

Evidence for centers in the central nervous system that regulate fat mobilization in dogs

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ABSTRACT Infusions of 2-deoxyglucose (2-DG) into intact, adrenalectomized, and adrenalectomized-hypophysectomized dogs caused increases in plasma free fatty acid (FFA) levels which could be reversed by infusing hexamethonium, or prevented by epidural anesthesia or destruction of the thoracic spinal cord. Similar infusions of 2-DG were given to adrenalectomized dogs after transection of the spinal cord. Lesions between C 4 and T 7 prevented the increase in FFA while lesions at T 8 or C 2-3 did not.

These results indicate that inhibition of glucose metabolism by 2-DG causes an increase in plasma FFA by a pathway involving the sympathetic nervous system and that there are centers regulating this activity in the cervical portion of the spinal cord.

KEY WORDS central nervous system · fat mobilization · hypoglycemia · 2-deoxyglucose · spinal cord · hypophysectomy · adrenalectomy · sympathetic nervous system · growth hormone · sympathetic blockade · free fatty acids · glucose receptors · adipokinetic factors · chemoreceptors · adrenal medullary secretion

LIPIDS ARE MOBILIZED from adipose tissue stores for transportation to peripheral tissues as free fatty acids (FFA) bound to albumin (1) and the nervous system has been shown to participate in the regulation of this process. Adipose tissue is richly supplied by autonomic nerve fibers: the evidence that they are important in the regulation of the metabolic activity of adipose tissue has been reviewed by several authors (2-4). The influence of the nervous system on fat mobilization is not limited to the emergency functions of the autonomic nervous system, particularly those associated with adrenal medullary secretion. Havel and

Goldfien (5) were able to demonstrate the presence of tonic sympathetic stimulation of adipose tissue during fasting in both normal and adrenalectomized dogs maintained on cortisone.

Participation of the central nervous system in the regulation of fat metabolism was first suggested by the work of Wertheimer (6) who showed that section of the spinal cord above the sixth thoracic segment prevented the formation of fatty liver in fasting dogs treated with phloridizin.

Since glucose is a vital substrate for the central nervous system, we postulated that a decrease in the availability of glucose would result in increased sympathetic nerve stimulation of adipose tissue and cause an increase in the level of circulating FFA. Glucose receptors increasing sympathoadrenal activity have been found in the central nervous system. Cantu et al. (7) have located centers in the spinal cord which are capable of augmenting adrenal medullary secretion during hypoglycemia.

The following studies were designed to examine the effect of lesions of the nervous system on the increase in plasma FFA levels induced by 2-deoxyglucose (2-DG), a competitive inhibitor of glucose (7a), which appears to stimulate the mobilization of glucose and fat through sympathoadrenal mechanisms (8-11). The results of these experiments support the hypothesis that a limitation of glucose metabolism in the central nervous system results in activation of the sympathetic nervous system leading to an increase in the level of circulating FFA, and also provide some information concerning the anatomical location of centers concerned with fat mobilization.

METHODS

Male mongrel dogs were anesthetized with pentobarbital (25-30 mg/kg) after a 12-24 hr fast. The fem-

Abbreviations: FFA, free fatty acids; 2-DG, 2-deoxyglucose.

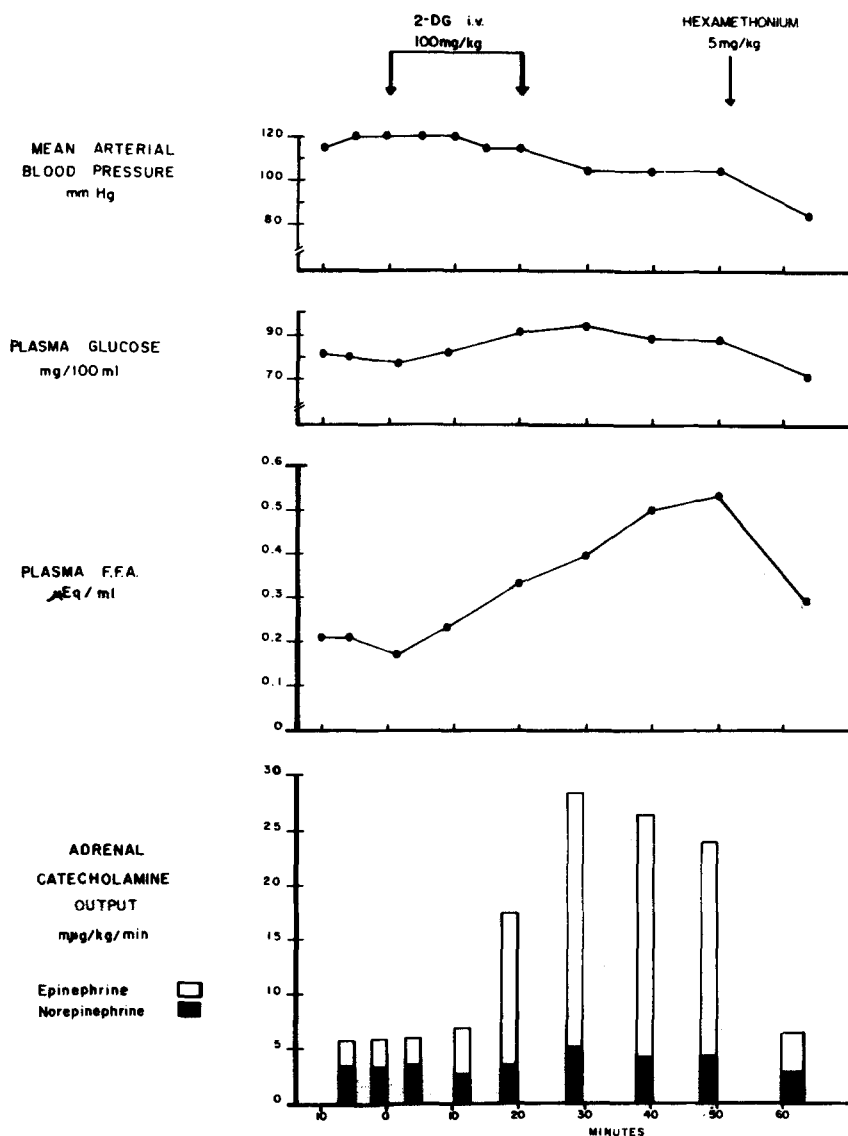


FIG. 1. "Intact" dog. Effects of 2-DG and hexamethonium on plasma FFA and glucose concentrations, blood pressure, and adrenal catecholamine secretion.

oral arteries were cannulated bilaterally for the purpose of measuring arterial pulse and blood pressure and for obtaining arterial blood samples to be analyzed for glucose by the method of Teller (12), FFA by the method of Trout, Estes, and Friedberg (13) and 2-DG by the method of Blecher (14). Tracheal cannulas were inserted and the animals were artificially respired by means of a pump. Concentrations of epinephrine and norepinephrine in adrenal venous plasma were measured by the method of Goldfiel, Zileli, Goodman, and Thorn (15). Additional procedures were performed as noted.

RESULTS

Intact Animals

When 2-DG (2-deoxy-D-glucose, A grade, Calbiochem, Los Angeles, Calif.) in a dose of 100 mg/kg was admin-

istered intravenously for 20 min to a normal animal, the plasma FFA increased from a level of 0.2 to 0.6 μ eq/ml by the end of the infusion and reached a peak of 0.75 μ eq/ml at 30–50 min. A similar experiment was performed in a normal animal in which the adrenal vein had been cannulated so as to allow the output of one adrenal to be collected. The infusion was started 2 hr after the completion of the surgical procedure. The results of this study are shown in Fig. 1. The increase in plasma level of FFA and glucose confirms the findings of Laszlo et al. (8) and the increase in adrenal medullary secretion is similar to that described by Hökfelt and Bydgerman (9). The above changes were reversed following the intravenous injection of 5 mg/kg of hexamethonium.

Adrenalectomized Animals

In order to determine whether the increase in FFA was

dependent upon the adrenal medullary secretion of catecholamines, we conducted similar experiments in adrenalectomized dogs. Bilateral adrenalectomy was performed in six fasting animals. At the completion of the surgical procedure, 50 mg of cortisone acetate and 25 mg of hydrocortisone hemisuccinate were injected intramuscularly and the animals allowed to rest for 2 hr. Samples of arterial blood were then collected before and during a 30 min period of saline infusion. 2-DG (50 mg/kg in 20 ml of saline) was injected intravenously through a constant infusion pump over a period of 20 min. Arterial blood samples were obtained 10, 20, 30, and 45 min after starting the infusion. A rise in the plasma level of FFA was observed in each experiment. The results of these studies are illustrated in Fig. 2, and show that the adrenal medulla is not required for this response. After the 45 min sample had been taken, hexamethonium in a dose of 5 mg/kg was given to each animal intravenously and an additional blood sample was obtained after 10 min. The resultant reduction in plasma FFA suggests that the change is mediated through the autonomic nervous system.

Effect of Growth Hormone

Roth, Glick, Yalow, and Berson (16) have shown that hypoglycemia markedly increases plasma levels of growth hormone, which acts as a potent stimulus to

fat mobilization (17, 18). Bovine growth hormone (generously supplied by Dr. C. H. Li) was injected intravenously in a dose of 0.1 mg/kg into two dogs, one of which was intact and the other adrenalectomized. In both instances an increase in FFA was found; this confirmed the more extensive studies by others (19, 20). The late onset of the rise in FFA as well as the lack of effect of two successive 5 mg/kg dose of hexamethonium is illustrated in Fig. 3 and indicates that augmented secretion of growth hormone is not the major cause of increased plasma levels of FFA in the previous experiments.

Adrenalectomized and Hypophysectomized Animals

In order to determine whether the rapid release of other pituitary adipokinetic factors was required for the response observed in the adrenalectomized dogs, we infused 2-DG into five dogs 3 hr after the completion of bilateral adrenalectomy and hypophysectomy. These studies were carried out in the manner described for the adrenalectomized dogs except that the rest period after surgery was slightly prolonged (3 hr). In each instance an increase in plasma FFA was noted, which was rapidly reversed by injection of hexamethonium, as illustrated in Fig. 4.

Intracarotid Infusions of 2-DG

To obtain information concerning the location of the

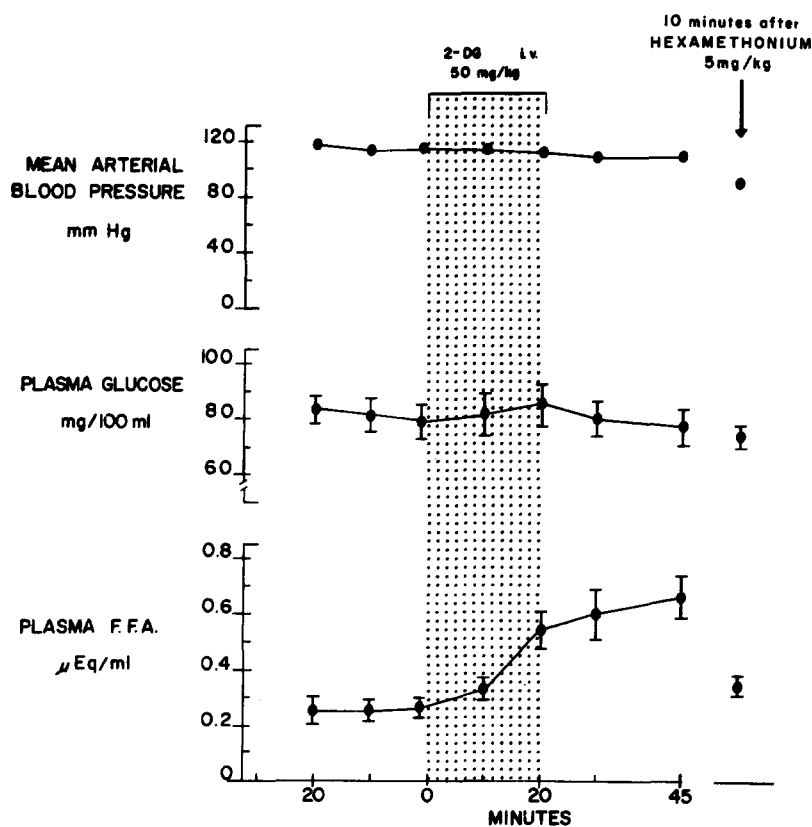


FIG. 2. Adrenalectomized dogs. Effects of 2-DG and hexamethonium on plasma FFA and glucose concentrations and mean arterial blood pressure. Values shown \pm SEM ($n = 6$).

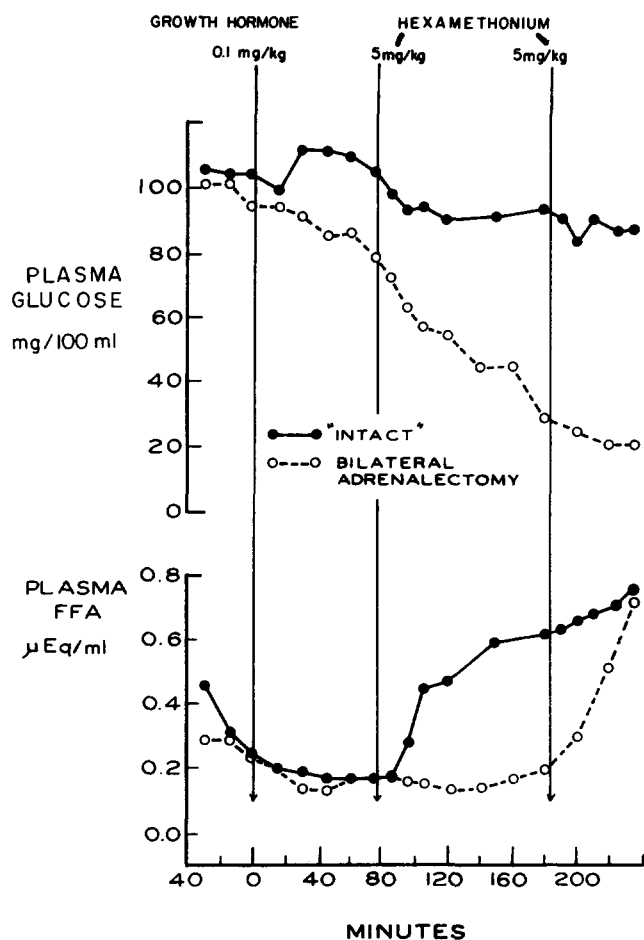


FIG. 3. Effect of bovine growth hormone and hexamethonium on the plasma glucose and FFA levels in an intact and an adrenalectomized dog.

receptors, we compared the response to intracarotid infusion with that produced by intravenous infusions of 2-DG. Fifteen experiments were done in dogs 2 hr after bilateral adrenalectomy. Cannulas were placed in the internal carotid artery on one side. Since the uptake of 2-DG is a function of its concentration, an attempt was made to reduce the cranial circulation by bilateral ligation of the common carotid arteries and jugular veins. Plasma levels of FFA increased in 11 of the 15 dogs. In the nine dogs receiving 50 mg/kg or more of 2-DG, the mean increase was 0.15 $\mu\text{eq/ml}$ compared to 0.80 $\mu\text{eq/ml}$ when the intravenous route was used. The difference is significant ($P < 0.02$). These results did not suggest the presence of intracranial receptors.

Interruption of Sympathetic Outflow in Adrenalectomized Dogs

Further evidence for the nervous origin of the increase in plasma FFA following 2-DG infusion was sought by the interruption of the sympathetic outflow. The thoracic spinal cord was removed from one animal and 2-DG

was infused for a period of 20 min in a dose of 100 mg/kg. The results of this experiment are shown in Fig. 5. FFA levels were extremely low. However, the severe hypotension might have limited the perfusion of adipose tissue with blood. We tried to minimize this complication by studying three animals during epidural anesthesia with 0.5% Pontocaine (Winthrop Laboratories, New York, N.Y.) which was administered at 30-min intervals through three cannulas placed at intervals in the epidural space. Although the mean arterial blood pressure was considerably higher in these experiments the infusion of 2-DG did not increase plasma FFA, as noted in Fig. 6. Although changes in the peripheral distribution of the cardiac output must occur during epidural anesthesia, it seems unlikely that they could be sufficient to have more than a minor influence on the changes observed.

Spinal Cord Transection in Adrenalectomized Animals

Intravenous infusions of 2-DG (100 mg/kg) were also given to 14 dogs 3 hr following division of the spinal cord at T 8-9, T 5-6, T 4-5, T 1-2, C 4-5, and C 2-3. A lesion at T 8-9 did not prevent the rise in FFA (Fig. 7), but lesions above T 8 and below C 4 abolished it (Fig. 8). Plasma glucose levels increased in some of these experiments. Similar infusions were given in eight animals after surgical transection of the spinal cord at the level of C 2-3. The plasma glucose levels were low in three of the animals and normal in the remaining five. The latter responded to the 2-DG infusion with an increase in plasma FFA (Fig. 9). This response and the fall observed after the injection of hexamethonium suggest the presence of intact centers below this level in the spinal cord which are involved in the sympathetic outflow to adipose tissue.

Hypoglycemia has been known to occur following injury of the spinal cord (21). Three adrenalectomized animals in which the spinal cord had been transected at C 2-3 were found to have a mean plasma glucose level of 23 mg/100 ml during the control period. The mean control concentrations of FFA were 0.72-0.82 $\mu\text{eq/ml}$ of plasma in these animals. This fortuitous observation shows that the adrenalectomized dog with high cord transection can have high levels of FFA during hypoglycemia as well as after infusion of 2-DG. The maintenance of FFA levels during infusion of 2-DG and the fall to levels similar to those found in the other five animals following injection of hexamethonium (Fig. 10) supports this interpretation of the data.

2-DG, when present in samples analyzed by the glucose oxidase method for determining true glucose, gives values corresponding to 12% of the concentration of 2-DG (22). Plasma levels of 2-DG were therefore measured during a typical infusion to permit more ac-

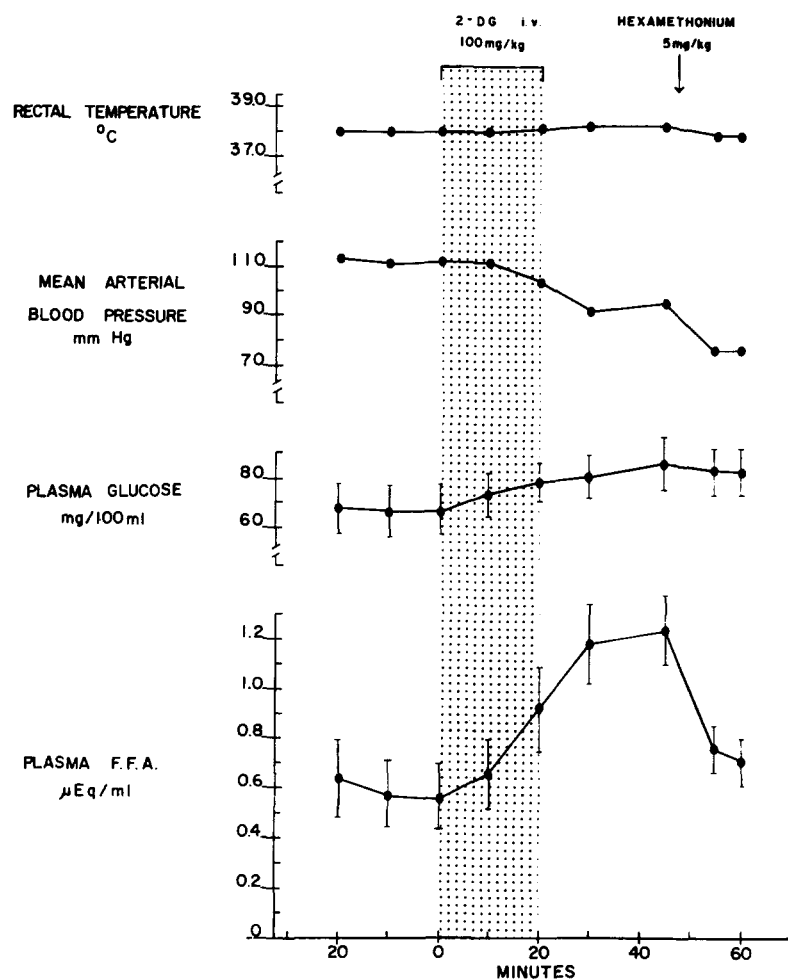


FIG. 4. Adrenalectomized and hypophysectomized dogs. Effects of 2-DG and hexamethonium. Means \pm SEM ($n = 5$).

curate interpretation of the changes noted in plasma glucose. Plasma concentrations of 2-DG observed in seven studies are shown in Fig. 11. The highest level observed in these studies (76 mg/100 ml) is sufficient to cause an error in plasma glucose of only 6 mg/100 ml at the end of the 2-DG infusion. The values shown on the previous figures have not been corrected for this error. Plasma levels of 2-DG observed with intravenous infusions did not differ from those found during and after intracarotid infusions.

In the course of these experiments it was noted that the plasma FFA levels at the start of the 2-DG infusions in cord-sectioned animals were considerably lower than those of the control adrenalectomized animals. Plasma samples were therefore obtained from three dogs 45 min after induction of anesthesia. Bilateral adrenalectomy and transection of the spinal cord at C 2-3 were then done and a second specimen obtained 3 hr after the completion of the procedure. The marked fall in the plasma FFA is shown in Fig. 12. The results of analysis of samples obtained before and 3 hr after adrenalectomy and hypophysectomy in five dogs are also illustrated. The

lack of a fall in the latter group suggests that the change following cord transection did not result from operative "stress" alone.

DISCUSSION

The rise in plasma level of FFA following infusion of 2-DG observed by Laszlo et al. (8) is confirmed by these studies, as is the observation that adrenalectomy minimizes the hyperglycemic response. The participation of the adrenal medulla in this response as shown by Hökfelt and Bydeman (9) was also confirmed. However, it is clear that in the absence of the adrenal medulla, the plasma FFA still increased, in contrast to the findings of Richardson and Hökfelt (23). These investigators found that in the rat, hypophysectomy and adrenalectomy abolished the marked rise in plasma FFA even when cortisone was administered. The differences observed might be related to differences in the experiments, particularly the longer time intervals between hypophysectomy and adrenalectomy and the administration of 2-DG. The longer interval might allow the dis-

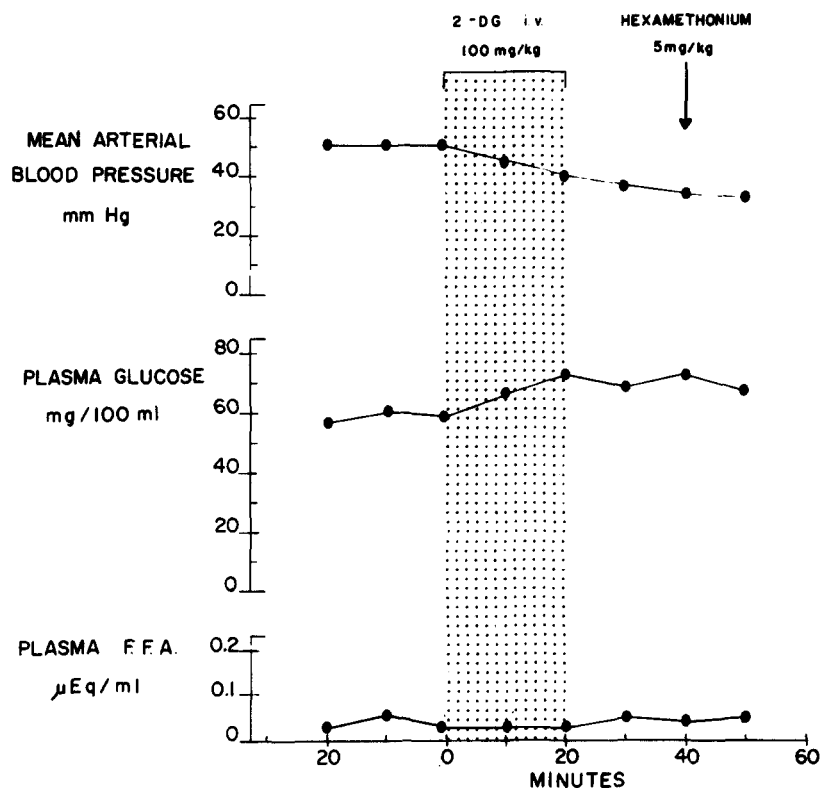


FIG. 5. Effects of removal of the thoracic spinal cord on action of 2-DG and hexamethonium (1 dog).

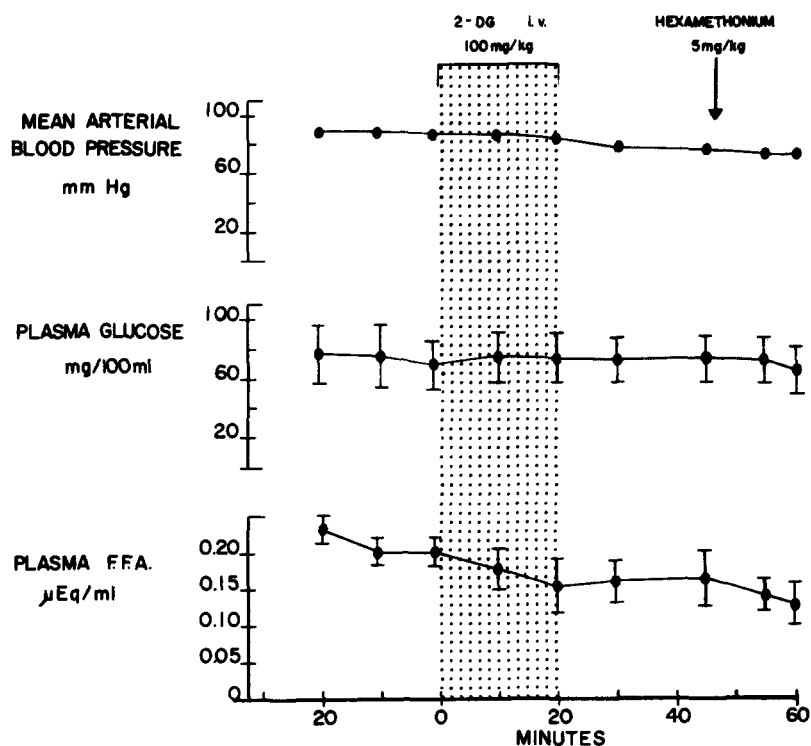


FIG. 6. Effect of epidural anesthesia (induced by Pontocaine). Action of 2-DG and hexamethonium during anesthesia. Mean \pm SEM ($n = 3$).

appearance of factors such as thyroxin which have permissive action in fat mobilization (24, 25). Major differences between the species could also explain the dissimilarities noted.

Infusion of 2-DG was selected as the means of limiting the availability of glucose to the central nervous system for several reasons. The effects of 2-DG on glucose turnover have been studied in the normal and adrenalecto-

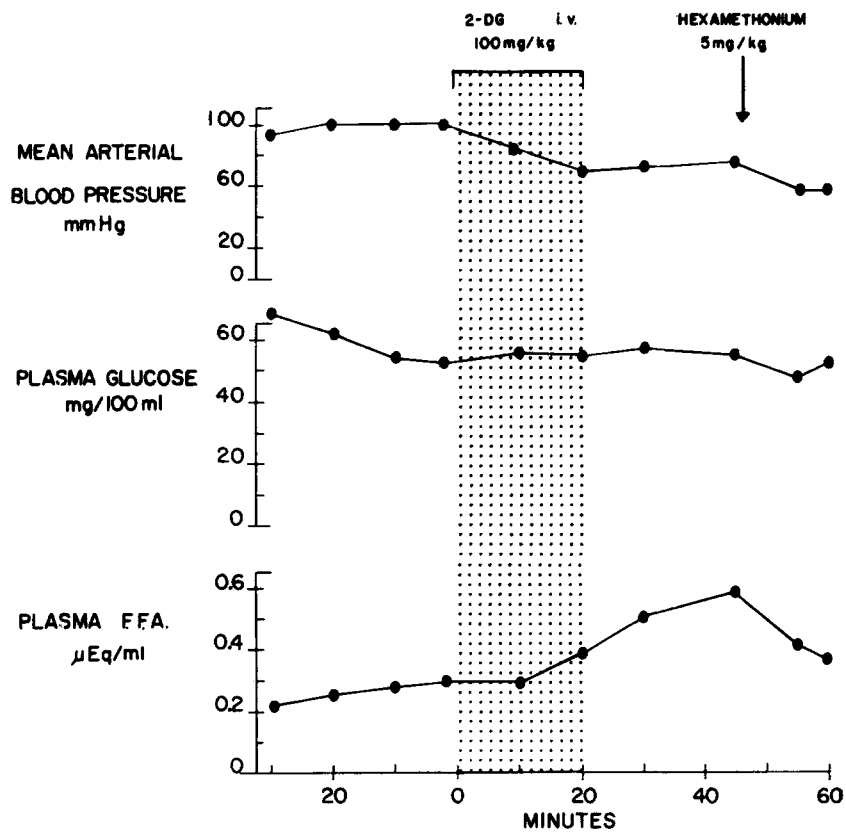


FIG. 7. Effect of transection of the spinal cord at T 8-9 in an adrenalectomized dog.

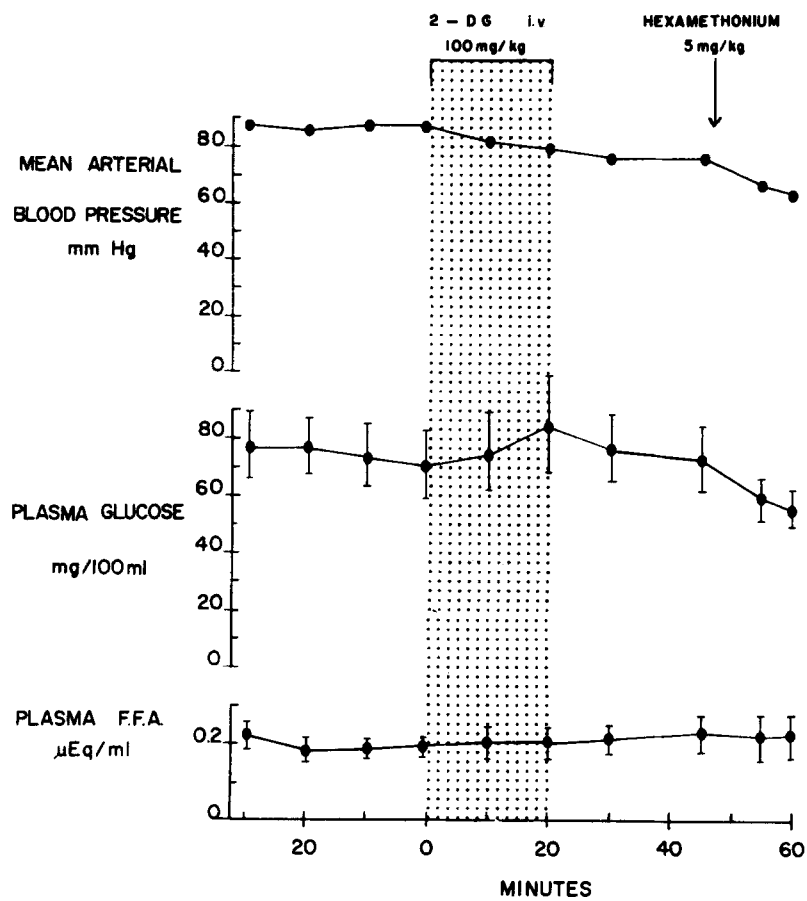


FIG. 8. Effect of spinal transection at T 5-6, T 4-5, T 1-2 (1), and C 4-5 (3) on response to 2-DG and hexamethonium in adrenalectomized dogs.

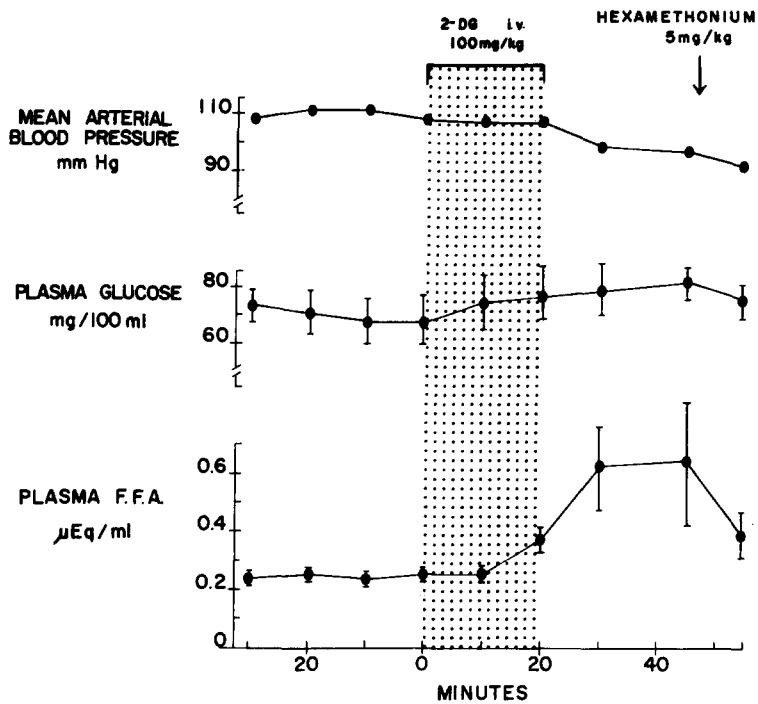


FIG. 9. Effects of 2-DG and hexamethonium in adrenalectomized dogs with normal plasma glucose levels after transection of the spinal cord at C 2-3. Means \pm SEM ($n = 5$).

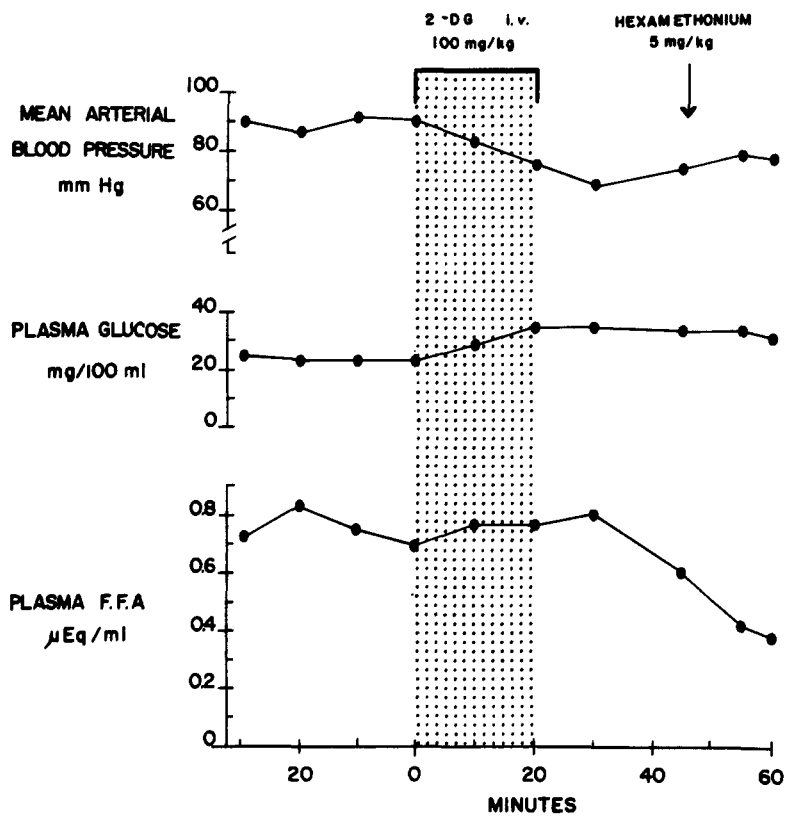


FIG. 10. Effects of 2-DG and hexamethonium in 3 adrenalectomized dogs with low plasma glucose levels after transection of the spinal cord at C 2-3.

mized dog. Altszuler, Dunn, Steele, Bishop, and de Bodo (11) showed that infusion of 2-DG inhibited glucose uptake in vivo. They also noted that adrenalectomized animals failed to become hyperglycemic and

that, in the normal animal, dehydroergotamine blocked the hyperglycemia. They concluded from the work of Hökfelt and Bydgerman (9), who showed that spinal transection in rats reduced hyperglycemic effects of

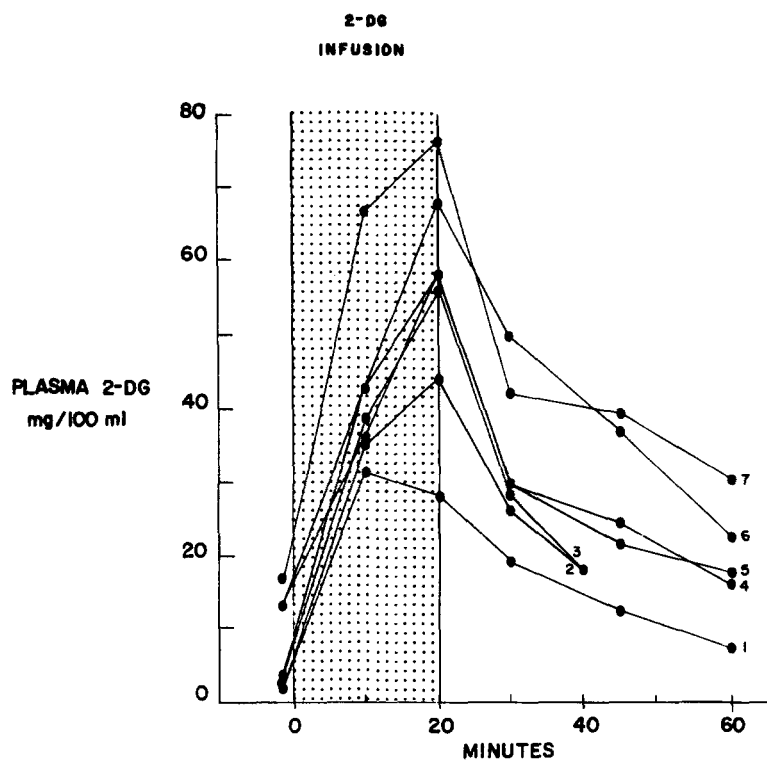


FIG. 11. Plasma concentrations of 2-DG in 5 adrenalectomized dogs. 1, 3: 100 mg/kg i.v. (C 2-3 cord section). 2: 100 mg/kg i.v. (thoracic spinal cord removed). 4: 50 mg/kg via internal carotid artery. 5: 100 mg/kg via thoracic aorta. 6: 100 mg/kg via internal carotid artery. 7: as for 6, followed by 100 mg/kg i.v.

2-DG, and from their own studies, that hyperglycemia was caused by epinephrine released by the adrenal medulla in response to central hypoglycemia.

2-DG has not been shown definitely to affect adipose tissue *in vitro* (25) and no evidence for an important effect in adipose tissue has been obtained *in vivo*. This is an advantage over insulin, which has a marked direct influence on the rate of release of FFA from adipose tissue (26).

Local perfusion of the central nervous system with hypoglycemic blood was also considered as a possible method. However, its use would have been complicated by the many problems of cross circulation in addition to the fact that heparin could not be used for anticoagulation (27).

That the increase in plasma FFA levels observed in these experiments is predominantly mediated via the sympathetic nervous outflow is supported by the inhibition or reversal when sympathetic outflow was blocked surgically or pharmacologically. The ability to block the response in this manner or by lesions in the spinal cord indicates that 2-DG does not elevate plasma FFA levels by a direct action on adipose tissue. These results also indicate that central nervous system centers are involved. The studies in animals with transections of the spinal cord show that centers which are activated by infusions of 2-DG exist below C 2-3 but the studies do not exclude the presence of higher centers. In fact, the decreased control level (before infusion) in the group

of animals with cervical cord lesions (Fig. 12) is consistent with, but does not establish, the hypothesis that a higher center which provides tonic stimulation to adipose tissue exists.

These studies do not localize the receptor cells responsible for fat mobilization. The sympathetic nervous system is known to be served by chemoreceptors located distally (such as the aortic and carotid bodies) and centrally (such as CO₂ receptors of the medulla). The presence of glucose receptors in the central nervous system has been suggested by the studies of Krulich (28) and of Sataka, Hayano, and Sloviter (29), who found that intracarotid infusions of glucose reduced peripheral glucose concentrations. Goodner and Tustison (30) reported that the fall in peripheral plasma FFA levels which followed the intravenous infusion of small amounts of glucose could be prevented by adrenergic blockade, but increases in glucose and insulin concentrations were noted in their studies. Dunér (31) reported that glucose injected into the hypothalamus reduced the output of epinephrine by the adrenal medulla. No increase was noted when Ringer's solution without glucose was injected.

Cantu et al. (7) have shown that the upper thoracic and cervical spinal cord contain centers responsible for adrenal medullary secretion in response to hypoglycemia and some of our unpublished observations suggest that the receptors for this response lie within the spinal cord itself. It seems likely that the receptors for the response

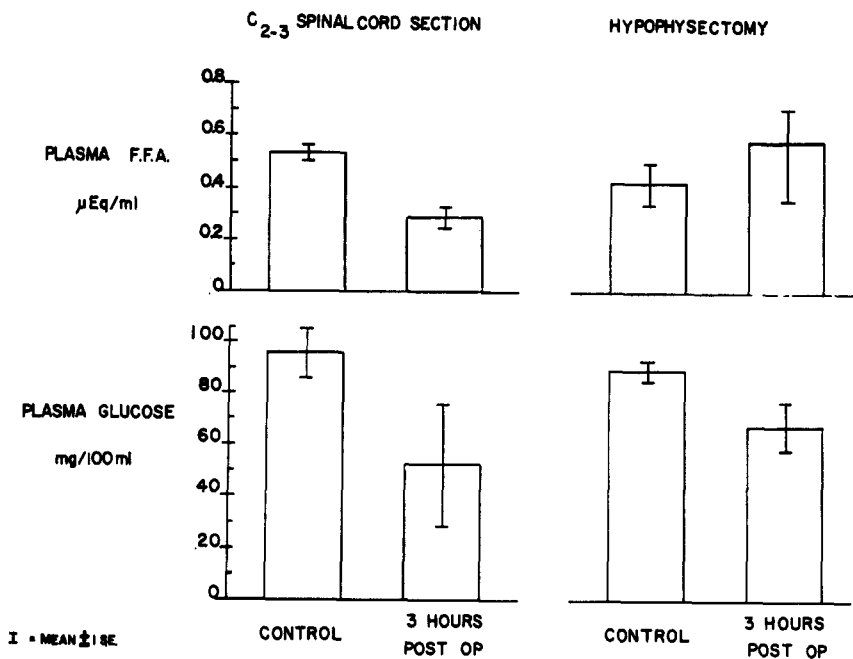


Fig. 12. Comparison of the mean plasma FFA and glucose concentrations obtained 45 min after induction of anesthesia (prior to surgery) and 3 hr after adrenalectomy and cord transection or adrenalectomy and hypophysectomy had been completed.

of FFA are also within the brain and upper cervical spinal cord. However, the existence of distally placed receptors is not excluded and in fact their presence might explain the less consistent increases in FFA when 2-DG was infused into the internal carotid artery, since the structures in the neck were extensively manipulated during the surgical procedure.

One cannot attribute the small rise in glucose level in the animals with cord transections to adrenal medullary secretion since they were adrenalectomized. It cannot be explained by the interference of 2-DG with the glucose oxidase method. It might be postulated that the rise in plasma FFA levels caused a decrease of glucose uptake into tissues. Fatty acids do inhibit glucose uptake by muscle *in vitro* (32). However, even if this occurs, it does not provide an explanation for the increase in plasma glucose observed in the cord-sectioned animals that did not respond with an increase in FFA. 2-DG might reduce the insulin output of the pancreas, which could lead to an increase in the plasma glucose. However, an associated increase in plasma level of FFA would also be expected under these circumstances.

Mobilization of fat during glucose deprivation does not appear to be a simple phenomenon. These studies as well as those previously published by Havel and Goldfien (5) clearly indicate that the sympathetic nervous system participates in this response. Goodman and Knobil (33) and Stern and Maickel (34) failed to prevent by sympathetic blockade increases in plasma level of FFA during fasting. Lewis and Page (35) in their studies of dogs with lesions of the spinal cord at the level of C 6 found a gradual increase in the level of

plasma FFA following a 16 hr fast as the fasting glucose concentrations fell over a 4 month period. In view of the normal increase in plasma levels of growth hormone during glucose deprivation (16) and the effect of growth hormone on fat mobilization (36), it is reasonable to assign an important role to growth hormone in the mobilization of fat during a fast. However, FFA levels increase in fasted hypophysectomized patients although the basal levels are low (37).

Present evidence suggests that mobilization of fat during fasting is a complicated response involving increased lipolysis and decreased esterification of triglycerides. It appears to be influenced not only by the central nervous system via the sympathetic outflow and the secretion of growth hormone by the anterior pituitary but also by the reduced secretion of insulin, and perhaps is reinforced by inhibition of glucose uptake in adipose tissue by the increasing level of FFA itself.

The technical assistance of Miss M. L. Aycock and Mr. John Richardson in the performance of these studies is greatly appreciated.

This study was supported by PHS Research Grants H-06285 and HD-00640, T1 HD 7-06 from the National Institutes of Health, United States Public Health Service.

Manuscript received 24 September 1965; accepted 30 December 1965.

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