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Effects of a Diazoxide Inhibition of Insulin Release on Food Intake of Normal and Hyperphagic Hypothalamic Rats

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LARUE-ACHAGIOTIS, C. AND J. LE MAGNEN. *Effects of a Diazoxide inhibition of insulin release on food intake of normal and hyperphagic hypothalamic rats.* PHARMAC. BIOCHEM. BEHAV. 9(6) 717–720, 1978.—In order to clarify the role of endogenous insulin in generating normal and pathological feeding behaviors, the effect of an anti-insulin drug, Diazoxide, on the meal pattern has been investigated in both normal and VMH rats. A dose-dependent reduction of food intake under Diazoxide treatment was found in normal rats. The same percentage reduction was obtained at night with a higher dose than required in the daytime. Conversely, hyperphagic VMH rats exhibited a dose-dependent reduction which was identical in the two portions of the diurnal cycle. The dose effective in entirely suppressing eating in VMH rats was twice that required for normal rats in the daytime period and identical at night. The results are discussed in relation to earlier findings concerning the diurnal insulino-secretory pattern in normal and VMH rats.

Insulin and food intake Hyperphagia and obesity Diazoxide

THE CRITICAL role of prandial insulin release in the regulatory control of food intake has been established [21]. However the exact mechanisms involved in the effects of prandial insulin release and exogenous insulin administration on food intake are not fully understood.

The meal pattern seems to be influenced by three of the metabolic effects of insulin. By increasing glucose utilization, the post-absorptive glucose-stimulated insulin release may act in the onset of satiety and contribute to the termination and size of the meal. Insulin, by controlling the post-absorptive level of glycogen storage, is involved in the control of the meal-to-meal balance between the outflow and inflow of fuel into the blood and thus may influence the duration of post-meal satiety. Finally, a low level of insulin contributes to a glucoprivic condition which may be involved in the stimulation of eating and the onset of a meal. In addition to these effects, a neurally mediated control of the β cell release is effective in a liporegulatory mechanism. This exerts an additional long term modulation of feeding. It has been shown that this liporegulatory modulation of feeding is in phase with the dark-light cycle [16]. In rats, hyperinsulinism, at night, induces an excessive depot of fats and consequently hyperphagia. Hypoinsulinism, in the daytime, is associated with a compensatory mobilization and oxidation of fats which, in turn, inhibit food intake. The lipostatic changes exhibited within the diurnal cycle have been also demonstrated after food restriction or forced-feeding [3, 5, 14, 28]. These changes are altered by ventro-medial hypothalamic (VMH) lesions which cause hyperphagia and obesity. VMH lesioned rats exhibit at night an exaggerated hyperinsulinism [7, 12, 13, 15] associated with the increased lipogenesis and hyperphagia [16]. Hyperinsulino-secretion persists in the daytime and results in fat storage and hyperphagia then replacing the regulatory lipolysis and hypophagia of normal rats. However, the exact role of a disfunction of the neural control of insulino-secretion in this failure of lipostatic mechanisms in VMH rats is obscure. In order to clarify this point, the present experiment was conducted to evaluate the dose dependent effects of an anti-insulin drug, Diazoxide, on meal patterns in normal compared to hyperphagic hypothalamic rats. Diazoxide is known to exert an inhibition on glucose-stimulated insulin release by pancreatic β cells [4,6]. This inhibitory effect has been suggested to result from the α-adrenergic stimulant property of the drug.

METHOD

Animals

Female Wistar rats, weighing approximately 260 g at the beginning of the experiment, were used. They had free access to water and food (Stock powdered diet—Sorolabo, 3.2 kcal/g). Their feeding patterns were continuously and graphically recorded using a food cup weighing device. They were individually housed in Plexiglas cages and placed in a quiet room in which a 12/12 dark-light cycle was maintained.

Procedure

Bilateral electrolytic lesions of the VMH nuclei were made under ether anesthesia by passing a 1 mA current for 30 sec through a stainless steel electrode which was insulated except for the tip. The stereotaxic coordinates were: 6 mm

anterior to the ear bar, 0.7 mm lateral to the sagittal sinus and 0.6 mm up from the base of the brain.

Initially, an attempt was made to infuse Diazoxide (Hyperstat-Cetrane) continuously in a solution of 15 mg/ml via a chronically implanted cardiac catheter. Such a continuous infusion was found to be technically impossible due to the formation of a clot in the catheter associated with the precipitate of the solution. A second preliminary experiment made on 7 rats showed that the effects on food intake obtained by Diazoxide injected in a single dose by the IV route (the catheter being rinsed afterwards by a saline solution) were identical to those obtained by using intraperitoneal administration. Thus, the latter route of Diazoxide injection was adopted.

Normal feeding patterns were at first tested in all rats during 3 days. Rats were then divided into two groups; a night group (10 rats) received the injections at the beginning of the dark period (6 p.m.); a day group (10 rats) received the injections at the beginning of the light period (10 a.m.). In the two groups, various doses of Diazoxide were used at 3 or 4 day intervals and given in random order. Rats of the night group were injected with 25 mg/kg, 50 mg/kg, 100 mg/kg of Diazoxide and the vehicle. The vehicle consisted of 10 ml of 1 N sodium hydroxide retitrated with 1 N hydrochloric acid to a pH of 10.5 and made up to 100 ml with distilled water. Rats of the day group were injected with 25 mg/kg, 35 mg/kg, 50 mg/kg of Diazoxide and the vehicle. Then all rats received VMH lesions. Eight rats in the night group and 9 rats in the day group were made successfully hyperphagic according to the criterion of at least a 50% increase in the 24 hr food intake. Following 5 days of recording of the hyperphagic patterns, the rats were again injected at the beginning of either the night or daytime periods depending on the group. In the two groups, Diazoxide doses were 25, 50, 75, 100 mg/kg and the vehicle. Thus, dose-dependent changes of various parameters of the meal pattern under the effect of Diazoxide injected either at night or in daytime were examined in the same rats both prior to and following VMH lesions.

RESULTS

Results on food consumption were analyzed in terms of the percentage reduction of the 12 hr cumulative intake. In normal rats, Diazoxide injected at the beginning of daytime produced a marked reduction in food intake (Fig. 1). The smallest dose (25 mg/kg) reduced the 12 hr intake by 58%. An almost total suppression was obtained with the dose of 50 mg/kg. At night, the same percentage reduction of control food intake required about two times the dose needed in the daytime. In contrast with the different responses observed in day and night before surgery, the dose-dependent reduction of food intake was identical in hyperphagic rats whether injected at the beginning of day or night. The smallest dose injected at night or in daytime was without significant effect and an almost total suppression was obtained only at a dose of 100 mg/kg. Thus, in the daytime, Diazoxide was 3 to 4 times less efficient in reducing the 12 hr intake than in intact rats during the same period. At night with the intermediary dose (50 mg/kg) Diazoxide was only 2 times less effective in reducing food intake of hyperphagic compared to intact rats. The response to the high dose (100 mg/kg) in hyperphagic hypothalamic rats was apparently biased by a stressing effect, manifested by drowsiness and stupor. The same effect was not observed in the intact or in the hyperphagic rats administered the same volume of vehicle.

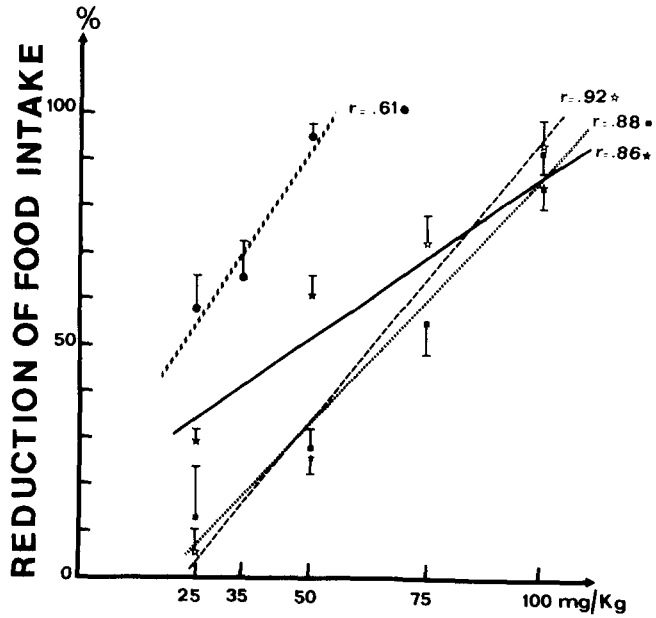

FIG. 1. Dose-dependent reduction of food intake (percentage of control food intake) following injection of Diazoxide at the beginning of either the night or daytime. ● ☆ ■ ★ mean ± S.E. ●●●●●● normal rats—day; ★———normal rats—night; ■-------hyperphagic rats—day; ☆ — — - hyperphagic rats—night.

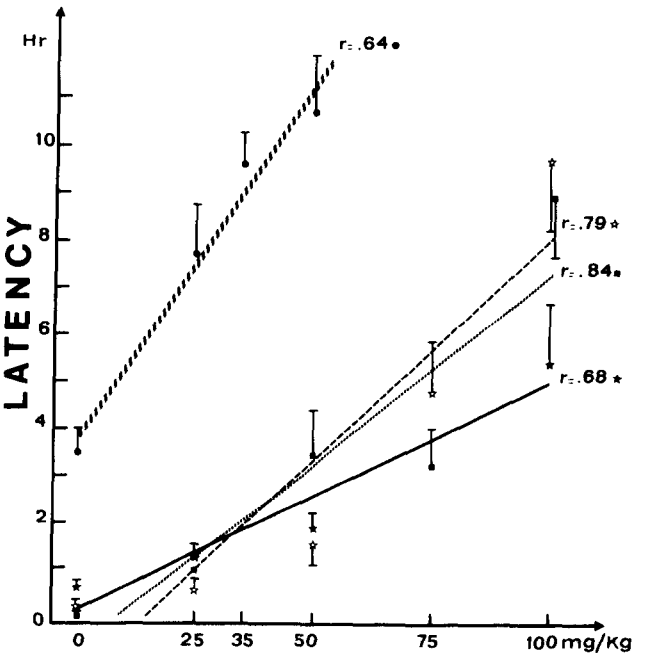

FIG. 2. Latency to the first meal after the Diazoxide injection as a function of dose ● ☆ ■ ★ mean ± S.E. ●●●●●● normal rats—day; ★———normal rats—night; ■-------hyperphagic rats—day; ☆ — — - hyperphagic rats—night.

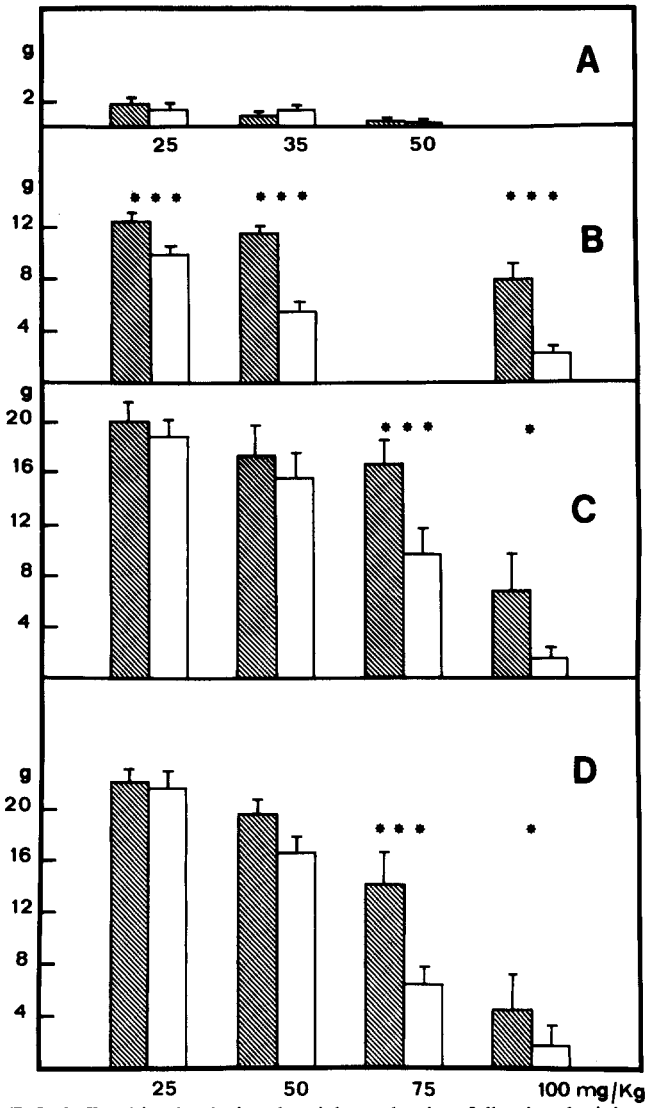

FIG. 3. Food intake during the night or daytime following the injections of different doses of Diazoxide (open bars) compared to the corresponding time period of eating in vehicle-treated rats (hatched bars). (A) Normal rats—day; (B) Normal rats—night; (C) Hyperphagic rats—day; (D) Hyperphagic rats—night.

An examination of the meal patterns within each 12 hr light or dark period showed that the decrease of the 12 hr food intake was related to 2 different and successive changes due to the initial single injection of Diazoxide:

(1) a total suppression of feeding or increased latency to eat, immediately following the injection (Fig. 2);

(2) a modified food intake when after this latency feeding was resumed. In normal rats in the daytime the latency to eat after the Diazoxide injection was increased in a dose-dependent fashion and was entirely responsible for the observed reduction of the 12 hr intake. With the 25 and 35 mg/kg doses the resumed food intake was identical to that observed in untreated rats during the corresponding time period (Fig. 3). At night, both a dose-dependent increase in latency and decrease in the later food intake were involved in the total 12 hr reduction. In hyperphagic rats at night and in the daytime a dose-dependent increase in latency was apparent. With the 50 mg/kg dose, the increased latency appeared to contribute totally to the 12 hr reduction of food intake. With higher doses both the increase of latency and decrease of the food intake explain the 12 hr reduction. During the period in which food intake reappeared after an initial suppression of feeding, the reduction of intake (when observed) was due to a significant decrease in the size and not the number of meals (Table 1).

An extension of the reduced food intake from night to the following daytime and from day to the following night-time was observed in hyperphagic rats only with the high dose. This effect was probably due to a persisting effect of the induced stress. In normal rats a 28.8% reduction at night with the small dose of Diazoxide produced a 28.6% increase in the following daytime intake. With the high dose, the effect of Diazoxide overlapped with the subsequent daytime. With the intermediary dose of 50 mg/kg the two opposite effects were apparently compensating.

DISCUSSION

Differences in energy metabolism, neuroendocrine and feeding patterns between the two parts of the diurnal cycle in rats have been demonstrated [16,17]. They have been also extensively studied and confirmed in man [1, 2, 8, 9, 10, 20,

TABLE 1

MEAL SIZE IN GRAMS

	Non-Treated	25 mg/kg	35 mg/kg	50 mg/kg	75 mg/kg	100 mg/kg
Normal Rats Day	0.95 ± 0.07	0.91 ± 0.15§	0.70 ± 0.12*	0.20 ± 0.12‡	—	—
Normal Rats Night	1.98 ± 0.12	1.41 ± 0.09‡	—	1.21 ± 0.14‡	—	0.85 ± 0.13‡
Hyperphagics Day	3.20 ± 0.16	3.22 ± 0.23§	—	2.66 ± 0.28*	2.26 ± 0.33†	0.43 ± 0.18‡
Hyperphagics Night	3.17 ± 0.18	3.17 ± 0.45§	—	2.51 ± 0.35*	1.74 ± 0.37‡	0.63 ± 0.32‡

*$0.01<p>0.05$.
†$0.001<p>0.01$.
‡$p<0.001$ (Wilcoxon t-test).
§Not significant.

22, 23, 24]. In rats, a rapid glucose uptake, a relative hypoglycemia and hyperinsulinism associated with lipogenesis are present at night. This metabolic pattern is correlated to the hyperphagia and hypophagia observed respectively at night and in the daytime.

The different dose-dependent effect of Diazoxide on food intake in the two portions of the diurnal cycle in normal rats may be explained by these opposite underlying metabolic conditions. In the daytime, a minimal dose of Diazoxide appears to be sufficient to exaggerate both the hypoinsulinism and the resulting hypophagia. At night a doubling of the dose required to identically reduce food intake is probably due to the hyperinsulinism related to the concomitant hyperphagia. This effect provides new evidence for the causal role of the diurnal metabolic pattern and particularly of the insulinosecretory responsiveness in the nocturnal hyperphagia and daytime hypophagia.

In hypothalamic hyperphagic rats the dose-dependent effects of Diazoxide on food intake in the dark and light periods may be explained by the neuroendocrine disruption produced by the VMH lesion. It has been shown that one of the effects of the lesion is to suppress the differences in the prevailing metabolic conditions of the two parts of the diurnal cycle [11, 16, 19]. Daytime glucose intolerance, hypoinsulinism and lipolysis are abolished in VMH rats. Thus, the daytime metabolic pattern becomes identical to that observed at night in the same rats. In relation to this constant metabolic condition during the 24 hrs, the exaggerated hyperphagia observed at night is extended to the daytime. A similar effect of Diazoxide on food intake at night and in the daytime, the minimal difference at night and the maximal difference in the daytime when compared to normal rats are found in the present study. This result is consistant with the assumption that the change of the diurnal insulinosecretory pattern induced by the lesion is mainly responsible for hyperphagia in VMH rats. The fact is striking that a particular dose of Diazoxide (50 mg/kg), given at the beginning of the night, and a higher dose (75 mg/kg) given at the beginning of the day, could suppress the 24 hr hyperphagia, i.e. could abolish the effect of the lesion.

However the meal pattern of normal rats was far from being reproduced under Diazoxide treatment in VMH rats. In these lesioned rats as well as in normal animals, Diazoxide injections caused an initial suppression of feeding. This early suppression effect following a single Diazoxide injection prevents the interpretation of the results in terms of the determined action of the prandial insulinosecretion on the meal size and meal frequency.

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