J. Pharm. Pharmac., 1968, **20**, 723–726 Received February 28, 1968

A note on insulin tolerance during mebanazine treatment

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In contrast to previous findings, mebanazine, a hydrazine monoamine oxidase inhibitor, administered for 21 days had no effect on insulin sensitivity in the rabbit. Insulin sensitivity was increased in mebanazine pretreated 24 hr starved rats but unchanged in mebanazine pretreated fed rats. Muscle and liver glycogen in the rat was unaffected by mebanazine treatment.

INCREASE in the hypoglycaemic action of insulin has been reported in rats (Barrett, 1965) and rabbits (Cooper & Ashcroft, 1966) during treatment with mebanazine (α-methylbenzylhydrazine), a hydrazine monoamine oxidase inhibitor. This phenomenon has been further investigated.

Experimental

Mebanazine oxalate 10 mg/ml and soluble insulin (Burroughs Wellcome & Co.) were made up in 0·9 g/100 ml saline immediately before use. Animals were 1·5–3·0 kg male rabbits and 200–300 g male Wistar rats. Blood glucose was estimated by glucose oxidase (Marks, 1959) and glycogen by the method of Krisman (1962).

Insulin tolerance in rabbits. Each experiment was on a different group of four animals. Blood was taken from an ear vein resting and at intervals after intravenous insulin. An initial insulin tolerance was undertaken (control 1), a second after treatment for 21 days with mebanazine (treated) and a third (control 2) 21 days after mebanazine withdrawal.

Insulin tolerance in rats. Rats treated with mebanazine 10 mg/kg subcutaneously for 21 days were housed individually and the weight of food consumed by each animal was given daily to a weight-matched individually housed control. The insulin tolerance of control and treated groups was determined on day 22. The animals were stunned, the abdomen opened rapidly and blood taken from the abdominal aorta. An equal number of treated and control rats were killed at each time interval to determine mean blood glucose.

Tissue glycogen. Glycogen was measured in liver and thigh muscle of mebanazine-treated and pair-fed control rats and in liver from normal rabbits.

Results

Unless otherwise stated, statistical comparison was by the Mann-Whitney U-test (Siegel, 1956).

Insulin tolerance in rabbits. Control and treated insulin tolerance curves for mebanazine (12 mg/kg s.c.), using insulin (0·24 unit/kg) after 2 hr food deprivation showed the insulin sensitivity to be unchanged

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during mebanazine treatment. Similar results were found with mebanazine, 3 mg/kg. In other experiments, hypoglycaemic stress was increased by doubling the dose of insulin and insulin tolerance was assessed in rabbits pretreated with mebanazine (12 mg/kg, i.m.) and then starved for 24 hr. In no experiment was insulin sensitivity increased during mebanazine treatment.

TABLE 1. MEAN BLOOD GLUCOSE, RESTING AND AT INTERVALS AFTER INSULIN IN RATS TREATED WITH MEBANAZINE (10 MG/KG S.C.) FOR 21 DAYS AND IN PAIR-FED CONTROLS

Experiment	Treatment	Food deprivation	Insulin (unit/kg, s.c.)	Mean blood glucose (mg/100 ml)				Number of deaths
				Resting	90 min	150 min	300 min	
1	Mebanazine	none	0·075	82·2 (5)	—	29·2 (5)	60·0 (10)	none (10)
	Pair-fed	none	0·075	75·9 (5)	—	24·9 (5)	51·7 (10)	none (10)
2	Mebanazine	24 hr	0·05	87·0 (8)	15·5 (4)	9·3 (4)	—	9 (9)
	Pair-fed	24 hr	0·05	62·8 (8)	21·8 (4)	19·5 (4)	27·6 (9)	none (9)
3	Mebanazine	24 hr	0·1	87·0 (8)	14·0 (4)	8·0 (4)	—	6 (6)
	Pair-fed	24 hr	0·1	62·8 (8)	19·0 (4)	15·5 (4)	15·0 (5)	1 (6)

Figures in parentheses are the number of rats from which the values are derived.

Insulin tolerance in rats (Table 1). In experiment 1 rats were allowed access to food until the insulin tolerance test. There was no significant difference between mebanazine-treated and pair-fed groups.

In rats starved for 24 hr (expt 2 and 3) fasting blood glucose was higher after mebanazine pretreatment ($P = 0{\cdot}003$); but after insulin 0·05 units/kg (expt 2), blood glucose was lower at 90 min ($P = 0{\cdot}029$) and 150 min ($P = 0{\cdot}029$) in the mebanazine-treated group. Of nine control and nine pretreated animals to be killed at 300 min, all pretreated rats died between 150 and 300 min after convulsions, and with a blood glucose in each animal of less than 9 mg/100 ml. No controls died and this difference in mortality is significant ($P < 0{\cdot}005$, Fisher exact probability test).

In starved rats given insulin 0·1 unit/kg (expt 3), blood glucose was again lower in the mebanazine-treated group at 90 min ($P = 0{\cdot}057$) and

TABLE 2. MUSCLE AND LIVER GLYCOGEN FOR MEBANAZINE-TREATED AND PAIR-FED RATS UNDER FED AND 24 HR STARVED CONDITIONS AND LIVER GLYCOGEN FOR FED AND 24 HR STARVED NORMAL RABBITS

Animals	Treatment	Food deprivation	Glycogen (mean)	
			muscle (mg/g)	Liver (mg/100 mg)
Rats (5)	Mebanazine	none	0·84	1·06
Rats (5)	Pair-fed	none	0·84	1·01
Rats (5)	Mebanazine	24 hr	0·62	0·055
Rats (5)	Pair-fed	24 hr	0·62	0·053
Rabbits (4) ..	Normal	none	—	9·0
Rabbits (4) ..	Normal	24 hr	—	0·040

150 min ($P = 0{\cdot}014$). Of six rats in each group to be killed at 300 min, all pretreated animals died between 150 and 300 min after convulsions, and with a blood glucose in each animal of less than 7 mg/100 ml. One control died and the difference in mortality is significant ($P < 0{\cdot}05$, Fisher exact probability test).

Tissue glycogen. Neither starvation nor mebanazine treatment caused a significant reduction in the muscle glycogen of rats ($P > 0{\cdot}05$). In mebanazine-treated and pair-fed rats as well as in normal rabbits, there was subtotal hepatic glycogen depletion after starvation for 24 hr (Table 2).

Discussion

Impaired glucose tolerance (Goldblatt & Ellis, 1932), insulin resistance (Tiitso, 1925) and hepatic glycogen depletion (Lawrence & McCance, 1931) have been described in animals deprived of food. For this reason control rats were pair-fed and the increased insulin sensitivity in mebanazine-treated starved rats cannot be due to changes in food intake brought about by treatment.

Barrett (1965) found increased tolbutamide hypoglycaemia in starved rats and increased insulin hypoglycaemia in fed rats after mebanazine treatment. I have found increased insulin hypoglycaemia only in starved animals. These animals have over 95% depletion of hepatic glycogen with sufficient remaining to produce less than 10 mg of glucose. The animals depend upon glucose production from other sources to restore blood glucose from hypoglycaemic levels.

Since mebanazine increased insulin hypoglycaemia under conditions where compensatory hepatic glycogenolysis can play little part, mebanazine cannot act by impairment of hepatic glycogenolysis. In the absence of liver glycogen, glucose production must be by muscle glycogenolysis or gluconeogenesis and mebanazine probably interferes with glucose production from these sources. In fed rats, hepatic glycogenolysis can predominate in restoration of blood glucose, impairment of muscle glycogenolysis or gluconeogenesis would be masked, and this could explain why mebanazine caused no detectable increase in insulin sensitivity in the fed animal.

Muscle glycogen was normal during mebanazine treatment but this may or may not be readily available. Conversion to glucose is at least in part dependent upon lactic acid dehydrogenase and *in vitro* inhibition of dehydrogenases by hydrazine monoamine oxidase inhibitors has been described (Redetzki & O'Bourke, 1961). These inhibitors also inhibit some pyridoxal requiring enzymes (Robinson, 1966) and it may be significant that transaminases, concerned in gluconeogenesis, require pyridoxal (Weber, Singal & others, 1964).

A possible explanation for the finding that mebanazine had no effect upon insulin hypoglycaemia in the rabbit would be that the larger animal always maintained its liver glycogen and so behaved in the same way as the fed rat, even after starvation. It can be seen from Table 2 that this is not so.

The present results in rabbits differ from the other report about this species (Cooper & Ashcroft, 1966). The discrepancy is difficult to explain. I used male rabbits and a sex difference in monoamine oxidase activity has been described (Zeller, 1966). This may result in sex differences in response to monoamine oxidase inhibitors. A strain difference in behaviour cannot be excluded and in man there is a genetically controlled variation in the rate of acetylation of the closely related drug isonicotinic acid hydrazide (Brodie, 1964). In the report of Cooper & Ashcroft (1966), mebanazine was administered to 3 rabbits. Twenty animals were used in the present investigation which should yield a more representative result.

The manner in which the insulin tolerance tests were made was entirely different in the two species but the results do raise the possibility of a species difference in response to mebanazine.

Acknowledgements. I am grateful to Dr. S. Oleesky and to Prof. H. Schnieden for their help and advice. Mebanazine was supplied by I.C.I. Ltd. and I am grateful to Dr A. M. Barrett of I.C.I. for his cooperation.

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