J. Pharm. Pharmac., 1970, **22**, 291–297 *Received October* 6, 1969

The mode of action of insulin potentiation by mebanazine

A. M. BARRETT*

Department of Pharmacology, Imperial Chemical Industries Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, U.K.

Chronic oral treatment of rats with mebanazine potentiated the hypoglycaemic response to insulin both in intensity and duration. A similar potentiation of intensity but not of duration was observed in pair-fed animals. In consequence, mebanazine-treated rats subjected to a 21 h fast before insulin died in hypoglycaemic coma whereas control and pair-fed animals did not. Intravenous glucose brought about a rapid return to consciousness in moribund mebanazine-treated rats in insulin coma but hyperglycaemic catecholamines did not. The observed hypoglycaemic unresponsiveness was not solely due to depletion of liver glycogen since both control and treated rats had undetectable levels of glycogen after fasting, yet only the treated animals died. Tissue levels of noradrenaline were raised by mebanazine treatment in brain, heart and liver but these enlarged stores were readily depleted by reserpine. A similar potentiation of hypoglycaemia was observed following chronic treatment of intact rats with tranylcypromine and of adreno-demedullated rats with guanethidine and bethanidine. No potentiation was observed with reserpine or α-methyldopa in either type of animal. Blood glucose was significantly raised by adrenaline and α-methylnoradrenaline, but not by octopamine. The observations support the hypothesis that hypoglycaemic unresponsiveness following chronic inhibition of monoamine oxidase is due to a replacement of noradrenaline in adrenergic neurons by octopamine. This substance is an ineffective hyperglycaemic agent and its physiological release in response to hypoglycaemia does not bring about a return to normoglycaemia.

Increased sensitivity to insulin hypoglycaemia in rats pretreated with mebanazine was first reported in 1965 (Barrett, 1965; Zor, Mishkinsky & Sulman, 1965). Attempts to define the mechanism of this effect have not been conclusive.

A specific inhibition of growth hormone activity, rather than monoamine oxidase inhibition, was considered by Zor, Dikstein & Sulman (1965a,b). Subsequent experiments (Adnitt, 1968a; Barrett, 1966, 1969) clearly showed that mebanazine possessed an anorexic action and that paired-feeding could produce very similar results in terms of weight gain, pituitary growth hormone content and the width of the tibial epiphysial cartilage. The increased insulin sensitivity was shown to be well correlated with inhibition of monoamine oxidase by Adnitt (1968a) after prolonged mebanazine-treatment and to occur both with hydrazine (mebanazine) and non-hydrazine (tranylcypromine) types of enzyme inhibitor. A single dose of mebanazine sufficient to produce a 98% inhibition of monoamine oxidase did not increase insulin sensitivity. Two pieces of evidence supported the hypothesis that the potentiation of insulin was related to the accumulation of a false neurochemical transmitter, β-hydroxytyramine (octopamine) after monoamine oxidase inhibition

* Present Address: N. V. Organon, Oss, Holland.

(Kopin, Fischer & others, 1964). First, the duration of hypoglycaemia was significantly prolonged, implying a failure of the normal response to low blood sugar levels. Second, the effect of mebanazine only occurred after chronic treatment (10–21 days). The present investigation was undertaken to help clarify the mechanism of increased insulin sensitivity in mebanazine-treated rats.

EXPERIMENTAL

The animals were male albino rats (190–230 g) from the specific pathogen-free strain bred at Alderley Park. They were maintained on a cubed diet and water *ad libitum* except where food intake was restricted for purposes of pair-fed controls. 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. Blood samples were obtained from the abdominal aorta after intraperitoneal pentobarbitone sodium anaesthesia, or by cardiac puncture under ether anaesthesia where indicated. There were five animals used in each experimental group and the rats were weighed daily, food and water consumption being recorded for groups of 5.

Blood glucose was determined by a kit glucose-oxidase method and glycogen by the method of Krisman (1962). The extraction of noradrenaline from tissues and its subsequent fluorimetric estimation was carried out by modifications of literature methods described by Iversen (1963). Octopamine does not interfere in the fluorimetric assay of noradrenaline since it is a phenol derivative.

RESULTS

The mean fall in blood glucose, 90 min after insulin (0·5 U/100 g, s.c.) was 37% in a group of control rats. Animals which had received mebanazine orally (15 mg/kg daily) for 3 weeks showed a significantly greater hypoglycaemic response, 61%, when given the same dose of insulin. A third group of rats which had been pair-fed with the mebanazine group also exhibited a potentiation of insulin hypoglycaemia (67%) which was not statistically different from the treated group. These results are summarized in Table 1.

Table 1. *The effect of insulin* (0·5 U/100 g, s.c.) *on blood glucose levels* (mg %) *in control rats, rats receiving mebanazine orally* (15 mg/kg daily) *for* 21 *days and rats pair-fed with those receiving mebanazine* (*blood sampled* 90 *min after insulin; means* ± *s.e.*, *n* = 5)

Treatment group	Initial value	90 min after insulin	Change	% fall	*P* value
Control	98·3 ± 6·4	64·7 ± 10·1	—37·5 ± 7·7	36·8 ± 8·4	—
Mebanazine	107·1 ± 5·5	41·2 ± 4·0	—65·9 ± 5·2	61·0 ± 4·3	<0·05
Pair-fed	92·5 ± 10·1	30·8 ± 5·2	—61·6 ± 5·8	67·0 ± 2·7	<0·01

From these results it might have been concluded that the potentiation of insulin hypoglycaemia was secondary to the reduction in food intake by mebanazine (Barrett, 1969). However, the pattern of eating behaviour in the three groups was quite different. The control group exhibited mainly nocturnal eating whereas the mebanazine-treated rats appeared to nibble their food more or less continuously with a lower over-all intake. The pair-fed animals consumed all their allowance within

3 h and thus spent 21 h daily without food. In an attempt to equalize these differences the experiment was repeated, fasting all three groups for 21 h before giving insulin. The results, summarized in Table 2, showed that fasting *per se* increased hypoglycaemic sensitivity in control rats. In the mebanazine-treated group the degree of hypoglycaemia was greater but the difference did not achieve statistical significance. Pair-fed animals were also more sensitive to insulin than were the controls, but again not significantly so. The results might be construed as showing an influence on food intake alone and that mebanazine had no specific effect on the sensitivity to insulin. However, in the afternoon following this experiment (blood samples having been obtained by cardiac puncture in the morning) the mebanazine-treated rats were moribund or dead. One pair-fed animal was dead, but all controls were alive and active. Post-mortem examination of the animals showed cardiac tamponade in the dead pair-fed animal but no damage in those rats receiving mebanazine. A blood glucose analysis on one of the moribund rats gave a value of 7%. This experience suggested that it would be worthwhile studying the time course of the response to insulin in the fasting state. The results of such an experiment are summarized in Table 3. In this case the maximum hypoglycaemic effect was significantly greater in the mebanazine and pair-fed groups compared with control rats, 90 min after insulin. Whereas at 3 h both the control group and the pair-fed values had returned to pre-insulin glucose levels, that of the mebanazine-treated group had dropped to 10 mg % and the animals were unconscious. Intravenous injection of glucose (2 ml of 10% solution) brought about a dramatic return to consciousness and the animals survived for a period of 2 weeks after which they were killed.

Table 2. *The effect of insulin* (0·5U/100 g, s.c.) *on blood glucose levels* (mg %) *in control rats, rats receiving mebanazine orally* (15 mg/kg daily) *for* 21 *days and rats pair-fed with those receiving mebanazine, after* 21 *h of fasting* (*blood sampled* 90 *min after insulin; means* ± *s.e., n* = 5)

Treatment group	Initial value	90 min after insulin	Change	% fall	*P* value
Control	74·4 ± 9·8	32·8 ± 4·8	—41·6 ± 5·0	57·2 ± 3·4	—
Mebanazine	69·0 ± 4·6	20·6 ± 3·4	—48·4 ± 8·0	69·0 ± 6·6	N.S.
Pair-fed	78·8 ± 8·0	26·0 ± 5·6	—52·8 ± 6·7	67·2 ± 5·4	N.S.

Table 3. *Time course of blood glucose response to insulin* (0·5 U/100 g, s.c.) *in control rats, rats receiving mebanazine orally* (15 mg/kg daily) *for* 21 *days and rats pair-fed with those receiving mebanazine after* 21 *h of fasting* (*means* ± *s.e., n* = 5)

Treatment group	Time after insulin injection (min)				
	0	60	90	120	180
Control	80 ± 6·1	61 ± 4·9	41 ± 3·7	60 ± 4·9	85 ± 8·1
Mebanazine	74 ± 6·2	43 ± 3·1	20 ± 4·1	18 ± 5·3	10 ± 4·1
Pair-fed	72 ± 8·4	45 ± 4·9	22 ± 3·9	41 ± 6·1	70 ± 6·4

It was possible that treatment with mebanazine altered either the amount of hepatic glycogen available to counteract the hypoglycaemic effect of insulin or the

ability of the treated animals to mobilize an adequate glycogen reserve satisfactorily. Determination of hepatic glycogen content in control rats which had been allowed to feed normally gave a value of 6·0 ± 0·6 mg % in comparison with 5·6 ± 1·0 mg % for rats given mebanazine orally for 21 days. In the pair-fed group the concentration was only 2·7 ± 0·6 mg %. Bearing in mind that pair-fed animals had a 21 h interval between consumption of the previous day's food, liver glycogen determinations were also made on animals in control and mebanazine-treated groups, deprived of food for 21 h. In neither case was there any detectable glycogen concentration. It was apparent, therefore, that both fasting control and pair-fed rats were able to survive a severe hypoglycaemic episode without measurable liver glycogen in the former case, but that mebanazine-treated animals could not do so. Absence of an adequate glycogen reserve could not therefore explain the effect of mebanazine in the present experiments.

Table 4. *Concentrations of noradrenaline* (μg/g) *in brain, heart and liver of control rats and rats receiving mebanazine orally* (15 mg/kg daily) *for* 21 *days* (*means* ± *s.e.*, *n* = 5)

Treatment group	Brain	Heart	Liver
Controls	0·32 ± 0·04	0·89 ± 0·09	0·08 ± 0·002
Mebanazine	0·56 ± 0·03	1·47 ± 0·08	0·10 ± 0·007
P value	<0·01	<0·01	<0·01

The 15-day period of treatment with mebanazine led to a statistically significant increase in the brain, heart and liver concentrations of noradrenaline (Table 4). These levels of noradrenaline were not significantly altered by the administration of insulin (0·5 U/100 g, s.c.). Treatment with mebanazine did not protect the enlarged stores of noradrenaline from depletion by reserpine (Table 5).

Table 5. *Effect of reserpine* (5 mg/kg, i.p.) *on noradrenaline concentrations in heart and liver of control rats and rats receiving mebanazine orally* (15 mg/kg daily) *for* 21 *days* (*samples taken* 24 *h after reserpine; means* ± *s.e.*, *n* = 5)

	Tissue noradrenaline content (μg/g)	
Treatment group	Heart	Liver
Control	0·970 ± 0·010	0·103 ± 0·040
Control + reserpine ..	0·032 ± 0·009	0·001 ± 0·001
Mebanazine	1·214 ± 0·012	0·114 ± 0·036
Mebanazine + reserpine ..	0·054 ± 0·010	0·001 ± 0·001

Attempts were made to determine whether or not other drugs affecting the storage and release of noradrenaline might also potentiate insulin hypoglycaemia and prolong sub-normal glucose levels. The experiments were duplicated in both intact and adreno-demedullated rats because of the insensitivity of medullary stores of catecholamines to depleting agents. The results are summarized in Table 6. After the administration of reserpine, guanethidine, α-methyldopa, bethanidine, tranylcypromine and mebanazine to intact rats only the latter two agents afforded potentiation.

In adreno-demedullated rats in addition to tranylcypromine and mebanazine, bethanidine and guanethidine also produced significant potentiation. The failure of reserpine to produce any potentiation even after de-medullation was most surprising. Although it produced almost complete depletion of tissue catecholamines there was no evidence of hypoglycaemic unresponsiveness despite the fact that the animals were in poor condition and not eating.

Table 6. *Effect of various drugs on the sensitivity of intact and adrenodemedullated rats to insulin hypoglycaemia* (0·5U/100 g, s.c.). A plus sign indicates significant potentiation at the 5% level.

Treatment	Daily dose (mg/kg) oral route	Duration	Potentiation of insulin: Intact rats	Potentiation of insulin: Demedullated rats
Reserpine	5 i.p.	2 days	—	—
Guanethidine	10 oral	10 days	—	+
Bethanidine	7·5 oral	10 days	—	+
α-Methyldopa	10 oral	21 days	—	—
Tranylcypromine	7·5 oral	21 days	+	+
Mebanazine	15 oral	21 days	+	+

It has been suggested that the administration of α-methyldopa or a monoamine oxidase inhibitor leads to the displacement of noradrenaline at adrenergic nerve terminals by α-methylnoradrenaline and octopamine respectively (see Kopin, 1968, for references). Intravenous administration of saline in fed rats had little effect on blood glucose, 15 min afterwards. Adrenaline (10 μg/kg, i.v.) raised the circulating glucose level to 155 mg per 100 ml compared with 168 mg per 100 ml 15 min after α-methylnoradrenaline (50 μg/kg). In contrast, octopamine at 100 μg/kg had no effect on blood glucose levels. These results are summarized in Table 7.

Table 7. *Blood glucose levels in rats given intravenous injection of saline or various drugs,* 15 *min later* (*values expressed as* mg/100 ml, *means* $\pm$ *s.e.*, $n = 5$)

Treatment	Dose	Blood glucose
Saline	0·1 ml/100 g	116 ± 2·0
Adrenaline	10 μg/kg	155 ± 13·2
α-Methylnoradrenaline	50 μg/kg	168 ± 18·0
Octopamine	100 μg/kg	110 ± 3·2

In a final experiment I tried to revive 21 h fasted rats, which had received mebanazine chronically for 3 weeks, from a fatal insulin hypoglycaemia with adrenaline and α-methylnoradrenaline, but was unsuccessful. Only the intravenous injection of glucose was effective in this condition.

DISCUSSION

Chronic administration of mebanazine has been shown to potentiate the hypoglycaemic response to insulin and to delay the recovery of blood glucose levels to normal. The former effect may well be associated with the reduction in food intake, since pair-fed animals showed a spontaneous recovery to pre-insulin glucose levels

comparable to that in control rats. The failure of the homeostatic response in mebanazine-treated rats could not be attributed to a depletion of liver glycogen or tissue noradrenaline. Mebanazine did not protect the enlarged noradrenaline stores from depletion by reserpine. Depletion of catecholamines by adrenergic neuron blocking agents also prolonged hypoglycaemia in adreno-demedullated rats.

Other workers (Adnitt, 1968a; Cooper & Ashcroft, 1966) have shown that enhanced insulin sensitivity was associated with the monoamine oxidase inhibition rather than any other property of this class of drugs. Further, clinical exploitation of the phenomenon has been reported in several different centres (Adnitt, 1968b; Cooper, 1966; Wickstrom & Pettersson, 1964). The present results help to provide a rational explanation of previously reported clinical findings.

It is well established that chronic inhibition of monoamine oxidase leads to an increase in tissue stores of noradrenaline. Yet at the same time there is a diminished sympathetic responsiveness in relation to neural release of the transmitter (Davey, Farmer & Reinert, 1963). Pre-treatment of rabbits with iproniazid increased the noradrenaline content of brain, heart and liver, but also elevated the octopamine content to a proportionately greater extent (Kakimoto & Armstrong, 1962). Pheniprazine has also been found to raise the level of octopamine in cat heart and spleen (Kopin, Fischer & others, 1965). These authors demonstrated release of octopamine from cat spleen by nerve stimulation and this, taken with other reports reviewed by Kopin (1968), may be taken as evidence of the presence of octopamine as a genuine "false transmitter". Inhibition of monoamine oxidase leads to an accumulation of tyramine; it is taken up by sympathetic nerve endings and β-hydroxylated to octopamine. Octopamine then replaces noradrenaline in the storage vesicles. In the present experiments mebanazine was shown to increase tissue noradrenaline levels, yet to diminish responsiveness to hypoglycaemia. Octopamine was found to have very weak hyperglycaemic activity. Depletion of catecholamines by guanethidine or bethanidine was also shown, in adreno-medullated rats, to produce a similar hypoglycaemic unresponsiveness to that seen with the monoamine oxidase inhibitors. It is logical to conclude that following mebanazine treatment the failure of blood glucose levels to return to normal after insulin is due to replacement of the normal transmitter by one which is inactive in the context of raising blood sugar.

Mebanazine and other monoamine oxidase inhibitors have been found to induce hypotensive effects in man (Pletscher, 1966). Experiments in man have shown equipressor doses of (—)-noradrenaline and (—)-α-methylnoradrenaline to be in the ratio of approximately 1 to 3, noradrenaline being the more potent (Mueller & Horwitz, 1962). Replacement of noradrenaline by its α-methyl analogue has been advanced as the explanation of the hypotensive action of α-methyldopa. Experiments in anaesthetized dogs have shown a 10 to 1 pressor potency ratio for (—)-noradrenaline and (—)-octopamine (Kappe & Armstrong, 1964). In contrast to these observations, Mueller & Horwitz (1962) found α-methylnoradrenaline and noradrenaline to be approximately equipotent in raising the blood sugar level whereas octopamine was virtually inactive both in the hands of Supniewsky (1929) and the present author. In his clinical study of mebanazine, Adnitt (1968b) found evidence of enhanced insulin sensitivity but no effect on pulse rate or blood pressure in response to hypoglycaemia. He considered it unlikely that monoamine oxidase inhibitors selectively antagonized carbohydrate changes without affecting cardiovascular changes but the evidence of the present study would support just such a viewpoint.

Acknowledgements

The author wishes to acknowledge the instruction and practical advice of Dr. B. A. Callingham on the determination of noradrenaline levels, through the courtesy of Prof. A. S. V. Burgen in the Department of Pharmacology at the University of Cambridge, and the expert technical assistance of Maureen Wain and Eoin Ferris.

REFERENCES

ADNITT, P. I. (1968a). *J. Endocr.*, **42**, 417–423.

ADNITT, P. I. (1968b). *Diabetes*, **17**, 628–633.

BARRETT, A. M. (1965). *J. Pharm. Pharmac.*, **17**, 19–27.

BARRETT, A. M. (1966). *Br. J. Pharmac. Chemother.*, **26**, 291.

BARRETT, A. M. (1969). *J. Pharm. Pharmac.*, **21**, 481–487.

COOPER, A. J. (1966). *Int. J. Neuropsychiat.*, **2**, 342–345.

COOPER, A. J. & ASHCROFT, G. (1967). *Diabetes*, **16**, 272–274.

DAVEY, M. J., FARMER, J. B. & REINERT, H. (1963). *Br. J. Pharmac. Chemother.*, **20**, 121–134.

IVERSEN, L. L. (1963). *Ibid.*, **21**, 523–537.

KAKIMOTO, Y. & ARMSTRONG, M. D. (1962). *J. biol. Chem.*, **237**, 422–427.

KAPPE, T. & ARMSTRONG, M. D. (1964). *J. mednl Chem.*, **7**, 569–571.

KOPIN, I. J. (1968). *Ann. Rev. Pharmac.*, **8**, 377–394.

KOPIN, I. J., FISCHER, J. F., MUSACCHIO, J. & HORST, W. V. (1964). *Pharmacologist*, **6**, 175.

KOPIN, I. J., FISCHER, J. F., MUSACCHIO, J., HORST, W. V. & WEISE, V. K. (1965). *J. Pharmac. exp. Ther.*, **147**, 186–193.

KRISMAN, C. R. (1962). *Analyt. Biochem.*, **4**, 17–23.

MUELLER, P. S. & HORWITZ, D. (1962). *J. Lipid Res.*, **3**, 251–255.

PLETSCHER, A. (1966). *Pharmac. Rev.*, **18**, 121–129.

SUPNIEWSKY, J. V. (1929). *C. R. Séanc. soc. Biol.*, **100**, 1147.

WICKSTROM, L. & PETTERSSON, K. (1964). *Lancet*, **2**, 995–997.

ZOR, V., DIKSTEIN, S. & SULMAN, F. G. (1965a). *J. Endocr.*, **32**, 35–43.

ZOR, V., DIKSTEIN, S. & SULMAN, F. G. (1965b). *Ibid.*, **33**, 211–222.

ZOR, V., MISHKINSKY, J. & SULMAN, F. G. (1965). *Biochem. Pharmac.*, **4**, 1059–1064.