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Simulation of the apparent effects of mebanazine on growth hormone by pair-feeding of control animals

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The degree and duration of insulin hypoglycaemia was potentiated by chronic oral medication with mebanazine in rats. Hypophysectomy alone increased sensitivity to insulin but did not abolish the potentiating effect of mebanazine. Chronic mebanazine treatment (15 mg/kg/day) for 6 weeks markedly reduced weight gain, food and water consumption and pituitary growth hormone content, but the results were not significantly different from those in unmedicated pair-fed controls. Similarly, immature rats treated with mebanazine had a significant reduction in the width of the tibial epiphysial cartilage but this was not different from that in pair-fed animals. After 18 h of fasting, acute administration of mebanazine had little effect on food consumption in the 2 h period following dosing but a significant effect over 24 h. In fed rats mebanazine in a single oral dose significantly reduced eating in the following 6 h. Treatment with mebanazine at 2·5 mg/kg for 15 days significantly reduced food intake but did not potentiate insulin hypoglycaemia. From the results it would appear that previous suggestions that mebanazine specifically interferes with growth hormone release are incorrect and the findings emphasize the importance of measuring food intake in experiments of long duration.

Long-term administration of the monoamine oxidase inhibitor mebanazine to rats has been shown to potentiate the hypoglycaemic effects of both insulin and tolbutamide (Barrett, 1965). The results were compatible with the hypothesis that mebanazine treatment interfered with the adrenergically mediated mechanisms for combating low circulating glucose levels. It was proposed that the pattern of insulin potentiation and hypotensive episodes during the clinical use of mebanazine (Wickström & Pettersson, 1964; Cooper & Keddie, 1964) might have a common origin. However, it was recognized that an alternative explanation of the increased sensitivity to insulin could derive from an alteration in the balance between insulin and pituitary growth hormone after the production of hypoglycaemia. Experimental evidence purporting to demonstrate that mebanazine does inhibit the secretion of growth hormone has been reported (Zor, Dikstein & Sulman, 1965a,b). The work now reported presents the results of experiments designed to discover the relation between the effects of mebanazine on growth hormone and insulin potentiation. A preliminary account of this study has been presented to the British Pharmacological Society (Barrett, 1966).

EXPERIMENTAL

The animals were male albino rats from the specific pathogen-free strain bred at Alderley Park. In most experiments they weighed between 190–230 g except where

immature rats were used for tibial tests (35–45 g), and were maintained on a cubed diet and water *ad libitum*. In some experiments food intake was restricted for purposes of pair-feeding controls. Hypophysectomy was performed by the parapharyngeal approach with subsequent maintenance on 5% glucose solution in place of water. The animals were used 2 weeks after surgery and completeness of hypophysectomy was checked visually after death. Blood samples were obtained from the abdominal aorta after intraperitoneal pentobarbitone sodium anaesthesia. Each experiment involved groups of 5 animals except for the initial experiment where there were only 4. Blood glucose was estimated by a kit glucose-oxidase method, and growth hormone by the tibial test (Papkoff & Li, 1962).

Mebanazine oxalate was administered orally or intraperitoneally in aqueous solution and soluble insulin subcutaneously. The rats were weighed daily and food and water consumption recorded for groups of 5 rats.

RESULTS

The hypoglycaemic response to insulin was significantly potentiated and prolonged by oral pretreatment with mebanazine (15 mg/kg daily) for a period of 3 weeks. The results are summarized in Table 1. In the control animals, blood sugar levels had returned to pre-insulin levels within 3 h whereas those of the mebanazine-treated rats had only recovered to 50% of the initial values in the same time interval. Chronic administration of mebanazine did not significantly alter the resting blood sugar concentration.

Rats which have been hypophysectomized are more sensitive to the hypoglycaemic actions of insulin and it was found that they were unable to tolerate the same doses of mebanazine as intact rats. It was possible however, to administer mebanazine

Table 1. *Effects of insulin* (1 *unit/kg s.c.*) *on the blood sugar level over* 3 *h of control rats and rats pretreated with mebanazine* (15 *mg/kg daily for* 3 *weeks*) (*means* ±*s.e.*). Four animals per group. Blood glucose values are expressed as mg/100 ml of blood

Time after insulin (min)	Control rats	Mebanazine treated rats	*P* value
0	106 ± 6	92 ± 7	N.S.
60	42 ± 4	30 ± 4	N.S.
90	48 ± 4	26 ± 4	<0·01
120	51 ± 3	36 ± 6	<0·02
150	61 ± 7	42 ± 3	<0·02
180	110 ± 6	48 ± 4	<0·001

Table 2. *Effect of insulin on blood glucose levels in hypophysectomized rats with and without pretreatment with mebanazine daily* (10 *mg/kg*). Four rats in each group. Means ±s.e. expressed as mg/100 ml blood

Dose of insulin (units/kg)	Control rats	Mebanazine treated rats	*P* value
0	128 ± 2	115 ± 8	N.S.
0·125	106 ± 3	57 ± 11	<0·01
0·250	77 ± 7	51 ± 2	<0·02
0·50	61 ± 4	47 ± 6	<0·05

daily at 10 mg/kg for 15 days without any overt signs of toxicity. An insulin tolerance test was made in hypophysectomized animals and the results are summarized in Table 2. Blood sugar levels are given before and 90 min after insulin, corresponding to the time at which a maximal response was observed in the first experiment. As in intact rats, mebanazine-treatment alone did not significantly affect the resting blood sugar values. The sensitivity to insulin was, however, significantly greater than in control hypophysectomized rats.

Since potentiation of insulin hypoglycaemia was observed in the absence of the pituitary gland it seemed unlikely that the phenomenon was a direct consequence of diminished reserves of growth hormone in the mebanazine-treated intact rats. In earlier experiments, it had been observed that rats receiving mebanazine daily at 15 mg/kg for a period of 6 weeks gained considerably less weight than animals receiving a daily oral administration of saline. Subsequent analysis of their pituitary glands showed that the treated group had only about 40% of the growth hormone content of the control group. It was possible that the decreased rate of growth in the mebanazine-treated rats was due to a decreased overall consumption of food.

In a new experiment, three groups of weight-matched rats (10 per group) were selected. One group received mebanazine daily at 15 mg/kg and the other groups were given saline, all by mouth. The drug treated group and one control group were allowed food *ad libitum* whilst the remaining control group acted as a pair-fed control for the mebanazine treated rats. Food and water consumption were recorded daily for 6 weeks when half the animals in each group were killed and various analyses performed. The growth curves for the animals not killed are illustrated in Fig. 1. Whereas the control rats showed a steady weight gain there was little change in the weight of animals receiving mebanazine or their pair-fed controls. When drug treatment was stopped and free feeding provided for all groups, the pair-fed animals gained weight more rapidly than the mebanazine-treated rats.

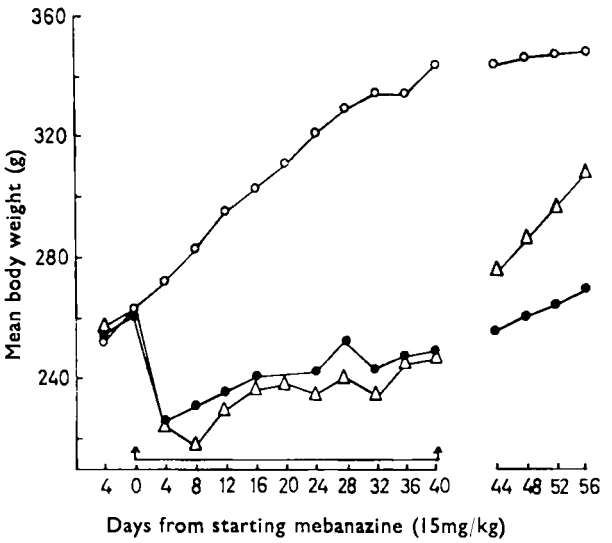

FIG. 1. Change in body weight for groups of 5 rats receiving saline (○—○), mebanazine daily 15 mg/kg (●—●) or saline with pair-feeding (△—△), during 40 days of treatment and 16 further days of free feeding.

Table 3. *Effects of mebanazine* (15 *mg/kg*) *and pair-feeding for* 6 *weeks in rats: body weight, food and water consumption, pituitary weight and growth hormone content* (*means* ±*s.e.*). Five animals in each group. An asterisk denotes a significant difference from control ($P < 0.05$)

Treatment	Change in body wt (g)	Daily consumption of food (g)	Daily consumption of water (ml)	Pituitary growth hormone Wt (mg)	Pituitary growth hormone (% control)
Control	+93 ± 6	19·9 ± 0·6	34·5 ± 1·6	8·1 ± 0·2	100
Mebanazine	−11 ± 12*	13·2 ± 0·4*	13·9 ± 1·1*	7·5 ± 0·4	60*
Pair-feeding	−10 ± 13*	13·2 ± 0·4*	24·0 ± 1·2*	8·2 ± 0·3	63*

Some of the results from the analyses of the rats killed after 6 weeks are summarized in Table 3. Control animals showed a net weight gain of 93 ± 6 g whereas both the mebanazine and pair-fed groups showed a net loss in weight. Both food and water consumption were significantly reduced by mebanazine-treatment although the pair-fed animals drank significantly greater volumes of water. Although there were no significant changes in pituitary weight, the growth hormone content was significantly reduced in both the mebanazine and pair-fed groups. Carcass analyses showed no significant differences between the mebanazine and pair-fed groups although both groups had approximately 50% less body fat than the control group. There were no significant differences between the mean weights of brain, spleen or adrenal glands for any of the groups.

Table 4. *The effects of mebanazine or amphetamine* (5 *mg/kg daily for* 5 *days*) *on body weight, food consumption and tibial epiphysial cartilage width in rats.* 5 rats per group

Treatment	Initial body wt (g)	Gain in wt (g)	Daily food intake (g)	Tibial cartilage width (μm)
Controls	40·7	13·0 ± 1·6	7·69 ± 0·75	352·8 ± 6·5
Amphetamine	41·9	13·3 ± 1·4	7·03 ± 0·75	348·3 ± 7·7
Pair-fed with amphetamine dosed	41·8	13·4 ± 0·8	7·03 ± 0·75	358·1 ± 12·8
Mebanazine	43·0	6·8 ± 0·6**	5·15 ± 0·67*	281·9 ± 6·1***
Pair-fed with mebanazine dosed	42·8	4·8 ± 1·2**	5·15 ± 0·67*	293·0 ± 8·1***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

In an earlier study (Zor & others, 1965a) it was shown that treatment of immature rats with mebanazine significantly reduced the width of the tibial epiphysial cartilage whereas amphetamine did not. This experiment has been repeated but with the inclusion of pair-fed control groups for both drug-treated groups of rats. The results are summarized in Table 4. Amphetamine did not reduce weight gain, overall food consumption or tibial epiphysial width. In contrast, mebanazine induced a highly significant reduction in epiphysial width, a lower overall food intake and a smaller weight gain. The results were very similar in the pair-fed controls which did not receive any drug-treatment. These experiments suggested that the reduction in the width of the tibial epiphysial cartilage was an indirect consequence of reduced

Table 5. *Food consumption in fasting and fed rats during various time intervals after oral dosing with amphetamine or mebanazine.* Five animals per group. An asterisk denotes significant difference ($P < 0.05$) from control group.

		Fasted 18 h				Fed overnight	
		2 h		24 h		6 h	
Treatment	Dose (mg/kg)	Consumed (g)	% Reduction	Consumed (g)	% Reduction	Consumed (g)	% Reduction
Saline ..	0·5 ml/100 g	4·9 ± 0·5	—	20·8 ± 0·2	—	4·0 ± 0·3	—
Amphetamine ..	2·5	4·0 ± 0·3	18	22·8 ± 0·2	0	4·6 ± 0·4	0
	5·0	2·0 ± 0·3	59*	20·2 ± 0·1	3	3·2 ± 0·3	20*
	10·0	0·3 ± 0·1	94*	20·8 ± 1·0	0	2·2 ± 0·2	45*
Mebanazine ..	2·0	4·6 ± 0·4	6	21·2 ± 0·6	0	2·6 ± 0·4	35*
	7·5	4·1 ± 0·1	16	17·6 ± 0·5	15*	1·4 ± 0·1	65*
	15·0	4·0 ± 0·4	18	15·2 ± 1·1	29*	0·0 ± 0·0	100*

food intake rather than to a specific reduction in growth hormone production by mebanazine.

Most tests for anorexic activity only measure acute effects in the immediate time interval after dosing. Whereas amphetamine, however, produced an acute suppression of appetite, the overall food consumption in a 24 h period following a single dose exceeded that of undosed animals (Table 5). In contrast, mebanazine had very little effect in the acute phase but significantly reduced the 24 h food intake. These results were obtained in animals which had been fasted overnight. When rats were allowed food up till the time of dosing, treatment with mebanazine had a proportionately greater effect than did amphetamine in the following 6 h. At the 15 mg/kg dose level, which was used in the 6 week experiment, there was a complete suppression of eating activity in the succeeding 6 h period. Doubling the single acute dose to 30 mg/kg suppressed food consumption for 24 h. In these rats there was a progressive fall in blood sugar level reaching its nadir at 24 h, the curve being superimposable on that for rats deprived of food but without drug.

It was observed in the previous experiment that even at the lowest dose of mebanazine tested (2·0 mg/kg orally) there was a statistically significant reduction in the food intake of fed rats. It was of interest therefore to determine whether or not this dose would also potentiate insulin hypoglycaemia. The results of an experiment utilizing three dose levels of mebanazine are summarized in Table 6. There was little change in body weight at 2·0 mg/kg per day compared with an increase of 49 ± 6 g in the controls. At the higher dose levels the animals showed a net loss in body weight. Food consumption was depressed in all the treated groups as was water intake. There were no significant differences in the resting blood sugar values after 15 days treatment at any dose level when compared to the controls. The fall in blood glucose 90 min after insulin was greater in all the treated groups but that seen after 2·0 mg/kg per day was not statistically greater than in the control group. The hypoglycaemia was potentiated significantly in the two higher dose-level groups. The results suggest that reduction in food and water intake alone does not entirely account for the potentiation of the hypoglycaemic response to insulin.

Table 6. *The effect of different doses of mebanazine on body weight, food and water consumption and response to insulin after* 15 *days oral treatment.* Five rats in each group: an asterisk denotes significant difference from controls.

	Saline 0·5 ml/100 g	Mebanazine 2·5 mg/kg	Mebanazine 7·5 mg/kg	Mebanazine 15·0 mg/kg
Body weight (g)				
Initial	240 ± 4	250 ± 3	251 ± 6	252 ± 1
After 15 days	289 ± 4	253 ± 7	236 ± 9	227 ± 10
Change	+49	+3	−15	−23
Food intake (g)				
Total	276 ± 19	192 ± 26*	153 ± 21*	137 ± 26*
% controls	100	70	56	50
Water intake (ml)				
Total	390 ± 21	259 ± 30	241 ± 41	161 ± 21
% controls	100	66	62	41
Blood sugar level (mg/100 ml)				
On day 15	121 ± 9	124 ± 3	114 ± 5	122 ± 12
90 min after insulin (10 u/kg s.c.)	66 ± 3	63 ± 3	38 ± 6	43 ± 3
Change	−55	−61	−76	−79
% control response	100	111	138	144

DISCUSSION

Potentiation of the hypoglycaemic response to insulin by mebanazine has been reported in rats (Barrett, 1965; Zor, Mishkinsky & Sulman, 1965; Adnitt, 1968a,b), in rabbits (Cooper & Ashcroft, 1966) and man (Wickström & Pettersson, 1964; Cooper & Keddie, 1964). A direct hypoglycaemic effect of mebanazine was observed by Zor & others (1965) but not by the other investigators. The dose of mebanazine used by Zor and his colleagues was 35 mg/kg, the maximum effect being at 24 h when the blood sugar averaged 72 mg/100 ml compared with a control level of 113 mg/100 ml. In the present study it has been shown that this dose of mebanazine is sufficient to depress food intake for 24 h and it may be concluded that the "direct" hypoglycaemic effect of the drug was, in fact, a secondary consequence of drug-induced fasting.

Subsequently Zor & others (1965a) demonstrated that mebanazine reduced the tibial epiphysial cartilage width whereas amphetamine did not. The results implied an inhibitory effect on growth hormone rather than on food intake. A further paper (Zor & others, 1965b) extended these findings in that mebanazine was shown to depress growth, that the effect was potentiated by hydrocortisone and only partially overcome by concomitant injection of growth hormone. It was implied that mebanazine specifically inhibited some enzyme involved in the release of growth hormone. In 1966, Zor, Winer & others found that mebanazine decreased hepatic DNA and total liver protein in immature rats. More specific evidence came from experiments in which chronic treatment of rats with mebanazine was observed to decrease glucose-6-phosphate dehydrogenase activity in the pituitary (Zor, Shore & others, 1967). They also noted a reduction in both pituitary RNA and DNA content, although the RNA : DNA ratio was unaltered. In none of these studies from Sulman's laboratory was there an adequate control allowing for the reduction in food intake demonstrated in this study. Physical limitation of food intake has been shown to have very similar effects both on weight gain and tibial epiphysial width to that of surgical excision of

the pituitary gland (Thompson & Crean, 1963). The results from the present experiments clearly show marked parallels between effects of mebanazine and pair-feeding and do not support the contention that the drug exerts a specific effect on growth hormone production, release or activity. The mechanism of potentiation of insulin hypoglycaemia is not solely dependent on the anorexic actions of mebanazine.

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