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Modification of the hypoglycaemic response to tolbutamide and insulin by mebanazine—an inhibitor of monoamine oxidase

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Following reports of clinical changes in the carbohydrate metabolism of diabetic patients receiving mebanazine, possible interactions between mebanazine, adrenaline, tolbutamide and insulin have been studied in rats. Mebanazine had no activity on blood sugar levels and was found to have no effect on the metabolic actions of injected adrenaline. In acute experiments, mebanazine appeared to reduce the net hypoglycaemic action of tolbutamide but had no effect on the response to insulin. In chronic experiments, mebanazine pre-treatment led to a significant potentiation of the hypoglycaemic responses both to tolbutamide and insulin. The possible mechanisms of action and the clinical implications are discussed.

CHANGES in carbohydrate metabolism have been observed during the trial of a new antidepressive agent, mebanazine (Actomol, α-methylbenzylhydrazine). Clinical findings, in patients exhibiting both diabetic and psychiatric symptoms, included an improved glucose tolerance and lower fasting blood-sugar levels (Wickström & Pettersson, 1964). Most of these patients were receiving conventional antidiabetic therapy in addition to mebanazine, which is an inhibitor of monoamine oxidase. In certain cases the dosage of insulin or sulphonylurea had to be reduced to avoid hypoglycaemic crises. The opposing effects of catecholamines and insulin on blood-sugar are well established, so that the effects of mebanazine are unlikely to be due to inhibition of amine oxidase and prolongation of catecholamine action. However, in addition to their central antidepressive effects, the monoamine oxidase inhibitors appear to possess peripheral pharmacological actions which are not readily interpreted as a consequence of amine oxidase inhibition. For example, they have been shown to antagonise the release of catecholamines from adrenergic nerve endings (Brodie & Beaven, 1963) and to decrease the entry of noradrenaline into previously depleted storage sites (Davey, Farmer & Reinert, 1963). These pharmacological effects would be associated with a reduction in sympathetic activity. This potential inhibition of adrenergic function due to monoamine oxidase inhibitors might be particularly important in relation to tolbutamide therapy since this drug has been claimed to have direct catecholamine-releasing activity (Dulin, Morley & Nezamis, 1956; Bander, 1959). The present experiments were designed to determine whether or not the peripheral actions of mebanazine modified the hypoglycaemic responses to tolbutamide and to insulin.

Experimental

METHODS

The experiments were made using male rats (190–230 g) from the colony of specific pathogen-free albinos maintained at Alderley Park.

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Blood samples were obtained from the abdominal aorta after intraperitoneal pentobarbitone sodium anaesthesia.

Each treatment was given to groups of four animals except where otherwise indicated. Hypoglycaemic responses to tolbutamide (25, 50 or 100 mg/kg orally) were measured in rats which had been fasted overnight, primed with a subcutaneous injection of 100 mg of glucose (0·2 ml) and dosed orally with either saline or substance(s) under examination. Blood samples for analysis were taken 2 hr after dosing. The difference in blood-sugar between control and test groups was calculated as a percentage of the control values. Hypoglycaemic responses to insulin were measured in fed rats. The animals received 0·5 U soluble insulin subcutaneously and were bled 90 min later, food being withdrawn during this period.

Adreno-demedullated rats were maintained on 0·9% saline in place of drinking water for 4 weeks and used in experiments 6 to 8 weeks after operation.

The following determinations were made: blood-sugar (Hagedorn & Jensen, 1923); plasma free fatty acids (Dole, 1956); plasma tolbutamide (Toolan & Wagner, 1959); plasma corticosterone (Zenker & Bernstein, 1958 as modified by Barrett & Stockham, 1963); heart rates were recorded in pentobarbitone anaesthetised rats during intravenous adrenaline infusion by means of a cardiotachometer triggered by the QRS complex of the electrocardiogram.

Results

EFFECTS OF MEBANAZINE ALONE, AND ON THE RESPONSES TO INJECTED ADRENALINE

The effects of monoamine oxidase inhibitors on carbohydrate metabolism of rats and on the metabolic action of injected catecholamines do not appear to have been described in the literature. The fasting blood-sugar level has been determined in rats after 12 daily oral doses of mebanazine ranging from 2·0 to 30 mg/kg, there being 18 hr between the last dose and testing. No significant changes were found under these conditions nor 2 hr after a single dose of 120 mg/kg (Table 1). Other animals either

TABLE 1. EFFECT OF MEBANAZINE ON THE BLOOD-SUGAR LEVEL COMPARED WITH THAT OF TOLBUTAMIDE

Treatment		Time (hr) between last dose and sampling	Blood sugar level (mg/100 ml)
Drug	Dose		
Saline	0·5 ml/100 g	2	79 ± 3·0 (4)
Tolbutamide	100 mg/kg	2	43 ± 1·0 (4)
Mebanazine	120 mg/kg	2	80 ± 2·5 (4)
Mebanazine	60 mg/kg	18	90 ± 8·5 (4)
Saline	0·5 ml/100 g	2	
Mebanazine daily for 12 days	2·0 mg/kg	18	82 ± 3·0 (4)
	7·5 mg/kg	18	81 ± 5·5 (4)
	30·0 mg/kg	18	83 ± 2·0 (4)

pre-treated with a single dose of mebanazine (120 mg/kg) or else chronically treated for 12 days (30 mg/kg) were given intravenous infusions of

adrenaline. In neither group were the adrenaline-induced changes in blood-sugar, plasma free fatty acids or heart rate different from those observed in untreated control animals (Table 2). The results are in accord with those of Vanov (1962) who found that the pressor effects of injected adrenaline were not potentiated by monoamine oxidase inhibitors.

TABLE 2. THE EFFECT OF ACUTE AND CHRONIC PRE-TREATMENT WITH MEBANAZINE ON THE RESPONSE OF THE BLOOD-SUGAR, PLASMA FREE FATTY-ACIDS AND THE HEART RATE TO AN INTRAVENOUS INFUSION OF ADRENALINE (2 μg/kg/min) FOR 15 MIN. Each value represents the mean of observations in four rats with the standard errors of the means.

	Plasma free fatty acid concentration (μ-equiv./l)			Blood-sugar level (mg/100 ml)			Heart rate (beats/min)		
Treatment	Controls	Infused	Difference	Controls	Infused	Difference	Initial	Final	Change
Controls—bled 15 min after pentobarbitone anaesthesia. Infused—bled 15 min after adrenaline infusion	353 ±33	630 ±42	+277	113 ±3·3	183 ±3·3	+70	409 ±18	514 ±11	+105
All animals received mebanazine (120 mg/kg) 150 min before proceeding as above	373 ±25	588 ±33	+213	100 ±5·2	175 ±11.0	+74	305 ±5	404 ±14	+99
All animals received mebanazine (30 mg/kg) daily for 12 days before proceeding as above	364 ±21	609 ±29	+245	107 ±4·9	179 ±4·8	+72	401 ±21	517 ±16	+116

The lack of effect of mebanazine on the blood-sugar level or on the responses to adrenaline is consistent with the suggestion of Axelrod (1960) that monoamine oxidase plays little part in the termination of the actions of catecholamines outside the central nervous system.

EFFECTS OF TOLBUTAMIDE OR INSULIN, ALONE

The hypoglycaemic effects of tolbutamide and insulin in the rat are well known. For control purposes, in each experiment standard doses of either tolbutamide (50 and 100 mg/kg) or insulin (0·5 U/100 g) were always given. In 20 experiments, the mean fall in blood-sugar to the lower dose of tolbutamide was 28 and 40% at the higher dose. Six weeks after adreno-demedullation, the hypoglycaemic response to tolbutamide was markedly enhanced. At a dose level of 100 mg/kg, demedullated animals became comatose within 1 hr of dosing and falls of up to 80% in blood-sugar were recorded. The mean fall in blood-sugar of intact rats given insulin was 40% in two experiments.

ACUTE EFFECTS OF MEMBANAZINE ON THE RESPONSE TO TOLBUTAMIDE AND INSULIN

Possible interactions between mebanazine and hypoglycaemic agents and the role of the sympathetic nervous system in the regulation of blood-sugar levels were therefore examined. When tolbutamide and mebanazine

were given together, the fall in blood-sugar was significantly smaller than that observed when tolbutamide was given alone (Fig. 1). There was also a reduction in the slope of the dose-response curve, for those animals receiving both drugs. The effect was not solely due to adreno-medullary stimulation as the antagonism was also found in demedullated rats (Fig. 2). Indeed, the effect was more dramatic since simultaneous administration of mebanazine prevented the blood-sugar dropping to fatal levels after tolbutamide. These animals did not become comatose and showed no observable signs of distress.

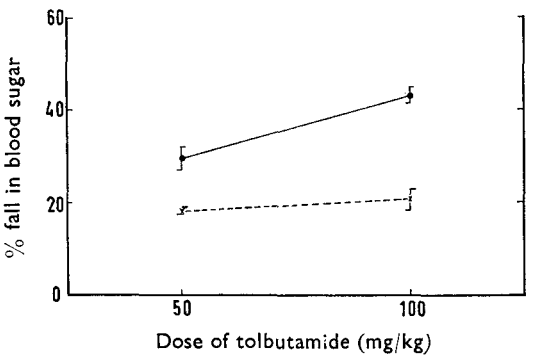

FIG. 1. The effect of tolbutamide on blood-sugar when given alone (O——O) or simultaneously with mebanazine, 120 mg/kg (X - - - X).

On the other hand, the degree of hypoglycaemia produced by insulin was the same in both control and mebanazine-treated rats, 2 hr after a single dose of the inhibitor (Table 3).

TABLE 3. THE EFFECT OF INSULIN ON BLOOD-SUGAR IN CONTROL RATS AND ANIMALS PRE-TREATED WITH A SINGLE DOSE OF MEBANAZINE 30 MIN BEFORE INSULIN. Blood samples were taken 90 min after insulin (i.e. 2 hr after mebanazine)

Dose of insulin	Dose of mebanazine	Blood-sugar level (mg/100 ml)	Fall in blood-sugar %
0	0	107 ± 9·5 (4)	—
0·5U/100 g	0	62 ± 5·2 (4)	42
0·5U/100 g	120 mg/kg	62 ± 1·5 (4)	42

It was likely that the antagonism between mebanazine and tolbutamide, under these conditions, resulted from the potentiation or unmasking of some property of tolbutamide rather than from an enhanced adrenergic response to hypoglycaemia *per se*. Evidence concerning the catecholamine-releasing potential of tolbutamide has been cited earlier (Dulin & others, 1956; Bander, 1959). Further, it is known that the pharmacological actions of substances which act by releasing noradrenaline, e.g., tyramine (Burn & Rand, 1958) are potentiated by monoamine oxidase inhibitors (Blackwell & Marley, 1964).

EFFECT OF CHRONIC MEBANAZINE TREATMENT ON THE HYPOGLYCAEMIC RESPONSES TO TOLBUTAMIDE AND INSULIN

The clinical improvements in diabetes mellitus were noted only after several weeks of treatment with mebanazine. To study the effects of

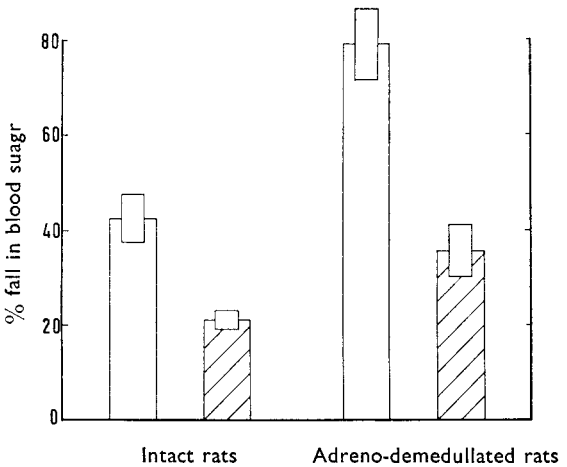

FIG. 2. The antagonistic effect of mebanazine, 120 mg/kg, on the hypoglycaemic response to tolbutamide, 100 mg/kg, in intact and adreno-demedullated rats. Hatched columns, both drugs. Open columns, tolbutamide alone.

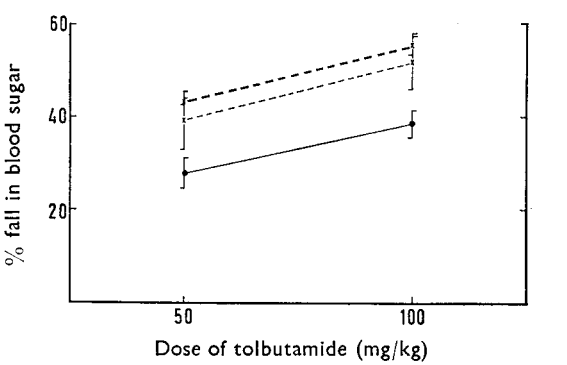

FIG. 3. The effect of tolbutamide on blood-sugar in control rats and 18 hr after the last of 21 consecutive daily doses of mebanazine. ●——● tolbutamide alone. X---X tolbutamide and mebanazine 15 mg/kg/day. X- - -X tolbutamide and mebanazine, 30 mg/kg/day.

chronic mebanazine treatment, rats were dosed orally for 3 weeks. After an overnight fast, without further mebanazine, the hypoglycaemic response to tolbutamide was assessed. The chronically-treated animals showed a significantly greater fall in blood-sugar after tolbutamide than that found in rats which had been dosed with saline for 3 weeks. There was no significant difference between the potentiation at 15 or 30 mg/kg of

mebanazine (Fig. 3). Similar potentiation occurred after 12 days of treatment at 7·5 to 30 mg/kg daily. Although the potentiation increased with dosage, the results at 7·5 and 30 mg were not statistically significant from one another (Table 4).

TABLE 4. THE EFFECT OF A SINGLE DOSE OF TOLBUTAMIDE ON BLOOD SUGAR, 18 HR after the last of twelve consecutive daily doses of mebanazine

Daily dose of mebanazine (mg/kg)	Dose of tolbutamide (mg/kg)	Fall in blood-sugar % (mean ± s.e.)	P value
0	100	37·7 ± 1·2 (4)	—
2·0	100	38·8 ± 2·7 (4)	>0·05
7·5	100	48·4 ± 4·0 (4)	<0·05
15·0	100	51·9 ± 5·7 (4)	<0·05
30·0	100	53·0 ± 2·4 (4)	<0·01

If the dose of mebanazine was increased to 60 mg/kg, given 18 hr before tolbutamide, there was as great a potentiation of hypoglycaemia as that seen after prolonged dosing at lower levels.

Similar results were obtained in groups of 9 rats chronically treated with mebanazine after receiving insulin in place of tolbutamide. Although only one dose level of insulin, 0·5 U/kg, was used the increase in hypoglycaemic effect was statistically significant after 12 days of treatment with mebanazine (15 mg/kg). The control value was 38 ± 4·9; treated value 51·0 ± 2·3: $P < 0·05$.

The results are compatible with the hypothesis that mebanazine produces partial inhibition of catecholamine release which normally occurs as a consequence of a fall in blood-sugar.

EFFECT OF CHRONIC DOSAGE OF MEBANAZINE ON GLUCOSE TOLERANCE

In an attempt to relate the present findings to the clinical observations, oral glucose tolerance curves were constructed for both control and chronically treated rats (Fig. 4). In both groups, the fasting blood-sugar level was the same and there was little difference in the 10 min peak value. However, the rate of fall in blood glucose was greater in the mebanazine-treated group, although only at the 20 min time interval was the difference between the two curves statistically significant ($P < 0·05$). Attempts to follow changes in intravenous glucose tolerance in rats were not successful owing to the rapidity with which blood-glucose falls after an intravenous injection of glucose.

CHRONIC EFFECTS OF MEBANAZINE ON HEPATIC AND ADRENOCORTICAL FUNCTION

Exaggerated hypoglycaemic responses have been reported after liver damage (Dall & Melrose, 1964). Hydrazines in general are potentially hepatotoxic and it is possible that mebanazine (which is, of course, a hydrazine) treatment might have affected the rate of hepatic inactivation of tolbutamide. Blood levels of tolbutamide 2 hr after dosing were not significantly different in either control or mebanazine-treated rats (Table 5).

In another experiment, the rate at which the blood tolbutamide level fell after a single 100 mg/kg dose was similar both in control rats and in animals which had received 30 mg/kg mebanazine daily for 3 weeks.

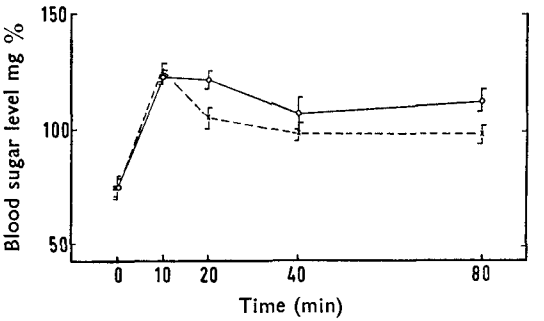

FIG. 4. The blood-sugar level in control and chronically mebanazine treated rats following an oral dose of glucose (10 g/kg). The animals were fasted overnight. O——O control rats. ×- - -× mebanazine, 15 mg/kg/day.

TABLE 5. THE EFFECT OF CHRONIC TREATMENT WITH MEBANAZINE ON THE BLOOD LEVELS OF TOLBUTAMIDE, 2 HR AFTER ORAL ADMINISTRATION

Daily dose of mebanazine (mg/kg for 12 days)	Blood tolbutamide level (μg/ml)	
	After 50 mg/kg	After 100 mg/kg
0	282 ± 29 (4)	477 ± 38 (4)
2·0	230 ± 51 (4)	432 ± 51 (4)
7·5	328 ± 17 (3)	481 ± 31 (5)
30·0	281 ± 45 (3)	453 ± 46 (5)

The same mebanazine regimen produced a slight adrenal hypertrophy but no change in the resting plasma corticosterone concentration (controls 4·1 ± 1·3 μg/100 ml). The plasma corticosterone response to ether anaesthesia was not reduced by mebanazine treatment (controls 33·8 ± 4·7 μg/100 ml; mebanazine 46·6 ± 9·5 μg/100 ml).

Discussion

The results of these experiments in the rat afford a basis for the interpretation of the clinical observations in diabetic patients receiving mebanazine. Various mechanisms have been proposed to explain the orthostatic hypotension found as a side-effect of therapy with monoamine oxidase inhibitors. Apart from bretylium-like effects (Gessa, Cuenca & Costa, 1963), monoamine oxidase inhibitors with a hydrazine structure have been found to inhibit dopa decarboxylase and dopamine α-hydroxylase, both of which are important in the biosynthesis of catecholamines (Gey, Pletscher & Burkard, 1963). Preliminary studies suggest that mebanazine also possesses such properties. These pharmacological actions would serve to decrease both the stores and release of adrenergic transmitter resulting in reduced sympathetic activity. It is possible that the same

mechanisms are involved both in orthostatic hypotension and potentiation of hypoglycaemic agents. Co-existing hypotension and hypoglycaemia in a previously stable diabetic patient after mebanazine treatment was described recently (Cooper & Keddie, 1964).

Decreasing fasting blood-sugar levels and improved glucose tolerance in non-diabetic psychiatric patients was first noticed with iproniazid (Weiss, Weiss & Weiss, 1959). The clinical studies of Wickström & Pettersson (1964) together with the present findings, suggest that a certain kind of tolbutamide- and insulin-insensitive diabetic patient may not only show an improved response with mebanazine but that in certain cases the conventional antidiabetic therapy may be withdrawn. Mebanazine would appear to possess an insulin-sparing effect rather than a direct insulin-like hypoglycaemic action. Perhaps, therefore, certain patients diagnosed and treated for diabetes mellitus may in fact have no primary insulin deficiency, but rather, an oversensitive adrenergic hyperglycaemic mechanism which responds to a high critical blood-sugar level. If this is true, then hypoglycaemic agents would make the situation worse rather than better by stimulating further increases in adrenaline output which in turn would raise the blood-sugar level even higher. In the presence of a normal renal threshold to glucose, hyperglycaemia and glycosuria would persist in spite of rational antidiabetic treatment and only be antagonised by some form of adrenergic blockade.

A further implication of these experimental findings is that exaggerated hypoglycaemic responses may occur in patients receiving both antidiabetic therapy and mebanazine. It remains to be seen whether or not this applies to other monoamine oxidase inhibitors and adrenergic neurone blocking agents. Potentiation of insulin hypoglycaemia by ganglion blocking drugs has been reported in man, dog and rabbit (Laurence & Stacey, 1951; Schachter, 1951). Moreover, if the tolbutamide diagnostic test for either diabetes (Boshell, Wilensky, Wayland & Carr, 1963) or insulinoma (Fajans, Schneider, Scheingart & Conn, 1961) is conducted in persons also receiving drugs which interfere with adrenergic transmission, a false positive diagnosis is possible. Recently, there has been a report of erroneous diagnosis of insulinoma based on the intravenous tolbutamide test some years after sympathectomy (Cohn, Perlmutter, Silverstein & Numeroff, 1964).

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