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Lipomobilization effect of nonhydrazine type of monoamine oxidase inhibitors

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MONOAMINE oxidase (MAO) has the important function of degrading various biologically occurring monoamines such as epinephrine, norepinephrine, tyramine or 5-hydroxytryptamine. The administration of MAO inhibitors leads to the accumulation of these biological amines in different types of tissue.[1–4]

Some changes in glucose metabolism have been demonstrated after the administration of MAO inhibitors; these include a delayed and long-lasting hypoglycemia in animals,[5–8] as well as in humans,[9,10] and increased glucose uptake by peripheral tissues.[9–11] Relatively little is known about the effects of MAO inhibitors on lipid metabolism; however an elevation of plasma free-fatty acids and increased lipolytic rate of adipose tissue of rats treated with MAO inhibitors have been demonstrated.[12]

The drugs employed in all the above-mentioned experiments were primarily of the hydrazine type of MAO inhibitors. However, hydrazine itself has been shown to cause hypoglycemia and mobilization of lipid metabolism.[13–15] Therefore, metabolic changes observed after administration of MAO inhibitors might be due to their hydrazine moiety. The purpose of the present study was to investigate effects of non-hydrazine MAO inhibitors on carbohydrate and lipid metabolism *in vivo* and *in vitro*.

METHODS

Male Sherman rats weighing between 250–350 g were used. Before and during the experiments they were maintained on a commercial rat diet and water *ad libitum*. Two nonhydrazine types of MAO inhibitors were used: pargyline hydrochloride in doses of 60 or 90 mg/kg and tranylcypromine sulfate in doses of 20 or 40 mg/kg.

The MAO inhibitors were injected i.p. and rats were decapitated 1, 2 or 3 hr later. Blood was collected in cooled heparinized tubes. After centrifugation at 5000 rpm for 10 min at 4°, plasma was separated from blood cells and the concentrations of glucose, lactate, free-fatty acids (FFA) and glycerol were measured.

Another group of rats was reserpinized by injecting 2 mg/kg of reserpine intramuscularly daily for 3 days. Pargyline (90 mg/kg) or tranylcypromine (20 mg/kg) was given 1 hr after the last injection of reserpine. The rats were sacrificed 1, 2 or 3 hr after the administration of MAO inhibitors.

In propranolol-treated rats, the beta adrenergic inhibitor was injected i.p. 15 min before the administration of MAO inhibitors. The dose of propranolol was 2 mg/kg in pargyline and 4 mg/kg in tranylcypromine-treated rats.

Another group of rats was anesthetized i.p. with a mixture of sodium pentobarbital (50 mg/kg) and hexamethonium chloride (4 mg/kg). Half an hour later these rats were given pargyline and tranylcypromine in the same doses as in the reserpine or propranolol-treated rats, and were sacrificed 1 or 2 hr afterwards. The MAO inhibitors were replaced by saline in control animals of all different groups.

For studies *in vitro* epididymal fat pads were quickly removed from anesthetized male Sherman rats and 100-mg samples of tissue were weighed and transferred to test tubes containing 2 ml of Krebs–Ringer phosphate buffer with 5% bovine albumin fraction V and one-half of the recommended amount of calcium.[16] Glycerol concentrations in the media were determined at the end of 2-hr incubation. Pargyline in 10^{-2} to 10^{-5}M concentrations and tranylcypromine in 10^{-3} to 10^{-5}M concentrations were used.

Plasma glucose was estimated by the enzymatic oxidase method.[17] Plasma lactate was estimated enzymatically by lactate dehydrogenase. Plasma glycerol was measured enzymatically by using lactate dehydrogenase, pyruvate kinase and glycero kinase. The colorimetric method of Dalton and Kowalski[18] using a Technicon auto analyzer was used for the determination of plasma FFA. Enzymes were obtained from Boehringer Mannheim Corp.

All results were statistically evaluated by the Student *t*-test.

TABLE 1. EFFECTS OF PARGYLINE ON PLASMA GLUCOSE, LACTATE, FFA AND GLYCEROL CONCENTRATIONS IN RATS*

Time after injection:	60 min			120 min			180 min		
	C	Pa	Pb	C	Pa	Pb	C	Pa	Pb
Plasma glucose (mg %)	135·18 ±2·05 (10)	134·84 ±2·41 (18) NS	154·01 ±7·11 (8) P<0·025	131·01 ±1·99 (10)	136·23 ±2.15 (14) NS	139·07 ±3·58 (10) NS	135·94 ±1·38 (9)	139·88 ±3·47 (10) NS	130·38 ±4·87 (6) NS
Plasma lactate (mg %)	18·30 ±0·63 (10)	17·23 ±0·91 (17) NS	20·93 ±1·67 (7) NS	20·03 ±0·99 (10)	17·27 ±0·91 (14) NS	22·36 ±1·88 (10) NS	19·55 ±1·28 (10)	17·80 ±2·19 (10) NS	22·03 ±1·17 (8) NS
Plasma FFA (m-equiv./l.)	0·308 ±0·021 (10)	0·376 ±0·046 (17) NS	0·423 ±0·037 (8) P<0·02	0·318 ±0·025 (10)	0·423 ±0·025 (14) P<0·01	0·673 ±0·053 (8) P<0·001	0·291 ±0·025 (10)	0·413 ±0·035 (12) P<0·01	0·825 ±0·060 (7) P<0·001
Plasma glycerol (μmoles/l.)	57·59 ±6·31 (10)	74·52 ±5·96 (17) NS	139·37 ±7·54 (8) P<0·001	55·10 ±7·06 (10)	83·78 ±7·60 (14) P<0·02	160·11 ±8·36 (9) P<0·0001	50·42 ±6·86 (10)	72·49 ±5·94 (12) P<0·025	188·08 ±8·90 (6) P<0·001

* Values are averages ± S.E. The figure in parenthesis represents the number of experiments. C = control. Pa = 60 mg/kg of pargyline. Pb = 90 mg/kg of pargyline. NS = not significant.

TABLE 2. EFFECTS OF TRANYLCYPROMINE ON PLASMA GLUCOSE, LACTATE, FFA AND GLYCEROL CONCENTRATIONS IN RATS*

Time after injection:	60 min			120 min			180 min		
	C	Ta	Tb	C	Ta	Tb	C	Ta	Tb
Plasma glucose (mg %)	139·73 ± 2·59 (8)	125·76 ± 3·46 (8) P < 0·01	131·03 ± 4·87 (7) NS	137·34 ± 3·23 (7)	150·31 ± 5·32 (8) NS	133·59 ± 5·36 (6) NS	136·11 ± 1·50 (6)	155·01 ± 8·83 (5) NS	130·61 ± 5·74 (6) NS
Plasma lactate (mg %)	18·03 ± 0·60 (8)	28·89 ± 4·48 (8) P < 0·05	63·46 ± 7·55 (7) P < 0·001	21·32 ± 1·14 (7)	36·41 ± 5·76 (7) P < 0·025	61·11 ± 6·97 (6) P < 0·001	20·22 ± 1·45 (6)	48·87 ± 3·64 (5) P < 0·001	68·47 ± 2·44 (6) P < 0·001
Plasma FFA (m-equiv./l.)	0·382 ± 0·24 (8)	0·842 ± 0·064 (7) P < 0·001	0·653 ± 0·030 (7) P < 0·001	0·432 ± 0·026 (7)	0·726 ± 0·079 (8) P < 0·005	0·686 ± 0·030 (6) P < 0·001	0·328 ± 0·028 (6)	0·728 ± 0·083 (5) P < 0·005	0·673 ± 0·052 (6) P < 0·001
Plasma glycerol (μmoles/l.)	78·81 ± 7·01 (8)	238·03 ± 17·11 (8) P < 0·001	466·02 ± 38·11 (7) P < 0·001	88·15 ± 4·93 (7)	208·54 ± 16·07 (7) P < 0·001	419·48 ± 16·15 (6) P < 0·001	84·71 ± 7·46 (6)	201·18 ± 16.26 (5) P < 0·001	319·76 ± 36·56 (6) P < 0·001

* Values are averages ± S.E. The figure in parenthesis represents the number of experiments. C = control. Ta = 20 mg/kg of tranylcypromine. Tb = 40 mg/kg of tranylcypromine. NS = not significant.

TABLE 3. EFFECTS OF PARGYLINE AND TRANYLCYPROMINE ON PLASMA GLUCOSE, FFA AND GLYCEROL CONCENTRATIONS IN RESERPINIZED RATS*

Time after injection:	60 min			120 min			180 min		
	C	P	T	C	P	T	C	P	T
Plasma glucose (mg %)	158·00 ±8·21 (9)	199·90 ±35·50 (5) NS	150·54 ±6·42 (10) NS	143·09 ±7·82 (9)	158·17 ±13·23 (5) NS	132·58 ±2·75 (11) NS	143·23 ±9·64 (4)	166·29 ±12·58 (4) NS	
Plasma FFA (m-equiv./l.)	0·640 ±0·090 (9)	0·666 ±0·067 (5) NS	0·361 ±0·052 (10 P<0·02	0·649 ±0·050 (9)	0·773 ±0·094 (5) NS	0·526 +0·037 (11) NS	0·746 ±0·082 (4)	0·706 ±0·090 (4) NS	
Plasma glycerol (μmoles/l.)	132·81 ±7·56 (9)	130·60 ±12·91 (5) NS	195·25 ±12·77 (9) P<0·001	142·42 ±13·04 (9)	132·74 ±11·21 (5) NS	169·78 ±8·22 (11) NS	129·55 ±19·41 (4)	116·35 ±13·71 (4) NS	

* Values are averages ± S.E. The figure in parenthesis represents the number of experiments. C = control. P = 90 mg/kg of pargyline. T = 20 mg/kg of tranylcypromine. NS = not significant.

RESULTS AND DISCUSSION

In the present experiments the most remarkable changes were observed in plasma FFA and glycerol concentrations of rats after the administration of both nonhydrazine types of MAO inhibitors. Injection of pargyline (Table 1) resulted in a gradual increase in plasma FFA and glycerol during the 3 hr of the experimental period. Larger doses of the drug caused greater changes. Administration of tranylcypromine (Table 2) caused the highest increase in plasma FFA and glycerol in the first hour followed by a slow decline. A proportional increase in plasma glycerol but not in plasma FFA was observed when the dose of tranylcypromine was doubled. On the other hand, our present studies *in vitro* indicated that both pargyline and tranylcypromine lacked any direct stimulating effect on lipolytic rate of adipose tissue removed from the normal rats. The increase of plasma FFA and glycerol *in vivo* after the administration of pargyline or tranylcypromine must therefore have been mediated by mechanisms other than the direct lipolytic effect of the drugs. The possible role of endogenous catecholamines was investigated for two reasons: First, catecholamines were demonstrated to accumulate in different organs after the administration of MAO inhibitors[1-4] and second, catecholamines are recognized as strong lipokinetic agents.

Reserpine is known to cause alterations of catecholamine metabolism. In the cat, a single injection of reserpine completely depleted various organs of their catecholamine stores.[19] A dose of reserpine similar to the one used in our experiments temporarily depleted catecholamine stores in rat adrenals.[20] A depletion of amines from other organs, as adipose tissue, could also be expected. Under these circumstances pargyline failed to increase plasma FFA and glycerol in our reserpinized animals. Tranylcypromine produced a transient decrease of plasma FFA and a simultaneous increase of plasma glycerol in the first hour, with no change later in the experiment (Table 3).

TABLE 4. EFFECTS OF PARGYLINE ON PLASMA GLUCOSE, FFA AND GLYCEROL CONCENTRATION IN PROPRANOLOL PRETREATED RATS*

Time after injection:	60 min		120 min		180 min	
	C	P	C	P	C	P
Plasma glucose (mg %)	134·49 ± 3·28 (8)	144·61 ± 4·62 (8) NS	135·32 ± 3·26 (7)	141·11 ± 6·33 (9) NS	132·81 ± 2·33 (7)	129·00 ± 10·38 (7) NS
Plasma FFA (m-equiv./l.)	0·292 ± 0·018 (7)	0·378 ± 0·037 (7) P=0·05	0·302 ± 0·034 (7)	0·485 ± 0·053 (8) P<0·01	0·315 ± 0·030 (7)	0·620 ± 0·073 (7) P<0·005
Plasma glycerol (μmoles/l.)	68·57 ± 9·24 (8)	113·28 ± 9·11 (8) P<0·005	76.24 ± 3·99 (7)	110·54 ± 10·38 (9) P<0·01	68·68 ± 9·79 (7)	137·01 ± 4·49 (7) P<0·001

* Values are averages ± S.E. The figure in parenthesis represents the number of experiments. C = control. P = 90 mg/kg of pargyline. NS = not significant.

Experimental data suggest that catecholamines stimulate lipomobilization by acting on adrenergic beta receptors in adipose tissue.[21-24] Propranolol, a pure beta adrenergic blocking agent, is able to block the lipolytic action of epinephrine in adipose tissue *in vitro*.[25] The rise in plasma FFA after administration of catecholamines was inhibited by administration of propranolol in anesthetized dogs and cats.[26] In our experiments (Table 4) an elevation of plasma FFA and glycerol after administration of pargyline was observed in both propranolol-treated and untreated rats. However, the elevations were significantly smaller in the propranolol-treated animals. Similarly, the elevation in plasma glycerol, but not FFA, was significantly smaller after administration of tranylcypromine in

animals treated with propranolol. In unanesthetized rabbits propranolol inhibited epinephrine-induced rise in plasma glycerol more than the rise of FFA.[27] The suggestion that propranolol itself promoted differential lipase activation in adipose tissue[27] may explain why propranolol diminished the rise of plasma glycerol but not the rise of FFA in our tranylcypromine treated-rats.

The role of adrenergic activity in FFA mobilization following fear, anger, trauma, catecholamine administration or stimulation of sympathetic nerves was clearly established in previous studies.[28–30] When adrenergic activity and the release of endogenous catecholamines were diminished by pentobarbital and hexamethonium in our experiments (Table 5), the rise of both plasma FFA and glycerol was abolished in pargyline- or tranylcypromine-treated animals.

TABLE 5. EFFECTS OF PARGYLINE AND TRANYLCYPROMINE ON PLASMA GLUCOSE, LACTATE, FFA AND GLYCEROL CONCENTRATION IN ANESTHETIZED RATS*

Time after injection:	60 min			120 min		
	C	P	T	C	P	T
Plasma glucose (mg %)	118·51 ±2·83 (6)	103·41 ±2·12 (5) P<0·005	110·27 ±3·30 (7) NS	145·55 ±3·04 (8)	127·67 ±4·32 (5) P<0·01	111·07 ±3·46 (6) P<0·001
Plasma lactate (mg %)	7·26 ±0·35 (6)	6·90 ±0·72 (5) NS	7·27 ±0·67 (7) NS	11·97 ±0·81 (8)	5·59 ±0·48 (5) P<0·001	5·88 ±0·30 (6) P<0·001
Plasma FFA (m-equiv./l.)	0·510 ±0·040 (6)	0·666 ±0·064 (5) NS	0·585 ±0·028 (7) NS	0·596 ±0·050 (8)	0·654 ±0·042 (5) NS	0·632 ±0·040 (7) NS
Plasma glycerol (μmoles/l.)	101·66 ±8·28 (6)	104·86 ±7·08 (5) NS	113·00 ±5·53 (7) NS	109·61 ±5·17 (8)	106·06 ±7·54 (5) NS	109·93 ±10·55 (7) NS

* Values are average ± S.E. The figures in parenthesis represent the number of experiments. C = control. P = 90 mg/kg of pargyline. T = 20 mg/kg of tranylcypromine. NS = not significant

Different responses of plasma glucose and lactate were obtained in our studies. After the administration of 20 mg/kg of tranylcypromine, a small decrease of plasma glucose was observed and no change occurred when the dose was doubled. Pargyline caused an increase in the plasma glucose level. only when the dose of 90 mg/kg was used.

Elevation of plasma insulin and decrease in plasma glucose after the tranylcypromine injection were demonstrated in fasted mice.[31] Our studies confirm the findings of a decrease in blood glucose after the administration of tranylcypromine. The mechanism for pargyline-induced elevation of blood glucose in our studies is unclear, but may be related to the fact that, unlike tranylcypromine, pargyline was reported not to increase plasma insulin.[31]

Plasma-lactate concentration increased gradually during the 3-hr period after the administration of tranylcypromine. A higher dose of drug resulted in a higher response in lactate concentration. Pargyline in both doses caused no change in lactate concentration.

Increase in blood lactate and pyruvate after the administration of tranylcypromine, as well as harmaline or iproniazid in rats, was attributed to an enhanced glycolysis of peripheral tissues.[32] An inhibition in the conversion of lactate to glucose was demonstrated in tranylcypromine-treated mice.[31] It seems likely therefore that both factors contributed to the elevated blood lactate in our tranylcypromine-treated animals.

On the basis of our findings, and those of others,[7,12,32] we conclude that metabolic effects observed after administration of MAO inhibitors are not due to their hydrazine moiety. The lipolytic response due to pargyline or tranylcypromine seems to be mediated by endogenous catecholamines. However, the involvement of other factors in the mechanism of action of these MAO inhibitors on glucose and lipid metabolism requires further studies.

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