Biochemical Pharmacology, 1965, Vol. 14, pp. 1059–1064. Pergamon Press Ltd., Printed in Great Britain.

THE HYPOGLYCAEMIC EFFECT OF MEBANAZINE (ACTOMOL) AND ITS MECHANISM*

U. Zor, J. Mishkinsky and F. G. Sulman

Department of Applied Pharmacology, School of Pharmacy,
Hebrew University, Jerusalem, Israel

(*Received* 19 *February* 1965; *accepted* 11 *March* 1965)

Abstract—The mechanism of the hypoglycaemic effect of the monoamine oxidase inhibitor mebanazine (Actomol) on the blood sugar has been studied in rats. Hypoglycaemia started as late as 4 to 8 hr after single injections of mebanazine, but continued for several days.

Mebanazine does not affect the activity of glucose-6-phosphatase, but slightly intensifies the activity of the phosphorylase system of the liver. It causes hypoglycaemia in adrenalectomized rats, too.

Mebanazine also reduces the blood glucose level in animals which receive simultaneously thyroxin, triiodothyronine or hydrocortisone. Mebanazine when injected simultaneously with adrenalin does not interfere with the hyperglycaemic effect of the latter.

The combined effect of mebanazine and insulin in reducing the blood glucose level is greater than the total sum of the individual effects when the drugs are given separately (augmentative synergism). Thus it becomes obvious that mebanazine potentiates the effect of insulin.

It is well known that some monoamine oxidase inhibitors (MAOI) cause hypoglycaemia in man.[1, 2] Mebanazine, a strong MAOI, also causes a certain degree of hypoglycaemia in man, but only few studies have been published on this subject.[3, 4] Thus, the mechanism of this effect is still unknown. It seemed possible that the effect of mebanazine on blood glucose is one of potentiating insulin, similar to the potentiating effect of MAOI on corticosteroids which manifests itself by raising blood pressure[9] and depressing growth.[10] In the present work we examined the hypothesis whether mebanazine acts on the blood glucose level *per se* or whether its effect is related to that of other hormones which regulate the blood glucose level, i.e. insulin, adrenalin, corticosteroids or thyroid hormones.

MATERIALS AND METHODS

Glucose estimation

Blood sugar was estimated by the glucose oxidase method as modified by Hestrin-Lerner *et al.*[5] The animals were killed by decapitation and 0·1 ml blood was taken for the assay.

Phosphorylase activity was tested as described by Cori *et al.*[6] Glucose-6-phosphatase (G-6-P) activity was assayed according to Harper.[7] Inorganic phosphate was assayed

* Aided by a generous donation from Mrs. Dora Teperman, Canada, in memory of her late husband, Joseph Teperman.

by the method of Fiske and Subbarow.[8] Protein was assayed in the manner described by Lowry *et al.*[12]

Adrenalectomized animals were prepared by the dorsal approach. The animals were kept at 24°, received 1% NaCl and 5% glucose in their drinking water and were used for assay 7 days after the operation.

The following substances were used for injections: mebanazine resinate containing 35% mebanazine base, hydrocortisone acetate, L-tri-iodothyronine sodium and L-thyroxin sodium. These were injected (s.c.) together with mebanazine resinate. Adrenalin bitartrate in ascorbic acid solution as 0·1 mg/ml was injected (s.c.) 23 hr after the injection of mebanazine resinate, and 1 hr before killing the animals. Insulin B.P. was injected (i.p.) 4 hr after the mebanazine resinate and 4 hr before killing the animals.

RESULTS

Figure 1 shows that mebanazine resinate decreases the blood glucose level after 4–8 hr, and that this effect continues for 2 days. The time response depends on the dose of mebanazine resinate.

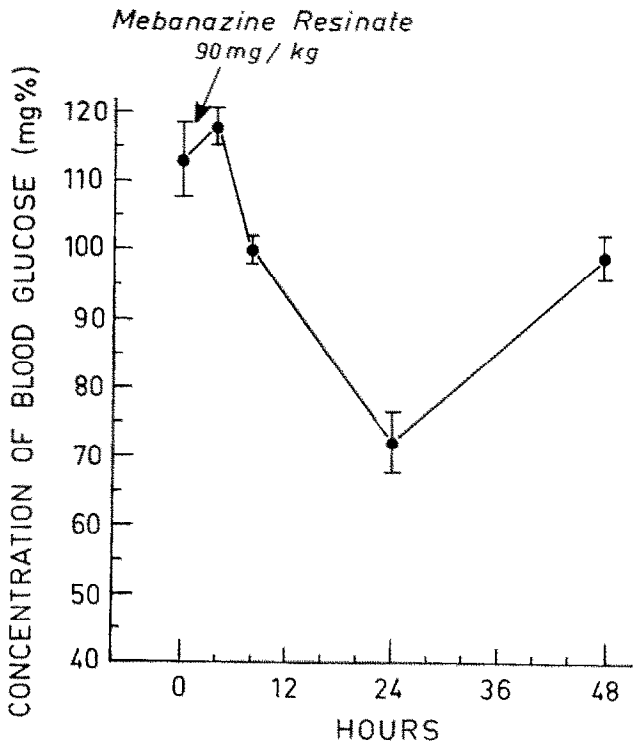

Fig. 1. Effect of mebanazine resinate treatment on blood glucose level of mature female rats.
Treatment: single i.p. injection of 90 mg/kg.
Mean values from one group of 5 rats with standard error of the mean.

Figure 2 demonstrates that the minimal dose of mebanazine resinate which lowered blood glucose after 12 hr was 60 mg/kg.

Table 1 shows that mebanazine resinate does not affect significantly glucose-6-phosphatase activity. There is a slight increase in the activity of phosphorylase.

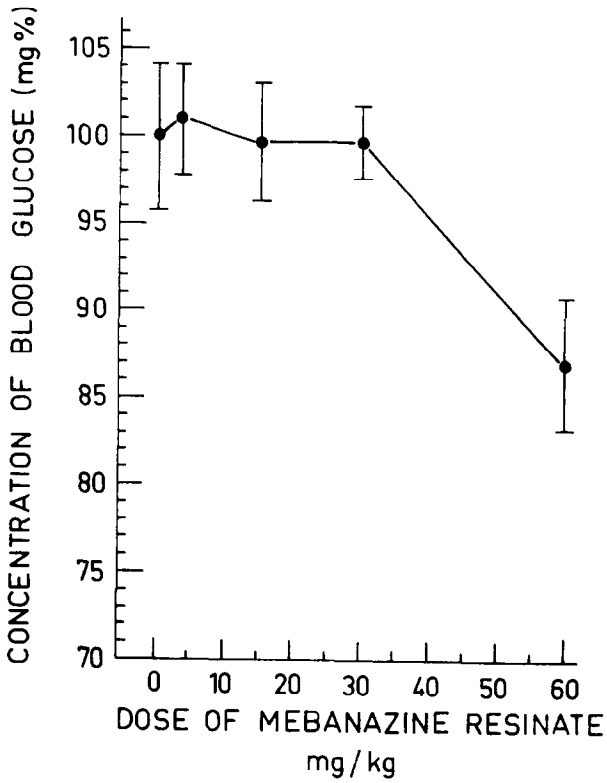

FIG. 2. Effect of different dose levels of mebanazine resinate on blood glucose level of mature female rats.

Treatment: single i.p. injections. The blood was taken 12 hr after the injection.
Mean values from five groups of 4 rats with standard error of the mean.

TABLE 1. EFFECT OF MEBANAZINE RESINATE ON THE ACTIVITY OF PHOSPHORYLASE AND GLUCOSE-6-PHOSPHATASE IN THE LIVER OF MATURE MALE RATS

Treatment	Phosphorylase μ mole H_3PO_4* gm liver/min	Phosphorylase μ mole H_3PO_4* gm protein/min	Glucose-6-phosphatase μ mole H_3PO_4* gm liver/min	Glucose-6-phosphatase μ mole H_3PO_4* gm protein/min
Mebanazine resinate	4·48 ± 0·14	67 ± 3	7·5 ± 0·57	86 ± 7·3
Control	3·5 ± 0·06	54·5 ± 2·1	8·8 ± 0·45	82 ± 7·2

Treatment: Single i.p. injection 90 mg/kg. The animals were killed after 24 hr. Mean values from two groups of 5 rats each, with standard error of the mean.

* Enzymatic activities are expressed as μ mole of H_3PO_4 liberated after splitting glucose-1-phosphate by phosphorylase or glucose-6-phosphate by glucose-6-phosphatase.

Table 2 shows that mebanazine resinate strongly decreases blood glucose in adrenalectomized rats. The fall in blood glucose level started after 4 hours.

Table 3 shows the effect of simultaneous treatment with mebanazine resinate and insulin. With the large dose of insulin (10 μ/kg) mebanazine resinate potentiates the insulin effect of reducing blood glucose level.

TABLE 2. EFFECT OF MEBANAZINE RESINATE ON BLOOD GLUCOSE LEVEL OF BILATERAL ADRENALECTOMIZED FEMALE RATS

Treatment	Blood glucose level in mg % after			
	0 hr	2 hr	4 hr	8 hr
Mebanazine resinate	85 ± 4	77 ± 2·5	70 ± 4·2	45 ± 4·2

Treatment: single i.p. injection 90 mg/kg. Mean values from one group of 5 rats with standard error of the mean.

TABLE 3. EFFECT OF SIMULTANEOUS TREATMENT WITH MEBANAZINE RESINATE AND INSULIN ON BLOOD GLUCOSE LEVEL OF MATURE FEMALE RATS

Treatment	Dose	Blood glucose level mg/%
Control	0·5 ml saline	113 ± 5·8
Mebanazine resinate	90 mg/kg	100 ± 3·9
Insulin B.P.	2 U/kg	90 ± 2·5
Mebanazine resinate + Insulin B.P.	90 mg/kg 2 U/kg	76 ± 7·7
Insulin B.P.	10 U/kg	94 ± 2
Mebanazine resinate + Insulin B.P.	90 mg/kg 10 U/kg	63 ± 5·3

Treatment: single i.p. injection. Mean values from six groups of 5 rats each, with standard error of the mean.

Table 4 shows the hypoglycaemic effect of mebanazine resinate when injected simultaneously with hydrocortisone acetate, adrenalin bitartrate, thyroxin sodium and triiodothyronine sodium. It is seen that mebanazine resinate does not inhibit the hyperglycaemic effect of adrenalin bitartrate, but mebanazine resinate reduces the blood glucose level when injected simultaneously with hydrocortisone acetate, triiodothyronine sodium and thyroxin sodium.

DISCUSSION

Some workers have suggested[1, 3] that the hypoglycaemic effect of MAOI is related to their ability to interfere with the release of catecholamines from their stores, followed by decrease of glycogenolysis and blood glucose level. In the present work we have shown that mebanazine increases the activity of phosphorylase in the liver, thus its overall effect is hypoglycaemic. On the other hand, mebanazine lowered blood glucose in adrenalectomized rats, thus proving that its action is not dependent on adrenalin or

corticosteroids; nor does it interfere with the hyperglycaemic effect of adrenalin. This clearly suggests that the hypoglycaemic effect of mebanazine and other MAOI is not related to the effect of these drugs on the release and hyperglycaemic action of adrenalin.

TABLE 4. EFFECT OF SIMULTANEOUS TREATMENT WITH MEBANAZINE RESINATE AND ADRENALIN BITARTRATE, HYDROCORTISONE ACETATE, TRIIODOTHYRONINE SODIUM AND THYROXIN SODIUM ON BLOOD GLUCOSE LEVEL OF MATURE MALE RATS

Treatment	Dose mg/kg	Blood glucose level in mg %
Control	0·5 ml saline	100 ± 4·2
Mebanazine resinate	90	83 ± 4·3
Adrenalin bitartrate	0·1	111 ± 7
Adrenalin bitartrate	0·1	
+ Mebanazine resinate	90	104 ± 4·5
Adrenalin bitartrate	1	229 ± 3·1
Adrenalin bitartrate	1	
+ Mebanazine resinate	90	200 ± 18
Hydrocortisone acetate	5	96 ± 3
Hydrocortisone acetate	5	
+ Mebanazine resinate	90	82 ± 4
Hydrocortisone acetate	50	105 ± 5
Hydrocortisone acetate	50	
+ Mebanazine resinate	90	80 ± 1·4
Triiodothyronine sodium	0·01	101 ± 4
Triiodothyronine sodium	0·01	
+ Mebanazine resinate	90	78 ± 4·4
Triiodothyronine sodium	0·05	110 ± 3
Triiodothyronine sodium	0·05	
+ Mebanazine resinate	90	81 ± 3·7
Thyroxin sodium	0·1	100 ± 4
Thyroxin sodium	0·1	
+ Mebanazine resinate	90	73 ± 5
Thyroxin sodium	0·5	117 ± 4·3
Thyroxin sodium	0·5	
+ Mebanazine resinate	90	81 ± 5·4

Treatment: single injection. Mean values from 18 groups of 5 rats each, with standard error of the mean.

It is known that MAOI potentiate corticosteroids to elevate blood pressure,[9] and to inhibit growth.[10] Here we have shown that mebanazine also potentiates exogenous insulin to reduce blood glucose. The potentiating effect of mebanazine on insulin is similar to the potentiating effect of the biguanides on insulin. Both require the presence of a certain amount of insulin.

Hydrocortisone and thyroid hormones which contribute to the metabolism of glucose did not inhibit the hypoglycaemic effect of mebanazine. Thus, it seems that the effect of mebanazine on blood glucose is mediated by insulin.

Acknowledgements—The authors gratefully acknowledge the generous supply of Actomol (mebanazine resinate) by I.C.I., Wilmslow, England; Insulin B.P. by RAFA Labs., Jerusalem; hydrocortisone acetate by Organon, Oss, Holland; L-epinephrine bitartrate by Sterling-Winthrop, Rensselaer, N.Y.; Triothyrone (triiodothyronine) by Assia-Zori Pharm. & Chem. Labs., Tel-Aviv; Eltroxin (thyroxin) by Glaxo, Greenford, England.

REFERENCES

1. D. LEAK and T. L. DORMANDY, *Proc. Soc. exp. Biol. Med.*, **108**, 597 (1961).
2. J. WEISS, S. WEISS and B. WEISS, *Ann. N.Y. Acad. Sc.* **80**, 854 (1959).
3. A. J. COOPER and K. M. G. KEDDIE, *Lancet*, **1**, 1133 (1964).
4. H. M. VAN PRAAG and B. LEIJNSE, *Lancet* **2**, 103 (1964).
5. S. HESTRIN-LERNER and S. BEN JONAH, *Proc. Microbiol. & Chem. Assoc. of Israel*, **1**, 27 (1962).
6. G. T. CORI and B. ILLINGWORTH, *Biochem. biophys. Acta*, **21**, 105 (1956).
7. A. E. HARPER, *Methods of Enzymatic Analysis*, Ed. by H. U. BERGMEYER, Academic Press, N.Y. 688 (1963).
8. C. H. FISKE and Y. SUBBAROW, *J. biol. Chem.* **66**, 375 (1925).
9. A. L. SCHERBEL, *Arch. Int. Med.*, **107**, 37 (1961).
10. U. ZOR, S. DIKSTEIN and F. G. SULMAN, *J. Endocrin.*, **32**, 35(1965).
11. W. CREUTZFELDT, H. D. SOELING and Z. ZARDÁY, *Metabolism*, **12**, 264 (1963).
12. O. H. LOWRY, N. Y. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.*, **193**, 265 (1951).