Possible Role of Hydrazine Group in Hypoglycemia Associated with the Use of Certain Monoamine-oxidase Inhibitors (MAOI's)

William Z. Potter, M.S., Daniel S. Zaharko, Ph.D., and Lyle V. Beck, Ph.D., Bloomington, Indiana

SUMMARY

It has been reported that some patients treated chronically with certain monoamine-oxidase inhibitors (MAOI's) exhibit as a complication hypoglycemia, or potentiation of insulin or sulfonylurea induced hypoglycemia. This effect has been attributed to interference by the MAOI with the hyperglycemic role of the sympathoadrenal system in glucose homeostasis, possibly through replacement of endogenous stored catecholamines by impotent "false transmitter." These hypotheses lead to certain expectations, none of which were realized in the present experiments. Instead, evidence was obtained that it is the hydrazine group present in certain MAOI's, rather than their MAOI activity, to which hypoglycemic action should be attributed. Hydrazine and the hydrazine MAOI, phenelzine, induced similar decreases in plasma glucose and insulin levels of overnight fasted rats; phenelzine induced increases in plasma FFA levels. The nonhydrazine MAOI, pargyline, induced marked increases in plasma glucose levels. DIABETES *18*:538-41, August, 1969.

Certain monoamine-oxidase inhibitors (MAOI's) used chronically have been reported to induce potentiation of insulin or sulfonylurea induced hypoglycemia in certain patients.[1-4] Used alone, these MAOI's were found to induce moderate decreases in fasting blood glucose levels.[5,6] Various workers[1,2,7,8] suggest the hypoglycemic effects of MAOI's are secondary to their ability to interfere with the hyperglycemic role of the sympathoadrenal system in glucose homeostasis. Kopin et al.[8] have presented evidence that the hydrazine MAOI, pheniprazine, rapidly induces replacement of stored norepinephrine in rat heart and salivary gland by the almost impotent catecholamine, octopamine, termed by them a "false transmitter." Cooper and Ashcroft[1,2] have therefore proposed that hypoglycemia occurring in association with use of an MAOI might be due to a similar replacement of epinephrine stores in the adrenal medulla by a "false transmitter." No evidence for such replacement was presented. While Cooper and Ashcroft recognized that among the MAOI's "the hydrazines seem to carry the greater risk, potentiating as well as prolonging (insulin-induced) hypoglycemia" they failed to comment on the fact that hydrazine, not an MAOI, is itself a potent hypoglycemic agent[9,10] or to consider the possibility that hydrazine MAOI's might induce and/or potentiate hypoglycemia, not as a result of possessing MAOI activity, but by the same mechanism(s) as hydrazine.

In the present experiments, comparative effects of hydrazine and the hydrazine MAOI, phenelzine, have been estimated on plasma glucose, insulin and free fatty acid (FFA) levels of fasted rats. Rats were fasted eighteen to twenty-four hours to lower liver glycogen and hence permit more rapid appearance of drug-induced hypoglycemia. Plasma insulin and FFA levels were selected for study because both epinephrine and norepinephrine have been reported to inhibit insulin release from the pancreas, to stimulate markedly FFA release from adipose tissue, and to oppose insulin inhibition of the FFA release.[11] Replacement of stored catecholamines by "false transmitters" should therefore lead to an increase, or at least no change in plasma insulin levels, and to a decrease, or at least no change in plasma FFA levels. In a further experiment, effects on plasma glucose levels of equimolar doses of phenelzine and a potent nonhydrazine MAOI, pargyline, were tested; in this case the "false transmitter" hypothesis leads to the expectation that all MAOI's will tend to induce hypoglycemia, while the "hydrazine" hypothesis suggests that hypoglycemia will be induced primarily by hydrazine MAOI's.

MATERIAL AND METHODS

Reagent grade anhydrous hydrazine was obtained from Matheson, Coleman and Bell, phenelzine sulfate

From the Department of Pharmacology, Medical Sciences Program, Indiana University, School of Medicine, Bloomington, Indiana 47401.

(Nardil) from Warner-Lambert Research Institute and pargyline (Eutonyl) from Abbott Laboratories. The 300-400 gm. body weight male rats employed were of the Cox Strain, Sprague-Dawley line, and were obtained from Laboratory Supply Co., Indianapolis, Indiana. Each rat was deprived of food but not water for eighteen to twenty-four hours before being used experimentally. Determinations made in duplicate on each rat plasma sample were as follows:

(a) *Free fatty acid (FFA)*: Fatty acids were extracted within thirty minutes after a given plasma sample was obtained, and the FFA determined colorimetrically as described by Novak[12] in his modification of Dole method.[13]

(b) *Glucose*: This was determined by the glucose oxidase method[14] using 25 μl. plasma aliquots from samples which had been held frozen to time of analyses.

(c) *Insulin*: This was determined by immunoassay as described by Zaharko and Beck[15] using 50 and 100 μl. aliquots of plasma samples held frozen to time of analyses.

EXPERIMENTAL AND RESULTS

In the first set of experiments each rat was anesthetized with 75 mg./kg. of sodium pentobarbital intraperitoneally. Within ten minutes after pentobarbital injection each rat was attached via tracheotomy tube to a respirator and the right carotid artery was cannulated with a heparinized tube for the blood collections. Each blood sample taken had an approximate volume of 0.7 ml. Samples were obtained at five to ten-minute intervals, two to three before and up to seven after the intraperitoneal injection of saline or drug solution. For each blood sample the hematocrit estimation was done immediately. The rest of the sample was held at 0° C. for up to thirty minutes, then centrifuged at 4° C. and the plasma removed. A portion of this was used for the FFA extraction; the rest was held frozen to time of glucose and insulin estimations. Each drug solution was neutralized and made up to a concentration of 0.4 M. in 0.9 per cent NaCl; each injection was made intraperitoneally, 0.005 ml./gm. to give a drug dose per rat of 2 mM./kg. Control rats were injected with 0.9 per cent NaCl, 0.005 ml./gm.

Table 1 data indicate that both hydrazine and phenelzine, 2 mM./kg., induced statistically significant decreases in both plasma glucose and plasma insulin levels of anesthetized rats. Phenelzine alone induced significant *increases* in their plasma FFA levels. The decreases in hematocrit values which occurred as result of the repeated blood withdrawals were not significantly different in the drug treated and control rats, and did not exceed 10 per cent red cells.

In the experiment which gave the data of table 2, the rats were not anesthetized and only two blood collections were made per rat, one at the beginning by tail vein and the other forty-five minutes after saline or drug injection, on sacrifice of the rat by decapitation. Each rat was injected intraperitoneally with 0.2 M. drug solution in 0.9 per cent NaCl or 0.9 per cent NaCl, 0.005 ml./gm., drug dosage zero or 1 mM./kg. As before, the hydrazine MAOI phenelzine induced marked, statistically significant *decreases* in plasma glucose levels; in addition it was noted that most of these rats went into convulsions shortly before the planned time of sacrifice. On the other hand, the nonhydrazine MAOI, pargyline, induced marked, statistically significant *increases* in the plasma glucose levels.

DISCUSSION

Adnitt[16] found that in humans dosage with the

TABLE 1

Effects of phenelzine and hydrazine on plasma glucose, insulin and free fatty acid (FFA) levels in eighteen-hour fasted, phenobarbital anesthetized rats

Drug injected intraperitoneally*	Plasma levels (Mean ± Standard Error)†					
	Glucose (mg./100 ml.)		Insulin (mμg./ml.)		FFA (μM./ml.)	
	Before injection	21-60 min. after injection	Before injection	21-60 min. after injection	Before injection	21-60 min. after injection
None (controls)	84±3.2	91±2.5	1.07±.12	.99±.12	.51±.077	.35±.036
Phenelzine, 2 mM./kg.	93±1.9	51±2.8‡	.70±.12	.42±.08‡	.56±.037	.76±.033‡
Hydrazine, 2mM./kg.	93±2.1	51±3.9‡	1.05±.20	.59±.06‡	.47±.10	.28±.01

*The two drugs were dissolved to 0.4 M. concentration in 0.9 per cent NaCl. Each mouse was injected intraperitoneally with drug solution or 0.9 per cent NaCl, 0.005 ml./gm.

†For the Before Injection Means, n = 8 (four rats, two plasma samples/rat); for the After Injection Means, n = 16 (four rats, four plasma samples/rat).

‡After Injection Mean judged significantly different from Before Injection Mean ($p < .05$).

TABLE 2

Effects of phenelzine and pargyline on plasma glucose levels of twenty-four-hour fasted, nonanesthetized rats

Drug injected intraperitoneally	n†	Plasma glucose levels (mg. per 100 ml.) Means ± Standard Errors: Before the intraperitoneal injection	45′ after intraperitoneal injection	Differences: "Before" vs. "After"	Drug "After" minus saline "After"
Controls	6	69±5.4	103± 6.2	+34± 8.3‡	—
Phenelzine, 1 mM./kg.	4	60±5.0	14± 3.1	−46± 5.9‡	−89± 6.9‡
Pargyline, 1 mM./kg.	5	62±5.5	144±10.2	+82±11.5‡	+41±11.9‡

*Each rat was injected intraperitoneally with 0.9 per cent NaCl or with 0.2 M. drug solution in 0.9 per cent NaCl, 0.005 ml./gm.

†The "forty-five-minute after intraperitoneal injection" blood samples were not obtained for two of the six rats injected with phenelzine because they went into convulsion and died appreciably before the planned sacrifice time.

‡p value for difference $< .01$.

hydrazine MAOI, mebanazine (structurally close to phenelzine) sufficient to induce hypoglycemia or to potentiate insulin induced hypoglycemia, did not lead to decrease in either arterial blood pressure or pulse pressure. He therefore concluded that "interference with the sympathoadrenal response to hypoglycemia is probably not the explanation for increased insulin sensitivity during mebanazine treatment. . . . It has never been demonstrated that monoamine-oxidase inhibition is responsible for the hypoglycemic effects. . . . The hypoglycemic action may result from inhibition of enzyme(s) other than monoamine-oxidase."

Again it is noted that marked hypoglycemic complications have been observed primarily in association with the use of hydrazine MAOI's.[1,2] Present findings support the hypothesis that hypoglycemia inducing and/or potentiating hydrazine MAOI's owe their hypoglycemic effects to whatever mechanisms enable the non-MAOI, hydrazine, to induce hypoglycemia. They fail to support either the general hypothesis that MAOI's have hypoglycemic actions because of a MAOI linked interference with the hyperglycemic role of the sympathoadrenal system, or the more specific hypothesis[1,2] that MAOI's owe such interfering ability to an action to induce replacement of stored catecholamines by "false transmitter(s)." This latter hypothesis implies that: (I) Interference with the sympathoadrenal system should result in decreased "braking" action of catecholamines on insulin release[11] and therefore in hyperinsulinemia; certainly there should be no decrease in plasma insulin levels. Table 1 data indicate, however, that the very similar hypoglycemias induced by the hydrazine MAOI, phenelzine, and the non-MAOI, hydrazine itself, were accompanied by equally similar, significant decreases in plasma insulin levels. (II) The postulated MAOI linked interference with the sympathoadrenal system should result in decreases in plasma FFA levels. Table 1 data, however, indicate that phenelzine had precisely the opposite effect. (III) Action to induce hypoglycemia should be correlated with MAOI activity. However, as shown in table 2, when each of two different MAOI's were administered to fasted unanesthetized rats, at an equimolar dose level of 1 mM./kg., only the hydrazine MAOI, phenelzine, induced hypoglycemia. The potent nonhydrazine MAOI used, pargyline, actually induced marked, statistically significant *increases* in plasma glucose levels.

And finally there is a proposed mechanism for hypoglycemia after hydrazine. The literature indicates that hydrazine inhibits gluconeogenesis[17]; perhaps this inhibition of gluconeogenesis is the result of the inhibition by hydrazine, etc. of glutaminoxaloacetic transaminase, which may play an essential role in gluconeogenesis.[18,19] In animals, such as the present rats, which have developed low liver glycogen levels as a result of prior food deprivation,[20] inhibition of gluconeogenesis may be expected to lead rapidly to hypoglycemia.[17] Further experimentation will be needed to determine whether those hydrazine type MAOI's which under appropriate conditions do induce hypoglycemia share with hydrazine the abilities to inhibit gluconeogenesis and glutamic-oxaloacetic transaminase.

ACKNOWLEDGMENT

Phenelzine sulfate (Nardil) was donated by Warner-Lambert Research Institute, Morris Plains, New Jersey, pargyline by Abbott Laboratories, North Chicago, Illinois, the guinea pig anti-insulin serum by Dr. Peter Wright of our Department, and the bovine unlabeled insulin by Eli Lilly Co., courtesy of Dr. Mary Root. This research was supported by U. S. Public Health Service Grants AM 05980 and 05981.

REFERENCES

[1] Cooper, A. J., and Ashcroft, G.: Modification of insulin and sulfonylurea hypoglycemia by monoamine-oxidase inhibitor drugs. Diabetes *16*:272-74, 1967.

[2] Cooper, A. J., and Ashcroft, G.: Potentiation of insulin hypoglycemia by MAOI antidepressant drugs. Lancet *7434*:407-09, 1966.

[3] Van Praag, H. M., and Leijnse, B.: The influence of some antidepressives of the hydrazine type on the glucose metabolism in depressed patients. Clin. Chim. Acta *8*:466-75.

[4] Van Praag, H. M., and Leijnse, B.: The xylose metabolism in depressed patients and its alterations under the influence of antidepressive hydrazines. Clin. Chim. Acta *11*:13-23, 1965.

[5] Pletscher, A.: Inhibitors of monoamine oxidase and decarboxylase of aromatic amino acids. *In* Handbook Exp. Pharm. XIX, O. Eichller and A. Farah (Eds.), Heidelberg, Springer-Verlag, 1966, pp. 593-646.

[6] Weiss, J., Weiss S., and Weiss, B.: Effects of iproniazid and similar compounds on the gastrointestinal tract. Ann. N. Y. Acad. Sci. *80*:854-59, 1959.

[7] Creveling, C. R., Daly, J. W., Witkop, B., and Udenfriend, S.: Substrates and inhibitors of dopamine-B-oxidase. Biochim. Biophys. Acta *64*: 125-34, 1962.

[8] Kopin, I. J., Fischer, J. E., Musacchia, J. M., Horst, W. D., and Weise, V. K.: False neurochemical transmitters and the mechanism of sympathetic blockade by monoamine oxidase inhibitors. J. Pharmacol. Exp. Ther. *147*:186, 1965.

[9] Barrett, A. M.: Modification of the hypoglycemia response to tolbutamide and insulin by mebanazine—an inhibitor of monoamine oxidase. J. Pharm. Pharmacol. *17*:19, 1965.

[10] Underhill, F. P.: The influence of hydrazine upon the organism with special reference to the blood sugar content. J. Biol. Chem. *10*:159, *1911*.

[11] Williams, R. H.: Secretion, fates and actions of insulin and related products. Diabetes *15*:623, *1966*.

[12] Novak, M.: Colorimetric ultramicro method for the determination of free fatty acids. J. Lipid Res. *6*:431-33, *1965*.

[13] Dole, V. P.: A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. Clin. Invest. *35*:150, 1956.

[14] Bergmeyer, H., and Bernt, E.: Enzymatic Analysis. New York, Academic Press, 1963, p. 123.

[15] Zaharko, D. S., and Beck, L. V.: Studies of a simplified plasma insulin immunoassay using cellulose powder. Diabetes *17*:444-57, 1968.

[16] Adnitt, P. I.: Hypoglycemic action of monoamine oxidase inhibitors (MAOI's). Diabetes *17*:628, 1968.

[17] Fortney, S. R., Clark, D. A., and Stern, E.: Inhibition of gluconeogenesis by hydrazine administration in rats. J. Pharmacol. Exp. Ther. *156*:277, 1967.

[18] Fortney, S. R.: Effect of hydrazine on carbohydrate metabolism in vivo and in vitro. Aerospace Med. *38*:727-31, 1967.

[19] Lardy, H. A., Paetkau, V., and Walter, P.: Paths of carbon in gluconeogenesis and lipogenesis: The role of mitochondria in supplying precursors of phosphoenolypruvate. Proc. Nat. Acad. Sci. USA *53*:1410-15, 1965.

[20] Amenta, J. S., and Johnston, E. H.: Hydrazine-induced alterations in rat liver. Lab. Invest. *11*:956, 1962.

Capillary Hemorrhage in Scorbutic Guinea Pigs

(*Continued from page 522*)

and lost weight during the study. In addition, the animals became quite lethargic and had a loss of hair. Capillary lesions and/or purpura are two types of lesions reported by the authors to be associated with the deficiency. The most frequent lesion consisted of endothelial junctional separation. This lesion was most severe after the third and fifth weeks of deficiency. Another lesion observed was cellular disruption.

There was a marked depletion of underlying collagen and a disappearance of basement membrane in the junctional separation. Erythrocytes, and sometimes tracer particles, were observed in the separations or in the cellular material around them.

In general, capillary lesions were somewhat localized at the site of the hemorrhages, with erythrocytes and colloidal particles concentrated in the same area. The electron microscope studies suggested that the gaps observed in both the endothelium and the underlying basement membrane are directly related to capillary hemorrhage. Colloidal markers passed the endothelium but were observed to accumulate along the basement membrane. The authors suggest that changes in the basement membrane may occur as a result of the action of leukocytic products.

While the number of animals observed over the five-week period was small, there appeared to be a progression in the degree of changes, with increasing loss of the basement membrane and more breakdowns of the cell and extravasation of blood constituents. In addition, endothelial cells were swollen in the scorbutic animals.

It is unfortunate that so few of the animals were on the diet for four or five weeks. The changes that seem to be most closely related to capillary hemorrhages became more evident after the third week, at which time only a small number of animals were available. With these limited data, it does appear that a partial answer can be given to the question of how erythrocytes accumulate in certain tissues in guinea pigs deficient in ascorbic acid.

From *Nutrition Reviews*, Vol. 27, No. 5, May 1969, pp. 158-59.