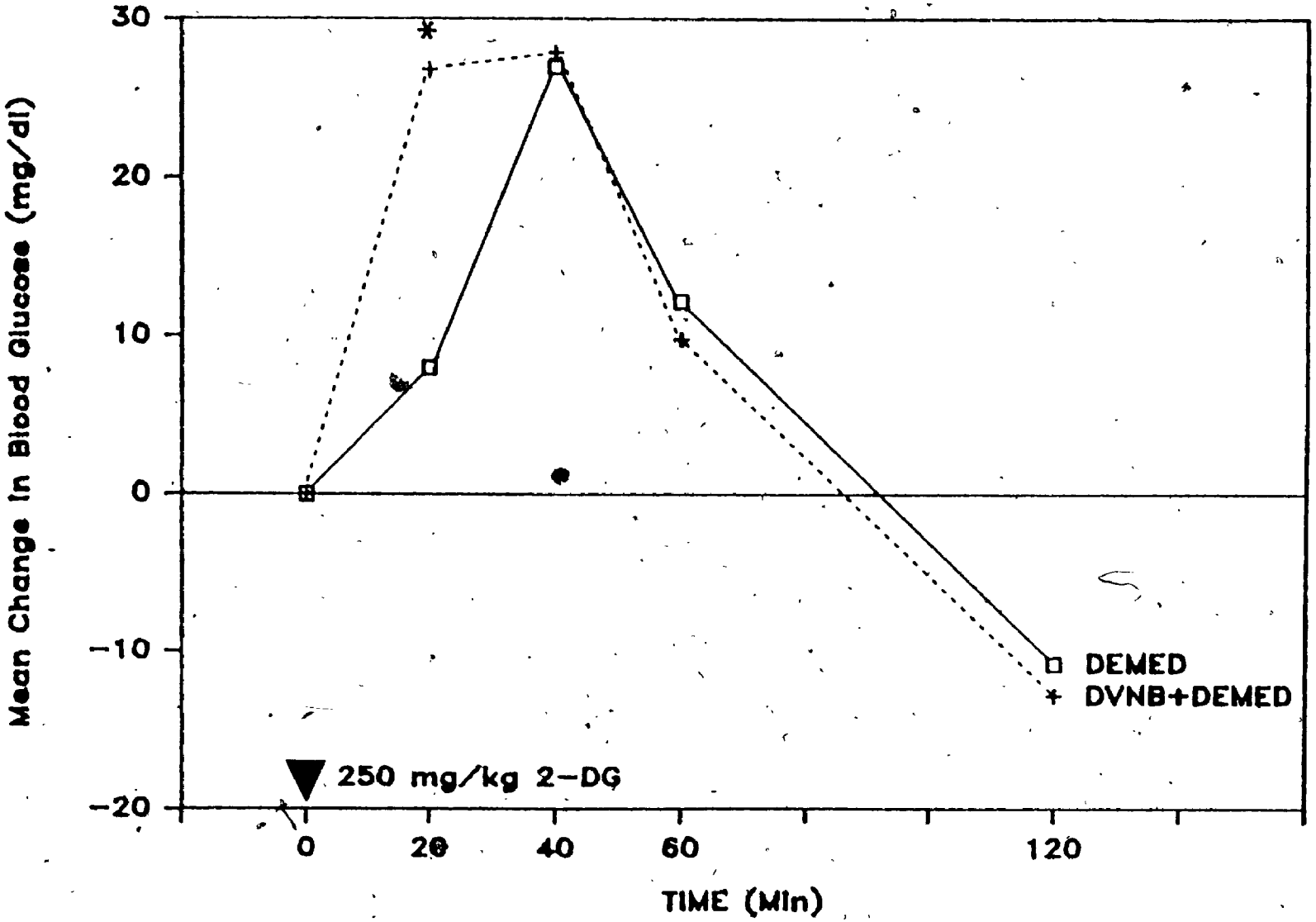
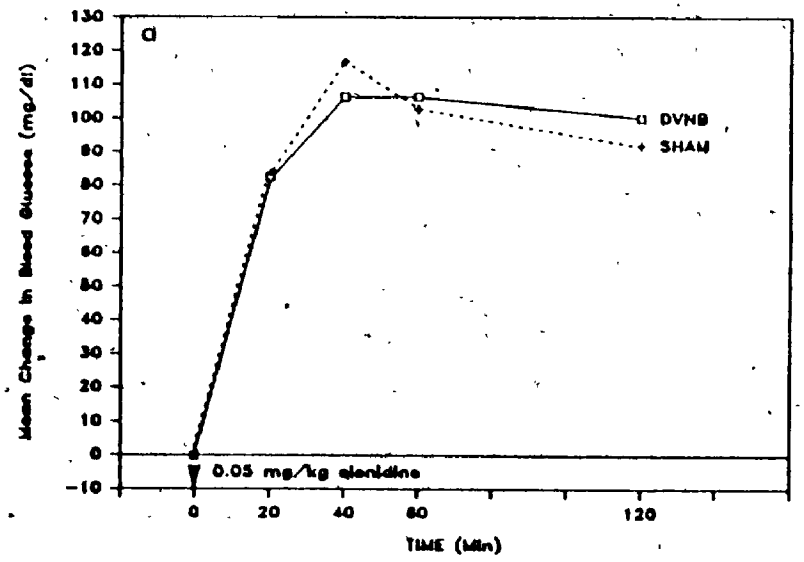
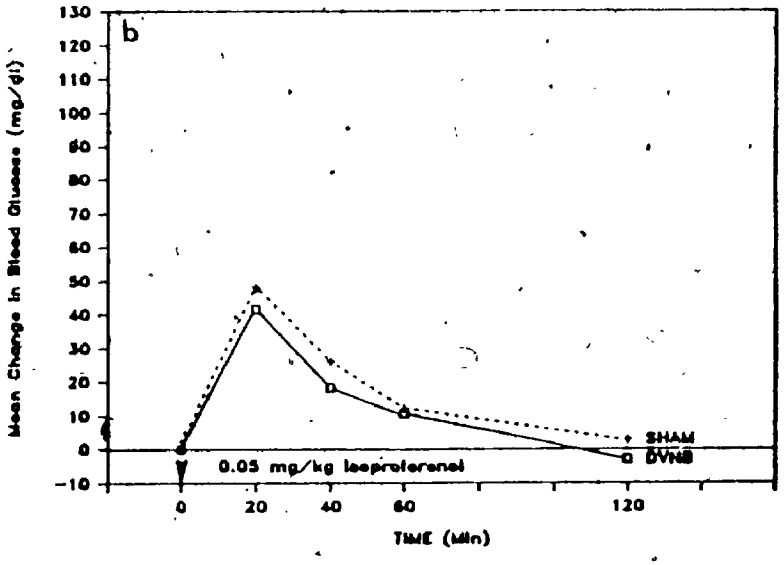
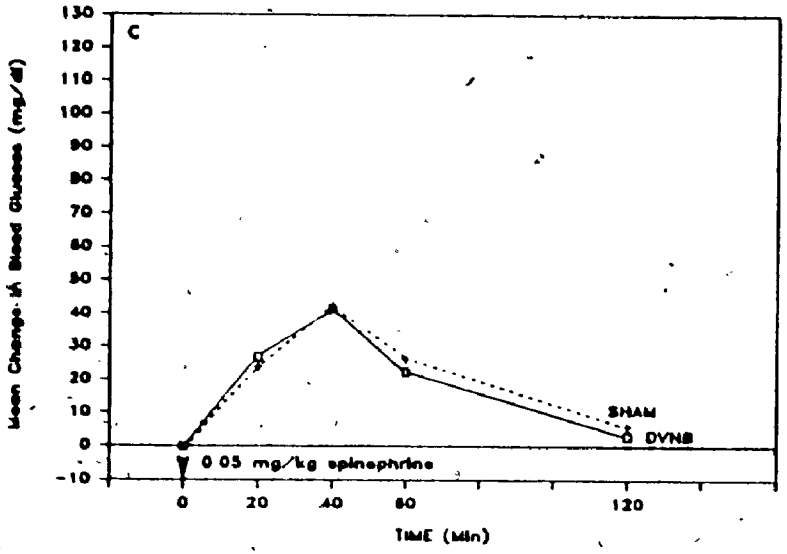

30
20
10
0
−10
−20
Mean Change In Blood Glucose (mg/dl)
0
20
40
60
120
TIME (Min)
250 mg/kg 2−DG
DEMED
DVNB+DEMED

Figure 9. Mean change in blood glucose levels at various times following the administration of adrenergic agonists in NE depleted (DVNB) and SHAM-operated rats (SHAM). The adrenergic agonists that were used were clonidine (0.05 mg/kg ip) [panel a], isoproterenol (0.05 mg/kg ip) [panel b], epinephrine (0.05 mg/kg ip) [panel c], and amphetamine (0.5 mg/kg ip) [panel d]. It is clear that the DVNB group responded to each of the adrenergic agonists in a similar manner as the SHAM group.

Figure 9


a
Mean Change in Blood Glucose (mg/dl)
DVNB
SHAM
0.05 mg/kg clonidine
TIME (Min)


b
Mean Change in Blood Glucose (mg/dl)
SHAM
DVNB
0.05 mg/kg isoproterenol
TIME (Min)


c
Mean Change in Blood Glucose (mg/dl)
SHAM
DVNB
0.05 mg/kg epinephrine
TIME (Min)

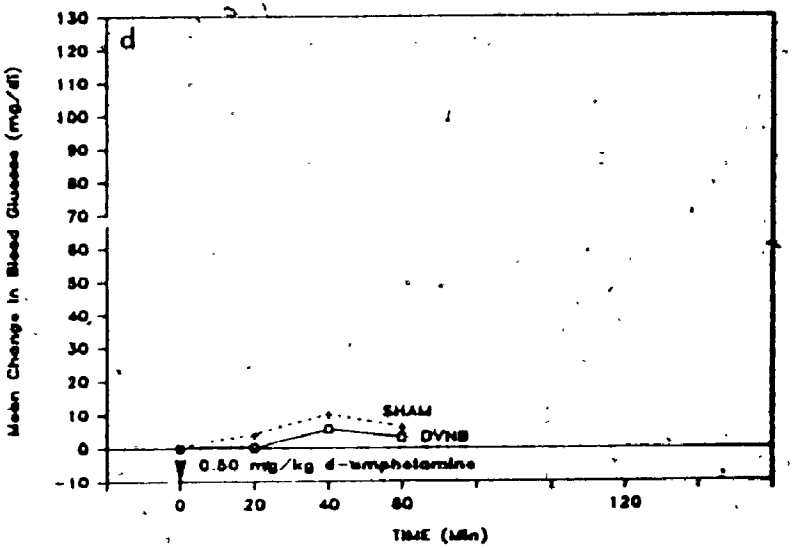

d
Mean Change in Blood Glucose (mg/dl)
SHAM
DVNB
0.50 mg/kg d-amphetamine
TIME (Min)

Figure 10. The number of rats reaching the retention criterion (See text for details of the criterion) following posttraining administration of various doses of glucose. Retention trials were carried out 48 hours following initial training in the "Y-maze" avoidance task. * indicates a significant difference from the VEH-injected group ($p<0.05$). Comparisons were made using the normal approximation to a binomial distribution (Mendenhall & Ramey, 1973).

FIGURE 10

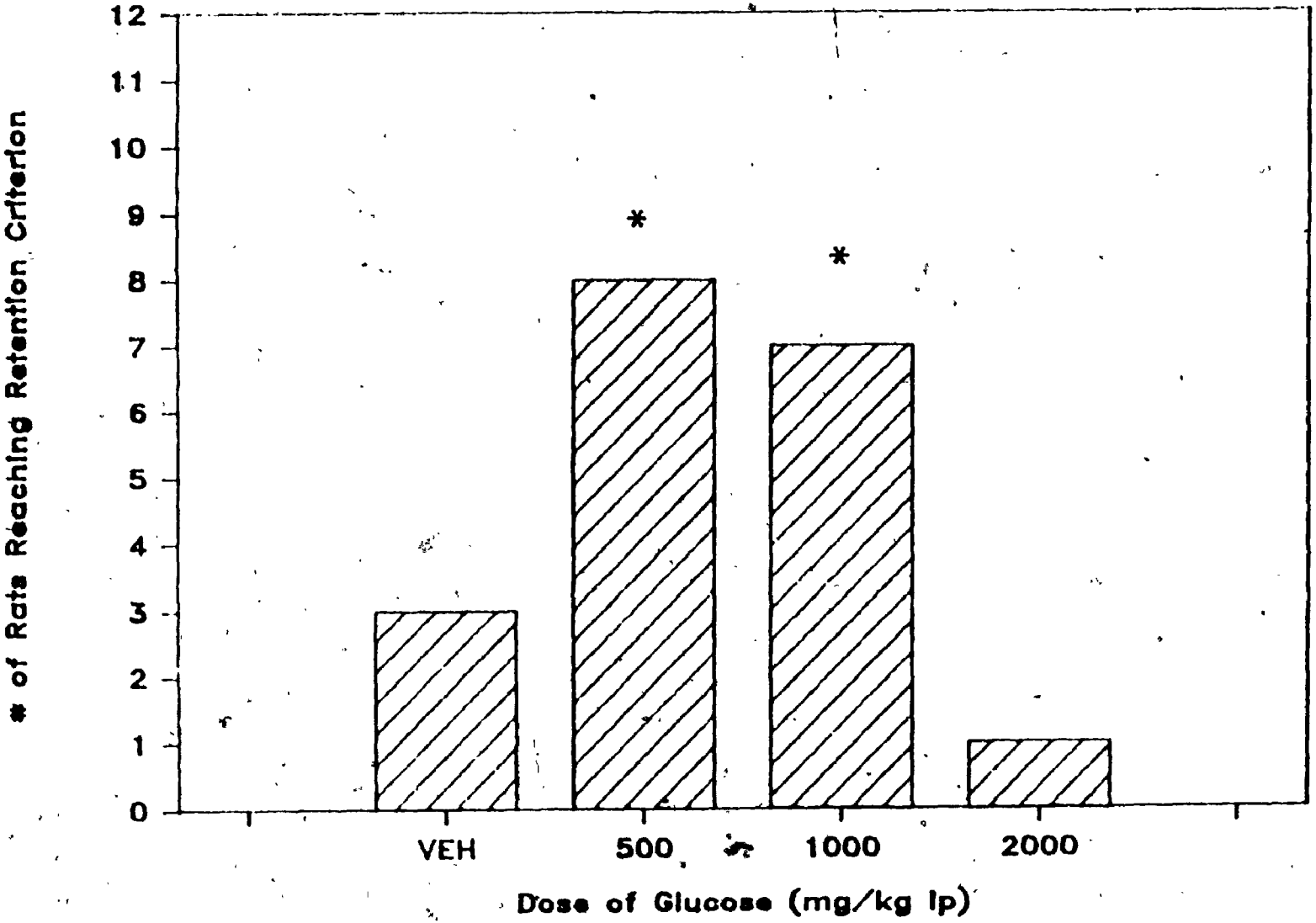
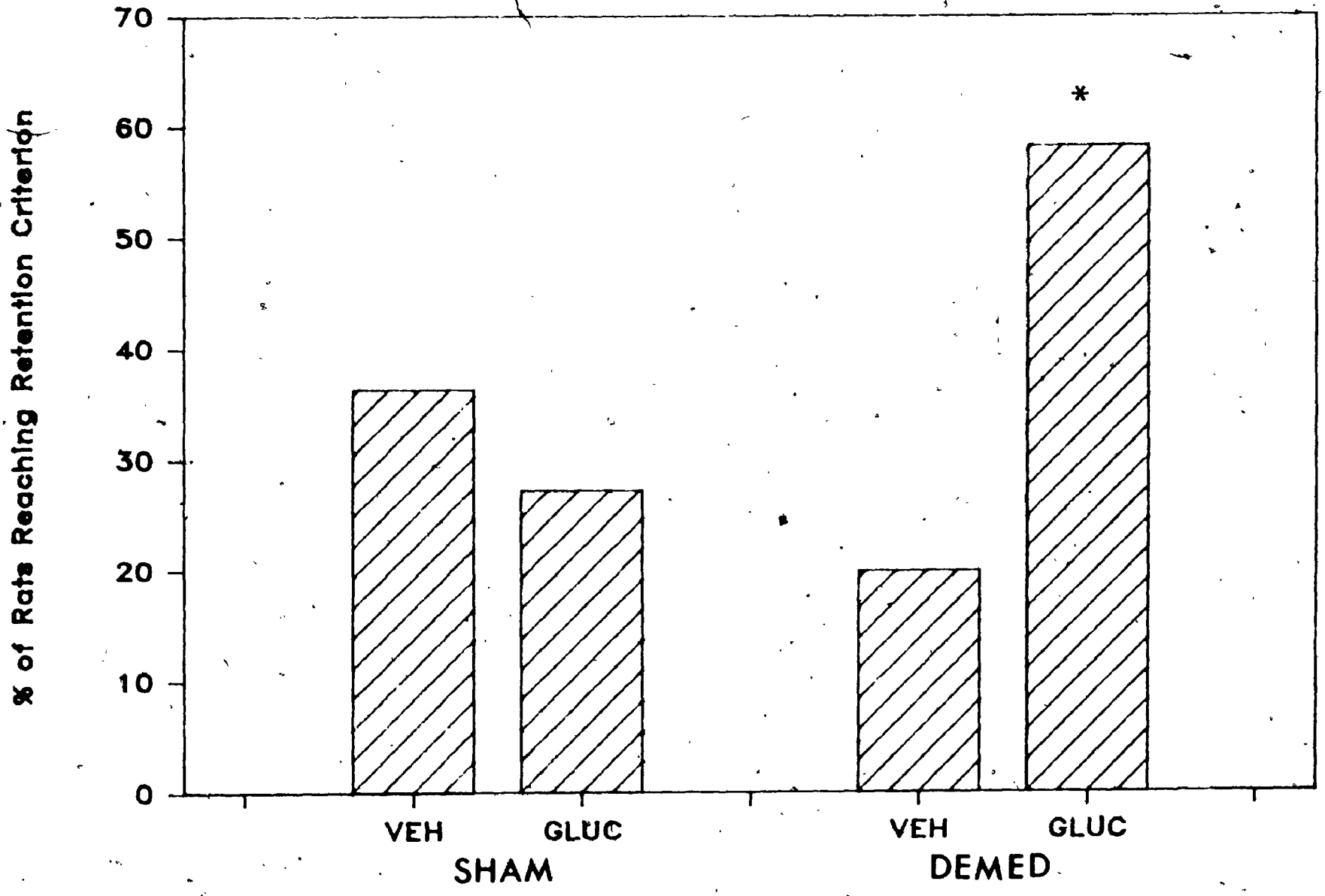

12
11
10
9
8
7
6
5
4
3
2
1
0
# of Rats Reaching Retention Criterion
*
*
VEH
500
1000
2000
Dose of Glucose (mg/kg ip)

Figure 11. The percentage of demedullated (DEMED) and sham-operated (SHAM) rats reaching the retention criterion (See text for details of the criterion) following posttraining administration of glucose (500 mg/kg ip). Retention trials were carried out 48 hours following initial training in the "Y-maze" avoidance task. * indicates a significant difference from the VEH-injected DEMED group ($p<0.05$). Comparisons were made using the normal approximation to a binomial distribution (Mendenhall & Ramey, 1973).

FIGURE 11


% of Rats Reaching Retention Criterion
70
60
50
40
30
20
10
0
*
VEH
GLUC
SHAM
VEH
GLUC
DEMED

APPENDIX A

State-Dependent Learning (SDL)

The suggestion that endogenous or exogenous substances that alter the physiological state of an organism at the time of training can function as cues to facilitate later retention, if these substances are administered again prior to the retention test, is not new (eg. Kety, 1974; Overton, 1978). Furthermore, if these cues are absent (or changed) during a retention test then retrieval may be inhibited to some extent. This view is often called a "State-Dependent Learning" model of memory processing.

With respect to the present hypothesis, future experiments will address the question of what role, if any, alterations in blood glucose levels during and following aversive conditioning play in normal and abnormal processing of memory for the conditioned response?

"Active" and "Inactive" Memory

An important advancement in the SDL view of memory processing is that cues associated with the original conditioning situation can "reactivate" the memory for the conditioned response when introduced at a later time (eg. Misanin, Miller & Lewis, 1968; Lewis, 1979; Gerson & Hendersen, 1978; Mactutus, Ferek, George & Riccio, 1982; Richardson, Riccio & Molenda, 1982; Richardson, Riccio & Mowrey, 1982). Proponents of this view believe that the state of activity of a memory rather than its age determines its susceptibility to disruption by an amnestic

agent.

The "active" vs "inactive" theory of memory processing is explained as follows. Learning always occurs in the presence of certain cues, and when similar cues are subsequently encountered the initial memory will be reactivated. Thus, memories are considered to be active during original learning and during reactivation. When memories are active they are susceptible to disruption by amnestic treatments. For example, an amnestic treatment has no effect when presented 24 hours following passive avoidance training, but this same treatment disrupts retention performance if cues present during training are presented with the amnestic treatment (See the following references for specific examples; Misanin et al, 1968; Lewis, 1979; Gerson & Henderson, 1978; Mactutus et al, 1982; Richardson et al, 1982).

An important finding with respect to the present hypothesis is that evidence has been reported that indicates that the release of E in response to an aversive stimulus (eg. footshock) is a major cue that is associated with the conditioned response in an aversively-motivated task (eg. Concannon, Riccio & McKelvey, 1980; Izquierdo & Dias, 1983). Furthermore, as would be expected by the present hypothesis, E can act as a reactivating cue to make the memory for a passive avoidance response susceptable to disruption by an amnestic treatment (Gold et al, 1982). That is, when electroconvulsive shock is paired with an injection of E 24 hours following initial training, performance on a subsequent retention test is poorer than for rats given saline paired with the electroconvulsive shock.

It has also been suggested that the effects of E on memory processing may be mediated by this hormone's effects on BG levels (Hall & Gold, 1985; Gold, 1986). These investigators found that posttraining injections of glucose enhanced memory, and behaviorally effective doses of E increased BG levels to approximately the same level as behaviorally effective doses of glucose (Hall & Gold, 1986). An important question that remains to be answered is whether an injection of glucose is sufficient to reactivate the memory for a conditioned response? This question will be the focus of future work.

END

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FIN