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CHRONIC EFFECTS OF GLUCAGON ON FOOD INTAKE AND BODY WEIGHT OF NORMAL, DIABETIC AND VENTROMEDIAL HYPOTHALAMUS LESIONED RATS*

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ABSTRACT

It has been reported that large doses of glucagon produce reductions in caloric intake and body weight. In the present paper, the effects of small doses of glucagon on daily food intake and body weight of normal, diabetic, or VMHx obese rats were studied. Glucagon (15 μg/kg) was subcutaneously injected twice daily nine days in 8 normal, 13 alloxandiabetic and 5 VMHx rats. As compared with the values obtained during a previous and a posterior period of nine days each, glucagon produced in normal rats mild but significant increases in food intake, body weight and energetic balance (change in body weight/total ingested food). The same values significantly decreased in diabetic rats. In VMHx rats only food intake decreased significantly but in a gradual way, the slope being negative during glucagon administration and positive during the two control periods. The results confirm that glucagon produces a negative energetic balance and reduction in food intake; at low doses these effects could be masked by its insulinogenic action. Besides, it is suggested that VMH receptors monitor pancreatic hormone concentration which could thus centrally influence feeding behavior.

RESUMEN

Ha sido descrito que la inyección de dosis grandes de glucagon produce disminución de la ingestión calórica y del peso corporal. En el presente trabajo, se estudiaron los efectos de pequeñas dosis de glucagon sobre la ingestión diaria de alimento y el peso corporal de ratas normales, diabéticas u obesas por VMHx. Se inyectó glucagon en dosis de 15 μg/kg, por vía subcutánea, dos veces al día durante nueve días, en 8 ratas normales, 13 diabéticas y 5 VMHx. Al comparar el periodo de inyección con valores obtenidos durante un periodo anterior y uno posterior, ambos de nueve días, se observa que el glucagon produjo, en ratas normales, aumentos ligeros pero significativos en la ingestión de alimento, el peso y el balance energético (cambio en el peso/cantidad total de alimento ingerido). Las mismas variables disminuyeron significativamente en

Abbreviations: VMH, ventromedial hypothalamus
VMHx, with bilateral lesions of ventromedial hypothalamus

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las ratas diabéticas tratadas con glucagon. En las ratas VMHx, sólo disminuyó la ingestión de alimento pero paulatinamente, presentándose una pendientes negativa durante la administración de glucagon, y positiva durante los dos periodos testigo. Los resultados confirman los efectos negativos del glucagon sobre el balance energético y la ingestión de alimentos; al emplear dosis pequeñas, esos efectos pueden quedar enmascarados por la acción insulinogénica de la hormona. Se sugiere, además, que receptores del VMH miden las concentraciones sanguíneas de las hormonas pancreáticas que, de esta manera, podrían tener una influencia central sobre el comportamiento alimenticio.

INTRODUCTION

Acute injections of insulin increase food intake (Booth and Brookover, 1968), and chronic ones increase also body weight (Hoebel and Teitelbaum, 1966, MacKay *et al.*, 1940). The effects of glucagon are less known. It was reported (Schulman *et al.*, 1957) that 1 mg of glucagon injected daily in man (approximately 15 μg/kg) produces significant reductions in caloric intake and body weight. Reduction in body weight was also obtained in rats (Salter, 1960) when glucagon was administered in a daily dose of 120 μg per rat (i.e. approximately 800 μg/kg). Recently, Martin *et al.* (1978) reported that the infusion of glucagon (55 μg/kg) into the portal vein of rats reduces short-term food intake.

It is generally considered that the effect of insulin on food intake and body weight is due to the hypoglycemia it produces (Bray, 1974). Insulin-induced hypoglycemia seems to act through the central nervous system since rats with lesions in the lateral hypothalamus do not increase their food intake after insulin injection (Epstein and Teitelbaum, 1967).

On the other hand, it was suggested that VMH contains glucose and insulin receptors (Debons *et al.*, 1970). According to Oomura (1976), insulin enhances the effect of glucose on the discharge of some VMH receptor. In terms of the glucostatic theory of the control of food intake (Mayer, 1955), the increment of Δ-glucose produced by insulin in the VMH would signal satiety. In other words, the direct VMH action of insulin would counteract somewhat its indirect effect on the lateral hypothalamus (through the hypoglycemia).

A hypothesis concerning a central action of glucagon was proposed in our laboratory (Racotta and Russek, 1974): glucagon would oppose insulin action upon the VMH glucoreceptors and in doing so it could produce hunger. Such an effect could somewhat counteract a peripherical action of glucagon: the hormone produces glycogenolysis and hyperpolarizes the hepatocyte membrane (Friedmann *et al.*, 1971) which, in accordance with Russek's model of food intake control (Russek, 1976) would determine satiety.

Glucagon is insulinogenic *in vivo* (Campbell and Rastogi, 1966) as *in vitro* (Devrim and Recant, 1966). For this reason, many glucagon actions will be masked *in vivo* by the peripheral effects of insulin (Cherrignton and Vranic, 1974). In the present work, the chronic effects of small doses of glucagon on food intake and body weight of normal and diabetic rats were studied, trying to separate the direct effects of glucagon from the indirect ones produced through insulin secretion.

A third group, of VMHx rats, was treated in the same way, in order to check up the possible participation of the hypothalamus in the energetic effects of glucagon.

METHODS

White adult male rats (250 to 350 g body weight) were housed in individual cages at constant temperature (21 ± 1°C) and 12 hr light — 12 hr dark. The light was shut on at 8.30 a.m. Food (ground Purina Lab Chow) and water were always available.

Normal rats. Eight rats were accustomed to the environmental conditions; the experiment started when they had reached a steady daily food intake. Further on, during a nine day control period (period C_1), food intake and body weight were measured daily. During a second period of nine days (period G) glucagon (Eli Lilly) was administered in two doses of 15 μg/kg each by subcutaneous injections of 1.0 to 1.5 ml under the skin of the back: food intake and body weight were also measured daily. A second control period of nine days (period C_2) followed period G.

Diabetic rats. Twenty rats were injected subcutaneously with 100 mg alloxan/kg (Sigma Chemical) in the morning, after 18 hr of fast. The diabetic condition was confirmed by glycemic tests (Glucose-Oxidase Method, Worthington), glycosuria tests (Clinistix), and by the elevation of the values of daily food and water intakes. Rats with a glycemia inferior to 300 mg/100 ml or which had reverted to previous food and water intakes were withdrawn.

The 13 diabetic rats obtained were submitted to an experimental schedule identical to the one used with normal rats, i.e. three periods of nine days each, injecting glucagon in the same conditions during the middle period.

VMHx rats. Hyperphagia and obesity were induced in rats by bilateral lesions of the VMH nuclei. The operation was performed under pentobarbital anesthesia in a stereotaxic device, by means of steel-wire electrodes insulated except for 0.5 mm at the tip and passing a current of 2.5 mA during 20 sec. Stereotaxic coordinates were AP 1.5, L 0.5, H 9.5 (Fifková and Maršala, 1967). Out of 12 lesioned rats 5 were obviously hyperphagic and eventually became obese. The rats gained between 1.5 and 4.8 g of body weight per day after the lesion (twice to sevenfold the daily weight gain of normal rats). The first control period (C_1) began 18 days after lesioning in one rat, and 45 to 55 days in the other four. Food intake and body weight were measured in the same manner as in the other groups, i.e. daily during three periods of nine days each, administering glucagon in the same doses during the middle period (period G).

RESULTS

Food intake. The mean food intake of each rat was calculated during each one of the three periods of nine days (Table I).

TABLE I. DAILY FOOD INTAKE

	Period C_1	*Period G*	*Period* C_2
Normal rats $n = 8$	21.59*	22.37	21.12*
Diabetic rats $n = 13$	31.27*	29.09	30.09*
VMHx rats $n = 5$	23.09*	21.44	19.51*

Values are mean intakes in grams. n = number of animals studied. *Period* C_1 and C_2 are the previous and posterior nine day periods, respectively, to *Period G* when two doses of 15 μg glucagon per kg were subcutaneously administered daily during nine days. Differences from mean food intake during *Period G* by two-tail paired *t*-test signicant at *$p<0.05$.

Normal rats ate on the average slightly more food during glucagon administration. Diabetic rats, on the contrary, ate less food during the same period.

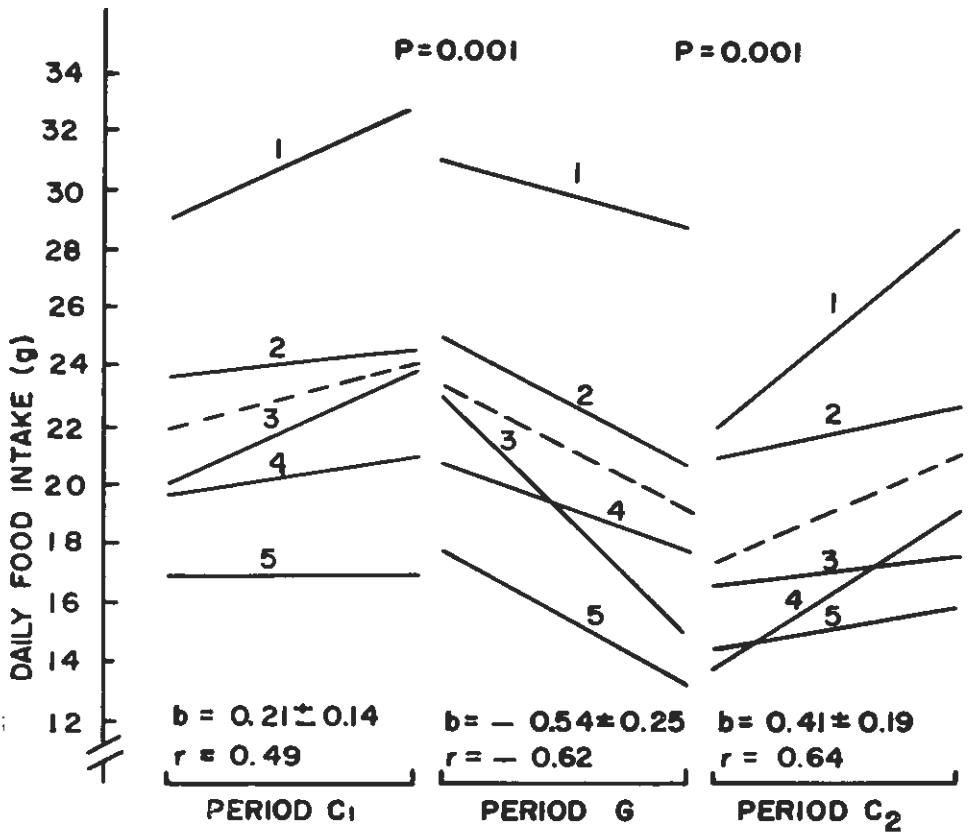

Fig. 1. Linear regressions of individual food intakes of VMHx rats during the periods of nine days (see text): the same numeral marks the slopes of the same rat. Dotted lines = mean linear regressions for each period; *b*'s = their slopes ± SE; r = their correlation coefficient. *P* values are related to the significance of statistical differences between *b*'s by Student non paired *t*-test.

VMHx rats ate less during period G but even less yet during the subsequent C_2 period. However, a more careful anlysis of the daily food intake of VMHx rats showed that food intake gradually lowered during period G and also gradually augmented during period C_2 (Fig. 1). Glucagon administration produced, therefore, a gradual reduction in daily food intake of VMHx rats.

Body weight. The changes in body weight were in the same sense as those of food intake (Table II). Normal animals gained significantly more weight during glucagon administration while diabetic rats lost weight in the same conditions. VMHx rats gained less weight on the average during administration of the hormone, but the difference was not statistically significant.

TABLE II. CHANGES IN BODY WEIGHT

	Period C_1	*Period G*	*Period* C_2
Normal rats $n = 8$	5.37**	15.48	12.05*
Diabetic rats $n = 13$	0.12**	—11.69	— 1.31**
VMHx rats $n = 5$	10.56	. 8.64	12.32

Values are means in grams. n = number of animals studied. Periods as in Table I. Differences from changes in body weight during *Period G* by two-tail paired t-test significant at *$p<0.05$; **$p<0.01$.

Linear regressions of daily changes in body weight were calculated (Table III). The slope of the change in body weight during period G was significantly different to the slope of period C_1 in each group of rats.

TABLE III. LINEAR REGRESSION OF BODY WEIGHT (b)

	Period C_1	*Period G*	*Period* C_2
Normal rats $n = 8$	0.69 ± 0.22**	1.66 ± 0.19	1.23 ± 0.13
Diabetic rats $n = 13$	-0.09 ± 0.23*	-1.35 ± 0.31	0.06 ± 0.19**
VMHx rats $n = 5$	1.82 ± 0.28*	0.87 ± 0.22	1.12 ± 0.38

Values of the slopes (b) are means $\pm$ SE n = number of animals studied. Periods as in Table I. Differences from b's of *Period G* by two-tail non paired t-test significant at *$p<0.01$; **$p<0.001$.

Energetic balance. By using the data of food intake and the changes in body weight, we calculated an index of the amount of ingested food that was utilized in augmenting weight (normal and VMHx rats), or the amount of body weight "consumed" in order to compensate for an insufficient intake (diabetic rats). The formula used was:

100 x Δ-Body weight (grams) / total food (grams)

During glucagon administration, normal rats stored in the form of body weight a significantly larger proportion of ingested food (table IV), presenting therefore a more positive energetic balance than during the control periods. Diabetic rats, on the contrary, had a negative energetic balance during period G. As for the VMHx rats, this index was smaller during glucagon administration but the differences were not statistically significant.

TABLE IV. RATIO BETWEEN THE CHANGES IN BODY WEIGHT AND THE AMOUNT OF INGESTED FOOD

	Period C_1	*Period G*	*Period* C_2
Normal rats			
$n = 8$	2.76**	7.69	6.34*
Diabetic rats			
$n = 13$ rats	0.04**	—4.46	-0.48**
VMHx rats			
$n = 5$	5.08	4.48	7.02

Values are means of the relation: 100 x body weight change/total ingested food. Periods as in Table I. Differences from the means of *Period G* by the Wilcoxon signed rank-test significant at $*p<0.05$; $**p<0.01$.

DISCUSSION

Rat pancreas secretes glucagon *in vitro* at a rate of 450 pg/min (Leclercq-Mayer *et al.*, 1975), i.e. 650 ng daily. We injected daily, two doses of 15 μg/kg each; considering a half-life of approximately 20 min (Federspil *et al.*, 1968) such a dose (4-5 μg per rat) would fall to the level of physiological secretion in about one hour.

In these conditions, glucagon administration to normal rats produced a mild but significant increment in daily food intake, in body weight and in the energetic balance. Such effects could be explained by the insulinogenic action of glucagon (Campbell and Rastogi, 1966; Devrim and Recant, 1966) and/or by a heretofore hypothetical action of glucagon at the hypothalamic (VMH?) level.

Experimentally insulin-induced hyperphagia is commonly related to the magnitude and duration of the hypoglycemia produced by this hormone (Lotter and Woods, 1977). Glucagon infusion in man, after raising the glycemia during the infusion, determines a mild hypoglycemia when the infusion is stopped (Unger *et al.*, 1976). Thus, the mild increase in food intake produced by glucagon could be related to a rebound of hypoglycemia due to insulin secretion, but this will have to be proven experimentally.

The increment in body weight and in the energetic balance produced by glucagon in normal rats could more easily be attributed to the insulinogenic action of the hormone. From the work of Hoebel and Teitelbaum (1966) it could be infered that the effects of insulin on food intake and body weight can be dissociated: insulin may produce increments in body weight without a steady concomitant hyperphagia.

The results obtained with glucagon-treated diabetic rats, i.e. significant hypophagia, reduction in body weight, and a negative energetic balance, suggest indeed that the contrary responses of normal rats to glucagon are related in some manner to the presence of insulin. Lack of insulin in alloxan diabetic animals seems to be the factor that shifts glucagon effects toward catabolism. This could explain the results obtained in other work showing reduction in body weight (Salter, 1960) and increase in heat production (Davison *et al.*, 1960) of normal rats with high doses of glucagon (between 25 and 1000 μg per rat of 200-250 g body weight).

All this suggests that insulin/glucagon balance is not only important for the regulation of blood glucose (Cherrington and Vranic, 1974) but also of energy balance. The negative energy balance of glucagon theated diabetic rats suggests a primary calorigenic action on the output of the energy (weight) regulation system, not compensated by an adequate alimentary input. Reduction in food intake suggests furthermore the existence of an independent negative effect of glucagon on the energetic input, when insulin is not available to compensate for it. This hypophagia could be explained by the known glycogenolytic (Sokal *et al.*, 1964) and lipolytic (Lefebvre and Luickx, 1968) effects of glucagon, according to the hypotheses of food intake control by hepatic glucoreceptors (Russek, 1976) or by fuel availability (Le Magnen, 1976), respectively.

The opposite effects of glucagon in diabetic and normal rats suggest therefore that the hormone has direct catabolic and hypophagic effects which are masked in normal animals by its insulinogenic action. How do the results obtined in VMHx rats fit into this picture?

The first control period began when the VMHx rats were eating normal amounts of food but when their body weight was still increasing. During the period of glucagon administration they presented a significant slope of decrease in food intake, yet no significant reductions in body weight and energetic balance.

VMHx rats present, among other metabolic changes, high levels of insulinemia and low levels of glucagonemia (Inoue *et al.*, 1977). It is possible that the exogenous glucagon we administered in relatively low doses could not elevate more the insulinemia: the anabolic effects obtained in normal rats, supposedly through an increment in insulin secretion, would thus not appear in VMHx rats. However, the only significant effect of glucagon in VMHx rats was the cumulative reduction of food intake.

If we consider that glucagon acts on the VMH receptors in an opposite manner to insulin, i.e. inhibiting glucose entry (Racotta and Russek, 1974), glucagon would signal insufficient glucose availability that would elicit a reflex secretion of insulin. This, added to the direct insulinogenic action, would cause the anabolic effects we obtained in normal rats. Besides, the same central signal, by inhibiting the VMH, could secondarily produce an increase in food intake (Mayer, 1955). In the VMHx rats, the central effect of glucagon cannot take place, and its direct insulinogenic action would be reduced because of an already very high insulin secretion. The reduction of food intake in VMHx rats could be due, as

in diabetic rats, to the glycogenolytic and/or the lipolytic effect of glucagon, but it would be less drastic in the first case because of the high insulin concentration.

In conclusion, the data presented in this work suggest the existence of general catabolic and hypophagic effects of glucagon already signaled by other authors (Davidson *et al.*, 1960; Salter, 1960; Schulman *et al.*, 1957), which would be compensated or even inverted, when low doses are administered, by the insulinogenic action of the hormone. This insuligenic action could also have a central component through an inhibitory effect of glucagon on the VMH receptors (Racotta and Russek, 1974).

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