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The Action of Adrenergic Anorexigenic Substances on Rats Recovered from Lateral Hypothalamic Lesions

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RUSSEK, M., A. M. RODRÍGUEZ-ZENDEJAS AND P. TEITELBAUM. *The action of adrenergic anorexigenic substances recovered from lateral hypothalamic lesions.* PHYSIOL. BEHAV. **10**(2) 329–333, 1973.–Adrenaline had no anorexigenic effect on rats in Stage 2 of recovery from bilateral lateral hypothalamic lesions at which time they were already eating wet palatable food but still incapable of caloric regulation. Once caloric regulation was recovered (Stages 3 and 4), and body weight was maintained without tube feeding, the anorexigenic effect of adrenaline reappeared. In contrast, in the recovered laterals (Stages 3 and 4) amphetamine produced less anorexia than in the normal rats and the hyperphagic effect of insulin was absent. There is evidence that adrenaline produces its effect via the hepatic glucoreceptors whose normal central pathways would traverse the lateral hypothalamus. Therefore, the lesion to these pathways might be, at least in part, cause of the aphagic syndrome and of the lack of adrenaline anorexigenic effect. Compensatory pathways that would become functional during the recovery process might account, at least in part, for the recovery of regulation and reappearance of adrenaline anorexia. On the other hand, amphetamine and hypoglycemia (insulin) might produce their effects on food intake by a direct action upon hypothalamic centers, and, therefore, the lateral lesions affect them permanently, reducing amphetamine anorexia and eliminating insulin hyperphagia.

Recovered laterals and adrenaline Adrenaline anorexia and recovered laterals Hypothalamic lateral lesions
Amphetamine and recovered laterals Insulin and recovered laterals

TEITELBAUM and Epstein [18] showed that rats made aphagic by bilateral lesions of the lateral hypothalamic area, if maintained alive by stomach tubing, recovered control of food intake following a definite sequence of events called the lateral syndrome. This syndrome can be divided into four consecutive stages: Stage 1, total aphagia and adipsia; Stage 2, adipsia and anorexia, rats nibble wet palatable foods but are adipsic, do not regulate food intake and require tube feeding to survive; Stage 3, adipsia and dehydration aphagia, rats regulate food intake on wet palatable foods and eat dry food if they are kept hydrated by stomach tubing but do not drink any water; Stage 4, prandial drinking, rats regulate on dry food and water, can still be adipsic, but drink enough water while eating.

The same authors observed that even in Stage 4, the rats did not respond to insulin with a compensatory increase in food intake and could die from the hypoglycemia [3]. As the recovered laterals did increase food intake in response to cold and to food dilution, it was concluded that they had selectively lost the hypoglycemic control of feeding.

It was interesting to investigate if the recovered laterals had also lost the hyperglycemic control of feeding, or rather the anorexigenic effect of intraperitoneal injections of adrenaline.

METHOD

In a total of 52 Wistar rats of either sex, weighing 250–350 g, the lateral hypothalamic area (A=6; L=1.8–2; V=8 from cortex, with lambda 1 mm below bregma) was electrolytically destroyed bilaterally (1–2 mA for 20–30 sec; anode in the brain), under pentobarbital anesthesia (40 mg/kg). Of these, 19 animals died during the operation or a few hours later; 6 animals had aphagia and adipsia for no more than one day and 10 were aphagic and adipsic for an average of 8.3 days (range 3–20), but died before passing to Stage 2. All the data reported were from the remaining 17 animals, which had an average Stage 1 duration of 4.3 days (2–11) and an average Stage 2 duration of 23.8 days (3–133). Two of them did not pass beyond Stage 2 and died after 24 and 133 days respectively; the other 15 progressed to Stage 3, but only 7 reached Stage 4. During Stages 1 and 2, the animals were maintained by stomach tube feeding of a liquid diet (eggs, sugar, evaporated milk and vitamins; for details of this diet see [17]). They were tube fed 10–15 ml, 2–4 times a day, as needed to keep them as close as possible to their preoperative weights. In addition, they were offered wet chocolate cookies. A rat was considered on Stage 3 when it maintained body weight

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on liquid food (the same used for tube feeding) or on wet cookies, without tube feeding, and in Stage 4, when it did the same on Purina Laboratory Chow pellets and water.

Nine animals were injected with adrenaline (0.6–1.0 mg/kg) in Stage 2, that is, as soon as the amount of wet cookies eaten in 24 hr exceeded 5 g (discounting the loss by evaporation, determined by a control dish with wet cookies outside of the cage). There was almost no spillage, but if some was observed, it was also discounted. A paired unoperated control of the same sex and approximately the same age and weight, was offered the same kind of food exclusively, and injected with the same dose of adrenaline at the same time. The large dose of adrenaline was necessary in order to have a clear cut anorexigenic effect on the 24 hr food intake of normal rats, because no feeding schedule could be used in the rats on Stage 2, as was used afterwards in rats on Stages 3 and 4. In the latter, much smaller doses of adrenaline, with a brief effect, could be used.

The other 8 rats progressed to Stage 3 before they were injected. These, plus 4 of the preceding group that recovered to Stage 3, were used to measure the effects of several other substances on the food intake of recovered laterals. First, adrenaline (0.6 mg/kg) was injected while the rats were eating ad lib. Afterwards, they were adapted to a 24 hr feeding - 24 hr fasting schedule, and the following substances were tried in them and in an equal number of paired unoperated controls; adrenaline 0.05 and 0.1 mg/kg (epinephrine hydrochloride, Parke Davis); amphetamine 1.0 and 2.0 mg/kg (D-amphetamine hydrochloride, National Biochemical Corp.) and DCI 10 mg/kg (1–3', 4'-dichlorophenyl -2-isopropyl aminoethanol hydrochloride, Aldrich Chemical Corp.). DCI is a specific beta-adrenergic stimulant blocker [5]. Isotonic saline was used as a control. At least 2 feeding days (4 days in total) elapsed between consecutive injections. The injections were administered 5 min before the beginning of a feeding day. The food intake was measured after 0.5, 1, 2, 6 and 24 hr, and compared with similar measurements done the previous feeding day. All rats were injected first with adrenaline then with amphetamine next with DCI and then again with adrenaline. This controls for the possibility that the different effects of adrenaline and DCI, on one hand, and amphetamine, on the other, were due to the order of injection or the degree of recovery.

Afterwards, the rats were changed again to ad lib feeding, and after a week they were injected with insulin, 10 and 20 U/kg (crystalline zinc insulin, Eli Lilly and Co.), with several days rest between the first injection of 10 U/kg and the second of 20.

RESULTS

Effect of Adrenaline on the Food Intake of Rats With Lesions in the Lateral Hypothalamus, in Stage 2 of Recovery (Anorexia and Adipsia)

A large dose of adrenaline (0.6–1.0 mg/kg) that reduced to less than half the 24 hr food intake of wet cookies in the

TABLE 1

THE EFFECT OF ADRENALINE (0.6–1.0 MG/KG) ON THE 24 HR FOOD INTAKE OF RATS WITH BILATERAL LESIONS IN THE LATERAL HYPOTHALAMUS

Type of Rat	Food (g/24 hr)		Water (ml/24 hr)		Average weight (g)	No. of injections	No. of Rats
	Control Day	Injection Day	Control Day	Injection Day			
Normal Controls	28.7	12.6 (−56%) $p<0.001$*	21.1	23.7	271.2	49	17
Lesioned Nonregulating (Stage 2)	18.5	15.0 (−19%) $p>0.1$*	0	0	275.8	37	9
Lesioned Regulating (Stages 3 and 4)	22.7	11.7 (−48%) $p<0.01$*	26.2†	25.0	299.0	12	12‡

*t-test Control day/injection day

t-test Normal/Stage 2 $p<0.01$

†The water was drunk by those rats in Stage 4 only

‡Four from preceeding group plus 8 new ones

Note: For each injection in a lesioned rat there was always an injection in a paired control.

normal controls, produced only a small, statistically non-significant (t-test) decrease in the intake of the recovering laterals in Stage 2 (Table 1). These rats ate substantial amount of wet cookies (about 2/3 of the amount normally eaten by the controls), in spite of being under tube feeding. As their weights were kept close to preoperative levels, no fasted controls were thought necessary.

Four of these rats were again injected after they had recovered regulation, and they showed the same response as another 8 that recovered before they were injected for the first time. That is, all these rats in Stages 3 and 4 showed an anorexic response to adrenaline that did not differ from that observed in normal rats. (Tables 1 and 2).

An interesting observation was that the water intake of the normal rats and those in Stage 4 was not affected by adrenaline. (Those in Stages 2 and 3 did not drink any water).

TABLE 2

THE EFFECT OF I.P. ADRENALINE (0.6–1.0 MG/KG) ON THE 24 HR FOOD INTAKE OF THE FOUR RATS THAT WERE TESTED IN BOTH STAGE 2 AND STAGE 3

	Food intake (g/24 hr) Control day	Injection day	No of Injections
Stage 2	16.7	17.1	17
Stage 3	20.5	7.8 (− 62%) p=0.02	8

Effects of Anorexigenic Substances on the Food Intake of Recovered Laterals (Stages 3 and 4) Under a 24 Hr Feeding–24 Hr Fasting Schedule

The effect of much smaller doses of adrenaline than the doses used in the previous experiment (0.05 and 0.1 mg/kg), was an anorexia lasting for 1–2 hr, similar in normal controls and recovered laterals. If anything, the percent decrease seemed to be larger in the recovered laterals (Fig. 1). Similar results were obtained with DCI which is known to produce substantial hepatic glycogenolysis and hyperglycemia (2).

On the other hand, amphetamine seemed to produce less anorexia in the recovered laterals than in the normal rats (Fig. 1). At the low dose (1 mg/kg), it reduced the amount ingested in 1 hr by the paired controls 32%, but did not affect the feeding of recovered laterals. At the high dose (2 mg/kg) it reduced the intake of both groups, but the effect in the laterals was somewhat less than in the normals.

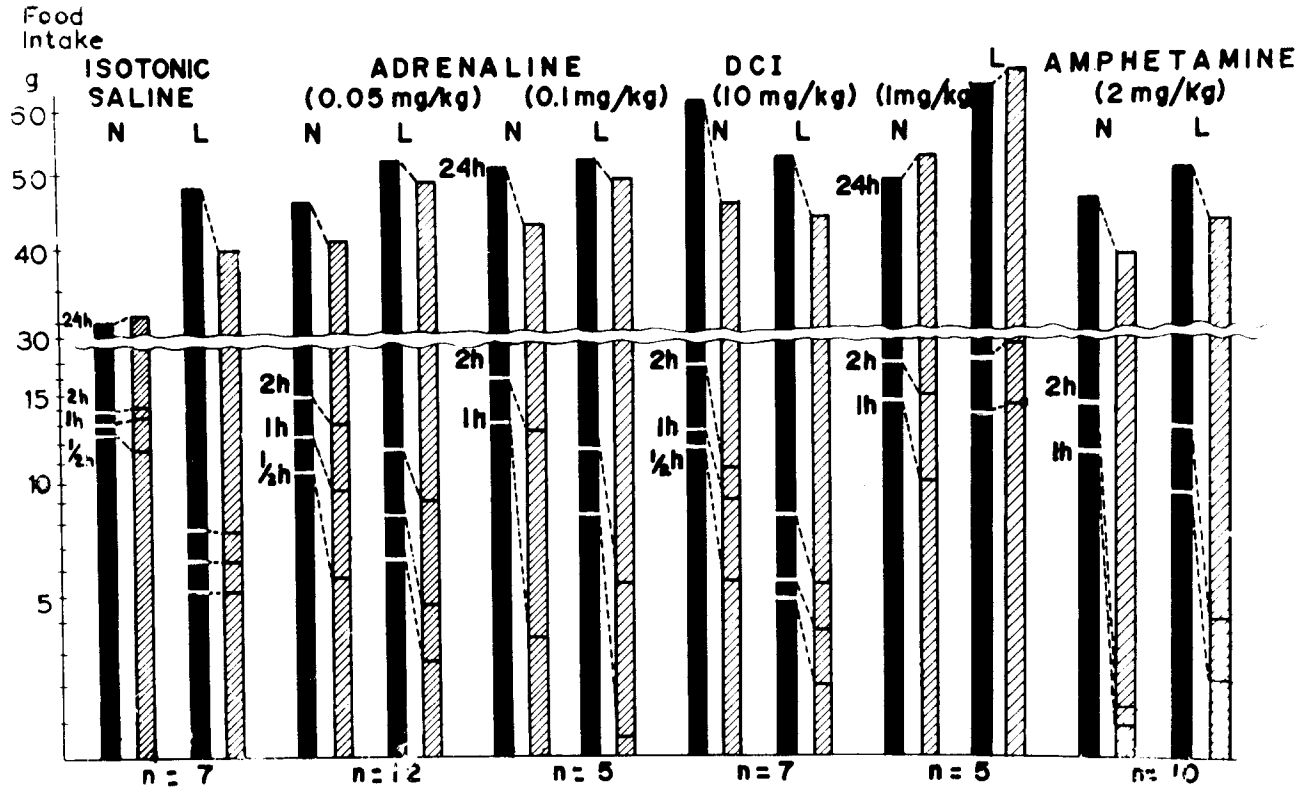

FIG. 1. Effect of several adrenergic substances on the food intake of recovered laterals (L) and their paired normal controls (N). The substances injected were adrenaline 0.05 and 0.1 mg/kg, DCI 10 mg/kg and amphetamine 1 and 2 mg/kg. Ordinate: Food intake in g, logaritmic scale, interrupted between 17 and 30 (undulated line). The rats ate on a 24 hr feeding 24 hr fasting schedule, and the amount ingested in the first ½ hr, 1 hr and 2 hr of the feeding days was measured. Black columns: control day. Hatched columns: injection day. Injections were administered intraperitoneally 5 min before the beginning of a feeding day. n= number of recovered laterals and of paired controls.

The Effect of Insulin on the Food Intake of Recovered Laterals

The injections were performed after the rats had food ad lib for only a week, so they were still accustomed to ingest a substantial amount of food during the morning hours (9–11 a.m.), a remnant of the 24 hr feeding–24 hr fasting schedule. Nevertheless, insulin increased the food intake of normal rats in the 2 hr following the injection while it produced a decrease or a much smaller increase in the ingestion of recovered laterals (Fig. 2).

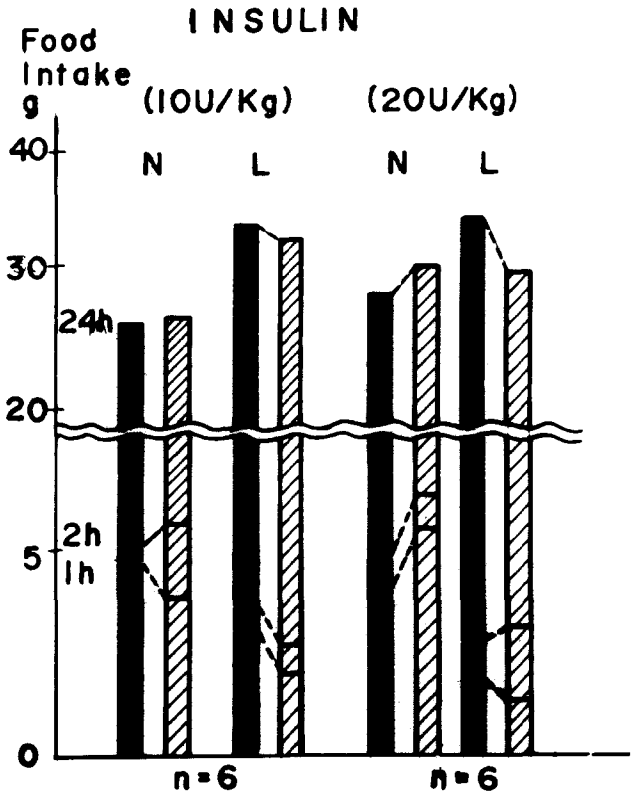

FIG. 2. Effect of insulin on the food intake of recovered laterals (L), and their paired normal controls (N). All symbols as in Fig. 1.

DISCUSSION

The anorexigenic effect of intraperitoneal adrenaline is lost after bilateral damage to the lateral hypothalamus, and reappears only after the rats have recovered the capacity to regulate their food intake. This lack of effect cannot be measured in Stage 1 (complete aphagia), but is clearly observed in Stage 2, when the rats are nibbling substantial amounts of wet cookies, but are still unable to regulate. In Stages 3 and 4, when the animals are already regulating and are not being tube fed any more, the anorexigenic effect of adrenaline is not only recovered but seems to be somewhat stronger than in the normal rats.

There are a number of facts strongly indicating that the anorexia produced by i.p. adrenaline is not due to its direct action on the central nervous system. (a) I.p. adrenaline elicits only negligible cardiovascular, respiratory and EEG changes, while the same dose injected i.v. produces strong tachycardia, strong hypertension, increased blood flow in head, liver and limbs, apnea, and EEG desynchronization and spiking [11]. This suggests that most of the adrenaline injected i.p. does not reach the general circulation but probably is absorbed and catabolized by the liver. (b) The amount of adrenaline in the blood of rats at the peak of the anorexia elicited by an i.p. injection of 0.1 mg/kg (one of the doses used in this study), is only 0.001 mg/100 ml higher than in the controls (unpublished results). This means that the total volume of blood contains about 0.2 μg (approximately 0.001 μM) more of adrenaline than normally. (c) It has been shown by many authors [4, 5, 6, 7, 8] that adrenaline injected directly into different areas of the brain, in doses up to 0.04 μM, produces an increase in food intake, and only doses of 0.1 μM produce a slight anorexia, much less than that observed with 0.1 mg/kg of i.p. adrenaline. (d) Adrenaline does not traverse the blood brain barrier [18] which makes it even more unlikely that the small amounts that appear in the blood after i.p. adrenaline would have any effect on the brain.

Of course the anorexia could still be caused by the action upon the central nervous system of the hyperglycemia resulting from the glycogenolytic effect of adrenaline on the liver, but this was shown not to be the case [11, 13].

Therefore, the most acceptable hypothesis is that adrenaline produces anorexia by the increase in intracellular glucose in the liver cells (as a consequence of glycogenolysis) which would abolish the hunger discharges from these intracellular receptors [12]. Electrophysiological evidence of these glucoreceptors [9] and electronmicrographic demonstration of intracellular nerve fibers in the hepatic cells [16] has been published recently. Moreover, a direct demonstration of the control of feeding by the liver has been obtained [14], and a high correlation between the changes in hepatic reducing sugars and in food intake, elicited by adrenaline and other substances, has been observed [15].

Therefore, the aphagic syndrome, as well as the lack of effect of adrenaline in the Stage 2 laterals might be due, at least in part, to the lesion of central pathways corresponding to the hepatic glucoreceptors, so the hunger signals that stimulate the lateral hypothalamus and initiate feeding responses are temporally ineffective. The recovery of the effect, once the rats are again regulating (and perhaps the recovery of regulation itself), might be the consequence of the appearance of compensatory pathways conveying information from the liver glucoreceptors. On the other hand, the weaker anorexigenic effect of amphetamine in these animals, already reported before [1], would agree with the generalized idea that this substance acts directly on the central nervous system, perhaps on the structures partially destroyed by the lesion [6]. The same could be said for the lack of effect of insulin on recovered laterals [3]. That is, the increase in feeding elicited by insulin could be a direct effect of the hypoglycemia upon the hypothalamic systems. The central action of hypoglycemia could be an emergency mechanism, not operating in the normal control of food intake, because no comparable hypoglycemia is ever developed under normal circumstances. Therefore, the normal control of feeding could be based mainly on the information arriving from the hepatic glucoreceptors. This glucostatic control would be present in the recovered laterals. The effect of insulin upon the hepatic glucoreceptors would produce anorexia, because it increases the glucose inflow into the hepatic cells. The early

anorexia produced by insulin in normal animals [2] would be due to its hepatic effect preceding the development of hypoglycemia. Therefore in the absence of the central hypoglycemic hunger effect of insulin, it should produce only anorexia, which was actually observed in the recovered laterals.

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