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

V. L. Teixeira,[1] J. Antunes-Rodrigues, and R. H. Migliorini[2]

Department of Physiology, School of Medicine, Ribeirão Preto, São Paulo, Brazil

Abstract The blood sugar and plasma free fatty acid responses to administration of 2-deoxyglucose were determined in normal rats and in rats subjected to adrenodemedullation and/or hypothalamic deafferentation, as well as in rats with bilateral hypothalamic lesions. Adrenodemedullation of both intact and deafferentated rats reduced the 2-deoxyglucose-induced increase of blood sugar but did not affect the plasma free fatty acid response to 2-deoxyglucose in normal rats. The increases in blood sugar levels induced by the drug in intact rats were not significantly affected by deafferentation, but, in marked contrast, plasma free fatty acid mobilization after 2-deoxyglucose administration was completely suppressed in deafferentated rats, both in the presence and in the absence of the adrenal medulla. These results confirm previous observations indicating that the sympathetic nervous system and adrenalin release from the adrenal medulla participate in the production of hyperglycemia by 2-deoxyglucose. They provide, in addition, evidence for the existence, in the anterior hypothalamus or in limbic structures, of centers that can specifically influence mobilization of free fatty acids through a direct activation of the sympathetic fibers of adipose tissue without intervening in glucose homeostasis. The experiments in animals with bilateral hypothalamic lesions, although small in number, seem to support the above conclusions.

Supplementary key words free fatty acids · 2-deoxyglucose · hypothalamic deafferentation · adrenodemedullation · electrolytic hypothalamic lesions · glucose receptors · sympathetic nervous system · anterior hypothalamus · limbic structures

The triglyceride stored in adipose tissue is mobilized for utilization by peripheral tissues as albumin-bound free fatty acids, and the regulation of this process is of great importance in caloric homeostasis. The role of hormones in FFA mobilization has been extensively investigated (1). There is abundant evidence that the nervous system also participates in the regulation of fat mobilization. Adipose tissue contains appreciable amounts of catecholamines and is richly supplied by autonomic nerve fibers, which are important in the control of its metabolic activity (2–4). There are indications that the influence of the autonomic nervous system is not limited to emergency functions but may complement hormonal effects in the mobilization of fat during fasting (5, 6). However, evidence for the participation of the central nervous system in the control of FFA release from adipose tissue is scarce. An experimental approach utilized to investigate the existence of a regulatory center(s) is the administration of 2-deoxyglucose (2-DG), a competitive inhibitor of glucose (7) that appears to stimulate the mobilization of glucose and FFA through induction of central glycopenia and activation of sympathoadrenal mechanisms (7–11). By administering 2-DG to adrenalectomized dogs after spinal transection, Goldfien, Gullixson, and Hargrove (11) obtained evidence for the existence, in the cervical portion of the spinal cord, of centers that influence FFA mobilization. These authors did not exclude the presence of higher centers that could provide tonic stimulation for adipose tissue.

The experiments reported here investigate the existence of regulatory centers in the central nervous system of the rat. The responses of plasma glucose and FFA levels to administration of 2-DG were examined in animals with hypothalamic deafferentation or with bilateral electrolytic hypothalamic lesions. The results obtained indicate that such centers exist and also provide some information concerning their locations.

METHODS

Male Wistar rats weighing 180–200 g, maintained on a stock diet with water ad lib., were utilized for all experiments.

Surgical procedures

All operations were performed on rats under ether anesthesia. Bilateral electrolytic lesions were produced by passing 1.5 mA for 10 sec through the uninsulated tip (approx. 0.5 mm) of a stainless steel electrode. The coor-

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

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[2] Correspondence and requests for reprints should be addressed to this author.

dinates utilized were those of De Groot's atlas (12, 13). The control rats underwent all steps of the surgical procedure except the application of the electric current. Hypothalamic deafferentation was done by cutting between the anterior and medial hypothalamus by means of a special knife assembly as described by Halász, Slusher, and Gorski (14). Adrenodemedullation was accomplished by first extirpating the whole gland on the right side and then incising the capsule of the left adrenal and removing the medulla by gentle pressure. The animals did not receive hormonal therapy or NaCl in the drinking water postoperatively. Deafferentated–adrenodemedullated rats were obtained by adrenodemedullating animals that had been deafferentated 15 days previously. All animals were utilized 3–4 wk after the last operation.

Administration of 2-DG and technique of blood sampling

Since preliminary experiments had shown that intraperitoneal injection of distilled water increased the plasma FFA levels, a 10% solution of 2-DG was prepared in physiological saline and administered intraperitoneally. All studies were done in the morning on unfasted animals. Rats received 0.5 g of 2-DG/kg body weight or an equivalent volume of saline. Measurement of plasma FFA concentration at several intervals after administration of the drug showed that maximal levels occurred after 30 or 60 min. In order to minimize manipulation of the animals that might affect the plasma levels of glucose and FFA, blood was obtained from each rat before 2-DG administration and at 30 or 60 min. Blood was collected by cutting the tip of the tail.

Chemical analyses

FFA levels were measured by the method of Dole and Meinertz (15), adapted to permit the utilization of small volumes of plasma. Blood glucose concentration was determined in 0.05 ml of blood by the glucose oxidase method (16). Since glucose oxidase has also a small affinity for 2-DG (17), the actual values of blood glucose concentration after administration of the drug, in all experimental groups, are a little lower than those actually observed.

Routine histological procedures were used to localize the sites of the lesions in all animals. Student's t test was applied to analyze the results, and $P < 0.05$ was taken as the criterion of significance.

RESULTS

Effect of adrenodemedullation

The administration of 2-DG (500 mg/kg) intraperitoneally to intact rats induced increases in the levels of blood sugar and plasma FFA. Blood glucose concentrations 30 and 60 min after administration of the drug averaged, respectively, 173% and 201% of initial values (Fig. 1); plasma FFA levels were 139% and 128%, respectively, of 0 hr values at these same intervals (Fig. 2). Adrenodemedullation reduced the blood sugar response to 2-DG administration, the hexose concentration averaging 125% and 127% of initial levels after 30 and 60 min (Fig. 1). By contrast, plasma FFA response to 2-DG was barely affected by adrenodemedullation; the concentrations of FFA at 30 and 60 min were not significantly different from those of intact rats (Fig. 2).

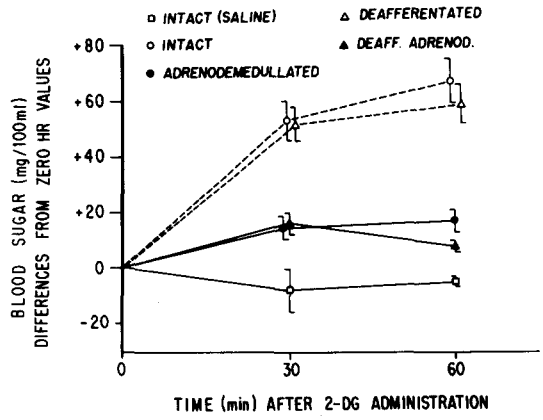

Fig. 1. Effects of 2-deoxyglucose administration on blood sugar levels of intact, adrenodemedullated, hypothalamic deafferentated, and deafferentated–adrenodemedullated rats. Blood sugar levels of intact rats after saline injection are also shown. Each point is the mean of 8–12 observations and the vertical bar represents the standard error. Initial values of glycemia (mg/100 ml, means ± SE): intact, 74 ± 5 (36 rats); adrenodemedullated, 68 ± 3 (19 rats); deafferentated, 79 ± 6 (22 rats); and deafferentated–adrenodemedullated, 70 ± 4 (17 rats).

Effect of hypothalamic deafferentation

Some of the rats with hypothalamic deafferentation had hyperphagia, which persisted for several days. Since histo-

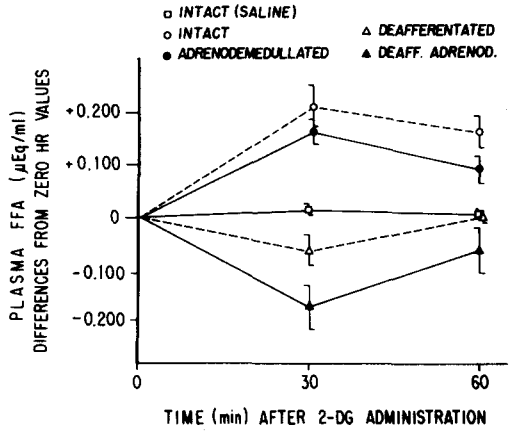

Fig. 2. Effects of 2-deoxyglucose administration on plasma free fatty acids of intact, adrenodemedullated, hypothalamic deafferentated, and deafferentated–adrenodemedullated rats. Levels of plasma FFA of intact rats after saline injection are also shown. Each point is the mean of 8–12 observations and the vertical bar represents the standard error. Initial values of plasma FFA (μeq/ml, means ± SE): intact, 0.46 ± 0.03 (36 rats); adrenodemedullated, 0.36 ± 0.03 (19 rats); deafferentated, 0.48 ± 0.04 (23 rats); and deafferentated–adrenodemedullated, 0.53 ± 0.05 (17 rats).

logical examination did not reveal injury of the medial hypothalamus in any of the animals, hyperphagia probably resulted from the blocking by deafferentation of inhibitory influences from more rostral structures. The increases in blood sugar levels induced by 2-DG were not significantly changed by deafferentation of the hypothalamus (Fig. 1). In marked contrast, however, FFA mobilization after administration of the drug was completely suppressed in deafferentated rats, the average levels of plasma FFA of these animals being even somewhat lower than 0-hr values after 30 min (Fig. 2). A similar suppression of the drug-induced FFA mobilization was obtained in deafferentated–adrenodemedullated rats, which also had a decrease in plasma FFA concentration after 30 min (Fig. 2). Contrary to what was observed in deafferentated rats, in the deafferentated–adrenodemedullated animals there was a significant reduction of the increase in blood sugar levels as a result of administration of 2-DG (Fig. 1).

Effect of electrolytic hypothalamic lesions

The effects of administration of 2-DG on the blood sugar and plasma FFA responses were determined in eight rats with electrolytic hypothalamic lesions and in four sham-operated controls. Although a larger number of animals must be studied, interesting observations were made. All hypothalamic lesions were located bilaterally in the medial and posterior hypothalamus (Fig. 3). None of the lesions interfered with the hyperglycemic response to 2-DG; the blood sugar levels of the animals after administration of the drug were 183% of initial values (Table 1). However, the increase in plasma FFA after administration of 2-DG was clearly reduced in three rats in which the lateral hypothalamic area was lesioned (Fig. 3 and Table 1). In these rats the plasma FFA response to 2-DG was only 30–40% of that obtained in sham-operated controls and in the rats with lesions located in other areas of the hypothalamus.

DISCUSSION

The present results confirm previous evidence (8–11) that the adrenal medulla is essential for the increase in blood sugar levels after administration of 2-DG. From this finding and from the verification that, in the normal animal, sympathicolytic agents (9, 11) or spinal transection (9, 11) block or reduce the hyperglycemic effects of 2-DG, it has been concluded that the hyperglycemia results from epinephrine release by the adrenal medulla in response to central glycopenia induced by the drug (10). In the present experiments, a small rise in glucose concentration was still observed after adrenodemedullation, even after allowing for the interference of 2-DG with the glucose oxidase method. Indeed, 2-DG, when present in samples analyzed by the glucose oxidase method, gives values corresponding to only 12% of the concentration of 2-DG (17), and blood levels around 150 mg/100 ml of the drug would be necessary to account for the increases in glycemia obtained. Such levels could be attained immediately after administration of 2-DG, assuming complete absorption of the injected dose into the extracellular space. However, since 2-DG rapidly moves into the cells and is phosphorylated but not further metabolized (7), the uptake by peripheral tissues and urinary loss reduce the plasma concentration of the drug at 30 and 60 min to levels too low to explain the hyperglycemia observed. In dogs given 2-DG, hyperglycemia has also been found to be markedly reduced, but not abolished, after adrenalectomy (11). Since this small response of blood sugar levels to 2-DG was also observed in the adrenodemedullated–deafferentated rats, in which there was a concomitant decrease of plasma FFA, it can be attributed neither to an FFA-induced inhibition of glucose uptake by muscle nor to decreased levels of circulating insulin. The possibility remains that the slight increase in blood sugar after administration of 2-DG, in the absence of the adrenal medulla, results from a direct inhibition by the drug of the peripheral utilization of the hexose similar to that found for another competitive inhibitor of glucose, 3-methylglucose (18). Mediation by the adrenal medulla is, however, essential for a normal hyperglycemic response.

The increase in plasma FFA induced by 2-DG, on the other hand, was hardly affected by adrenodemedullation. This finding is in agreement with the results of Goldfien et al. (11), who obtained increases in plasma FFA levels after administration of 2-DG to dogs adrenalectomized 3 hr earlier. These observations show that mediation by the adrenal medulla is not essential for the plasma FFA response to the drug. As indicated by its inhibition after surgical or pharmacological block of the sympathetic outflow (11), this response seems to be dependent upon sympathetic nerve stimulation of adipose tissue, which is richly supplied by autonomic nerve fibers (3–5). However, Richardson and Hökfelt (19) did not obtain an increase of plasma FFA after administration of 2-DG to rats adrenalectomized 3 days before and treated with cortisone to prevent cortical insufficiency. Since glucocorticoids markedly affect FFA mobilization from adipose tissue (20–23), it is possible that the administration of cortisone interfered in some way with the drug-induced release of FFA.

The complete block of the plasma FFA response to 2-DG observed here after hypothalamic deafferentation supports the view that the increase of plasma FFA after 2-DG administration results from a drug-induced limitation of glucose metabolism in specific areas of the central nervous system (11) and represents strong evidence for a central regulation of FFA mobilization in the rat. As performed in the present experiments, the hypothalamic deafferentation cut all connections between the anterior and medial hypothalamus (Fig. 4), thus completely suppress-

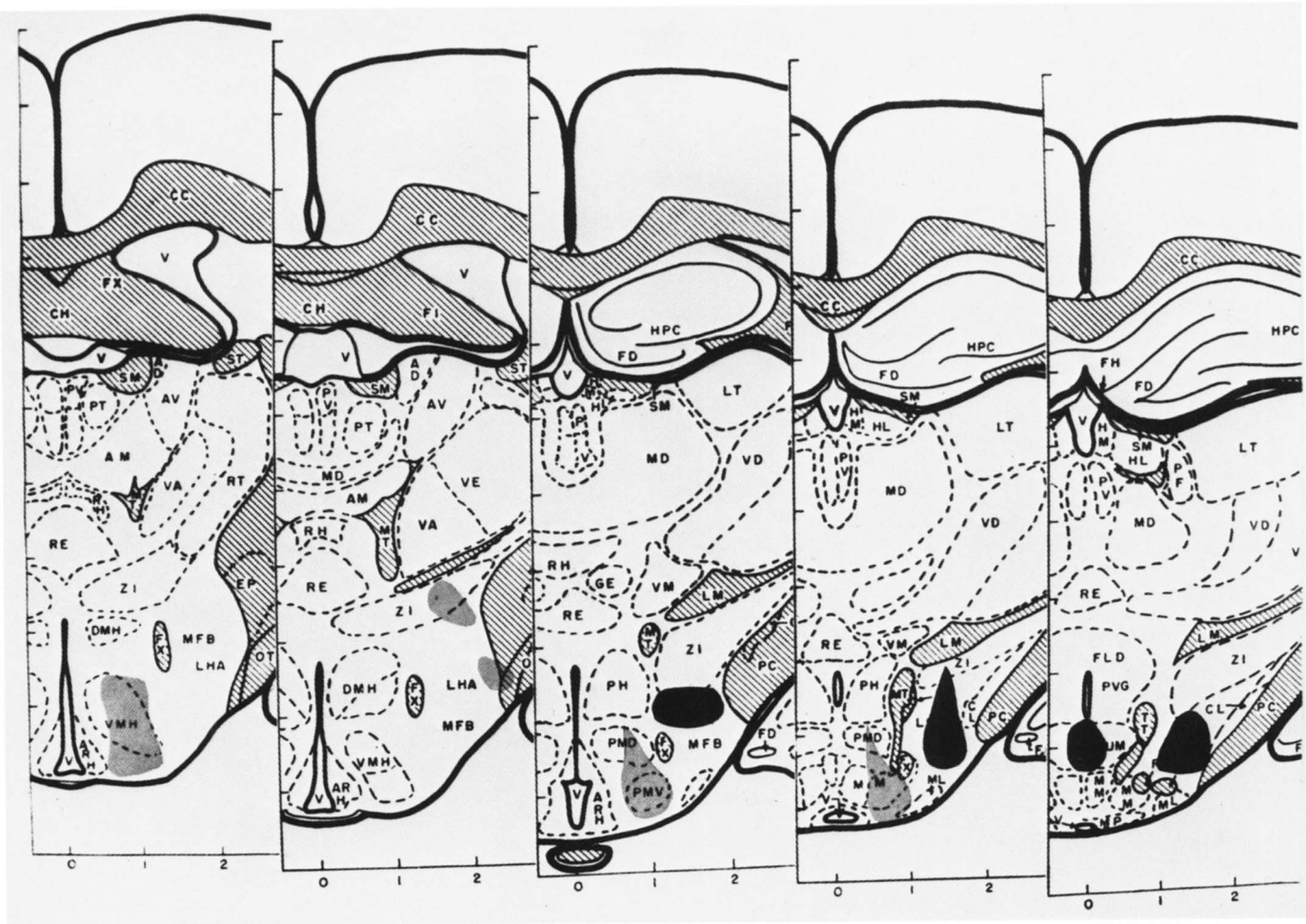

Fig. 3. Schematic frontal sections through the rat hypothalamus. Areas in black depict lesioned regions that determined impairment in FFA release after 2-deoxyglucose administration. Shaded areas represent lesions from rats that had a normal response to 2-DG. Each area corresponds to one animal, except that in the most caudal section the two black areas represent lesions from the same rat, in which the supramammillary region was also lesioned.

ing nervous influences from the anterior hypothalamic and preoptic areas and from the supraoptic, paraventricular, and suprachiasmatic nuclei. The lesions also partially interrupted the connection with the hypothalamus of limbic structures, such as septal area, amygdala, and hippocampus. Accordingly, any of these regions might, theoretically, be involved in the regulation of FFA mobilization. It is important to note that deafferentation suppressed the increase in plasma FFA after 2-DG without affecting, in the presence of the adrenal medulla, the drug-induced hyperglycemia. This indicates that the eventual center(s), although sensitive to changes in glucose concentration, may

TABLE 1. Blood sugar and plasma FFA responses to 2-DG in rats with electrolytic hypothalamic lesions and in controls

Location of Lesion	Blood Sugar Increase	Plasma FFA Increase
	mg/100 ml	*μeq/ml*
Lateral hypothalamic area	62	0.10
	65	0.13
	68	0.14
Other hypothalamic areas	58	0.34
	76	0.32
	66	0.38
	53	0.36
	64	0.43
Sham-operated controls	70	0.36
	53	0.33
	42	0.36
	56	0.42

Initial values (means ± SE, 12 rats): blood sugar, 77 ± 5; plasma FFA, 0.54 ± 0.02.

Fig. 4. Photomicrograph of a horizontal section through the hypothalamus of a rat brain. The arrows indicate the extension of anterior hypothalamic deafferentation.

regulate FFA release specifically, without intervening in glucose homeostasis. Evidence has been provided for the existence, in the ventromedial nuclei of the hypothalamus, of glucoreceptors that are involved in the control of food intake (24). The presence of glucoreceptors in the central nervous system that regulate blood sugar levels has been inferred from experiments showing that intracarotid infusions of glucose reduce peripheral glucose concentration (25). Studies by Cantu et al. (26) indicate that the upper thoracic and cervical spinal cord contain centers responsible for adrenal medullary secretion in response to hypoglycemia. As to glucoreceptors involved in FFA mobilization, Goldfien et al. (11) have suggested, from experiments in which 2-DG was given to adrenalectomized dogs after transection of the spinal cord, that there are centers in the cervical portion of this organ that may regulate the release of FFA from adipose tissue. They pointed out that the experiments did not exclude the presence of higher centers. In fact, they observed decreased levels of plasma FFA in the animals with cervical cord lesions before infusion of 2-DG, a finding that is consistent with the hypothesis that there exists a higher center that provides tonic stimulation to adipose tissue. The present results suggest that, in the rat, inhibition of glucose utilization by cells located in the anterior hypothalamus or limbic structures generates impulses that are relayed to the medial or posterior hypothalamus and from there to the sympathetic nervous system to stimulate lipolysis of adipose tissue. This view is corroborated by the results obtained in rats with electrolytical hypothalamic lesions. Although the number of animals studied was relatively small, it is interesting to note that inhibition of FFA response to 2-DG was observed only in rats with lesions in the lateral hypothalamic area, which consists mostly of fibers coming from more rostral areas of the central nervous system. Barkai and Allweis (27) recently reported that electrical stimulation of the premammillary area in the rat caused a 60% increase in the concentration of plasma FFA without a marked effect on the plasma glucose level (15% increase). Since in these experiments (27) the electrode position varied between sagittal planes 0.6 and 1.4 mm (the positions for individual animals were not presented), it is possible that the lateral hypothalamic area was stimulated in the four rats in which the effect was obtained. In this case, the results of Barkai and Allweis (27) would represent the counterpart of the inhibition of the plasma FFA response to 2-DG observed here in rats with lateral hypothalamic lesions. In cats, dissociation in the responses of blood sugar and plasma FFA was induced by electrical stimulation of the hypothalamus, but the effects obtained could not be related to a distinct hypothalamic locus (28). Another line of evidence pointing to a central nervous system control of FFA mobilization has been developed by Conway et al. (6) and by Goodner et al. (29–31) in studies of the regulation of lipolysis in human subjects and in the baboon. Conway et al. (6) showed that glucose infused into the carotid artery inhibited fasting lipolysis, while intravenous infusion at similar rates was ineffective. Also, inhibition of fasting lipolysis by glucose infusion (intravenously or via the internal carotid artery) could be prevented by adrenergic blocking agents (29–31). It was concluded from these experiments that sympathetic tone contributes to fasting lipolysis and is partially regulated by central glucoreceptors.

Finally, some comments should be made on the levels of plasma FFA of deafferentated rats, which actually decreased after administration of 2-DG. This effect was clearly evident at 30 min and was even more marked in the deafferentated–adrenodemedullated animals (Fig. 2). Since deafferentation supressed the sympathetic nervous system-mediated stimulation of FFA release, the most likely explanation for the above finding is a direct inhibition by the drug of adipose tissue lipolysis. Indeed, it has been repeatedly shown that 2-DG, added in vitro to fragments of adipose tissue incubated in glucose-containing medium, reduces the lipolytic activity of the tissue, probably as a result of a competitive inhibition of glycolysis (32–35). Also consistent with this hypothesis is the greater decrease of plasma FFA in the adrenodemedullated rats, in which the competition by 2-DG would be expected to be more effective by virtue of the lower concentration of circulating glucose.

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