Metabolic Effects of Nitroglycerin and Tranylcypromine in Unanesthetized Rabbits[1] (35248)

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Monoamine oxidase (MAO) inhibitors are compounds of different chemical structure which prevent the intracellular degradation of catecholamines and 5-hydroxytryptamine, and thus produce an accumulation of these amines in tissues such as brain (1), sympathetic ganglia (2), heart (3), and adipose tissue (4).

It is known that catecholamines regulate the rate of lipolysis and glycolysis in tissues through specific receptors. The increased availability of these amines for receptor sites following MAO inhibition might result in higher rates of these metabolic pathways. Increased glycolysis in rats treated with different types of MAO inhibitors was described by Gey and Pletscher (5). Recently, Kypson *et al.* (6) demonstrated higher lipolytic activity in rats injected with pargyline and tranylcypromine, both MAO inhibitors.

The possibility that the vasodilators of the nitrite type belong to the class of MAO inhibitors was suggested by Ogawa *et al.* (7), who observed inhibition of MAO activity in heart, liver, and brain of nitroglycerin-treated rats. The purpose of the present study was to compare the metabolic alterations caused by nitroglycerine with those of tranylcypromine in rabbits. Propranolol, an inhibitor of beta receptors and reserpine, a catecholamine depleting agent, were used to determine whether the effects of nitroglycerin or tranylcypromine were mediated by catecholamines.

Methods. Unanesthetized albino rabbits of both sexes, weighting 2.0–3.5 kg and starved for 16 hr before the experiment, were used. They were restrained in their supine position on an insulated pad in a noise-free room at 25°. Under sterile conditions, 5 ml of xylocaine hydrochloride (2%) was injected subcutaneously in a hindlimb, the femoral artery was isolated and a catheter was inserted. In rabbits infused with propranolol, a second catheter was placed in the isolated femoral vein of the opposite hindlimb, using similar local and sterile conditions. All incisions were covered with xylocaine-soaked gauze. Temperature was recorded with a Telethermometer. The animals were left undisturbed for at least 30 min before withdrawing the first sample of blood. Blood samples of 4 ml each were obtained from the femoral artery at regular intervals and were placed in ice-cold heparinized test tubes. After centrifugation of the blood samples at 4°, plasma was separated immediately from erythrocytes and kept frozen until it was used for determination of glucose, lactate, pyruvate, free fatty acids, and glycerol. The erythrocytes were preserved, mixed with a similar volume of dextran, and then returned to the same animal via the catheter.

One half hr after the first sample of blood was obtained, nitroglycerin (USP) dissolved in saline was injected ip to two groups of rabbits in doses of 40 and 100 μg/kg, respectively. Tranylcypromine in a dose of 30 mg/kg dissolved in saline was administered ip in another group of rabbits. Rabbits injected with saline served as controls. Reserpinized rabbits were prepared by the iv administration of reserpine (5 mg/kg) 16 hr before the experiment. In propranolol-treated rabbits the drug (3 mg/kg) was infused in the femoral vein for a 30-min period. The infusion started immediately after ip injection of nitroglycerin or saline. Each group consisted of 7 rabbits except the propranolol

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groups, which consisted of 6 rabbits each.

Specific methods of measurements of plasma glucose, lactate, and glycerol have been described in previous publication (6). Plasma pyruvate was measured enzymatically using lactate dehydrogenase. Plasma free fatty acids (FFA) were determined according to the method of Dalton and Kowalski (8).

All results were evaluated statistically, and significance of the differences was determined by Student's t test.

Results. 1. The effect of nitroglycerin on plasma glucose, lactate, pyruvate, FFA, and glycerol in rabbits (Fig. 1). The administration of 40 μg/kg of nitroglycerin caused an increase in plasma lactate to 22.04 $\pm$ 3.2 from 10.37 $\pm$ 1.6 mg/100 ml in control animals ($p<0.02$). Plasma FFA was elevated to 836 $\pm$ 103 from 484 $\pm$ 91 μeq/liter in controls ($p<0.05$) 90 min after the injection of the drug. At the same time a similar increase in plasma glycerol from 81.3 $\pm$ 6 to 108 $\pm$ 8.3 μmoles/liter ($p<0.05$) was observed after the administration of nitroglycerin.

The higher dose of nitroglycerin (100 μg/kg) elevated plasma lactate significantly from 10.37 $\pm$ 1.6 to 19.09 $\pm$ 2.5 mg/100 ml ($p<0.02$) 30 min after injection, and increased to maximal level of 33.6 $\pm$ 7.9 mg/100 ml as compared to control value of 12.09 $\pm$ 1.8 mg/100 ml ($p<0.025$) 90 min after its administration. Plasma pyruvate levels increased immediately and reached a maximal value of 1.62 $\pm$ 0.1 mg/100 ml as compared to 0.69 $\pm$ 0.1 mg/100 ml in controls ($p<0.005$) 2.5 hr after administration of the drug. Plasma FFA increased from control values of 448.2 $\pm$ 42 and 484 $\pm$ 91 μeq/liter to 851.5 $\pm$ 146 and 1002 $\pm$ 101 μeq/liter, 30 and 90 min after nitroglycerin administration ($p<0.05$; $p<0.005$). Thirty and 90 min after nitroglycerin injection, plasma glycerol was elevated to 138.4 $\pm$ 9 and 130 $\pm$ 12 μmoles/liter as compared to control values of 90.6 $\pm$ 10 and 81.3 $\pm$ 6 μmoles/liter ($p<0.005$; $p<0.001$). No change in plasma glucose level occurred in any group.

2. The effect of nitroglycerin on plasma lactate, FFA, and glycerol in reserpinized and propranolol-treated rabbits. In reserpinized animals, plasma lactate gradually increased, and the maximal elevation from 11.75 $\pm$ 0.9 to 32.3 $\pm$ 4.4 mg/100 ml ($p<0.005$) was observed 2.5 hr after the injection of nitroglycerin (Fig. 2). However, no change in plasma FFA and glycerol was observed in nitroglycerin-treated animals.

Plasma lactate gradually increased from 14.69 $\pm$ 1.1 and 15.3 $\pm$ 2.4 mg/100 ml in

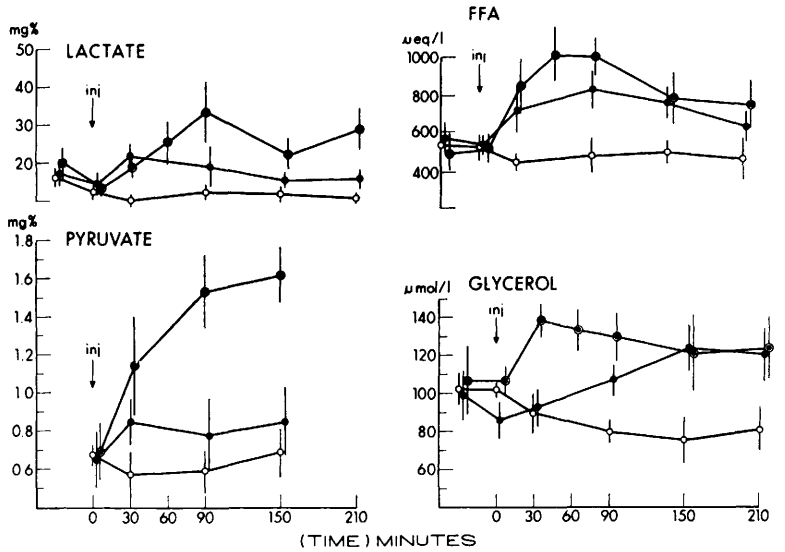

FIG. 1. Effect of 40 and 100 μg/kg of nitroglycerin on plasma lactate, pyruvate, FFA, and glycerol in rabbits. The drug was injected at 0 time. Each point is a mean value $\pm$ SEM; control (○); 40 (●); and 100 (⊙) μg/kg of nitroglycerin.

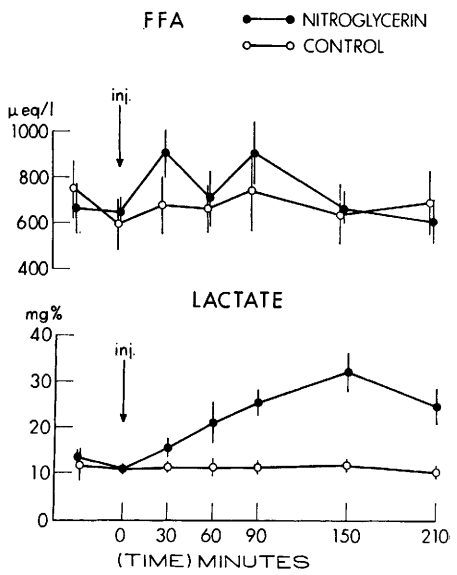

FIG. 2. Effects of nitroglycerin (100 μg/kg) on plasma FFA and lactate in reserpinized rabbits. The drug was injected at 0 time. Each point is a mean value ± SEM.

controls to 18.55 ± 0.8 and 30.99 ± 2.9 mg/100 ml ($p<0.05$; $p<0.001$) 90 min and 3.5 hr, respectively, after administration of nitroglycerin (100 μg/kg) in propranolol-treated rabbits. Plasma FFA and glycerol concentrations were unchanged during the entire experimental period (Fig. 3).

3. The effect of tranylcypromine on plasma glucose, lactate, pyruvate, FFA, and glycerol in rabbits. The administration of tranylcypromine caused no significant change in plasma glucose concentration during the entire experimental period. Plasma lactate gradually increased from control values of 10.37 ± 1.6 to 34.27 ± 5.1 mg/100 ml ($p<0.005$) at 30 min, and from 12.09 ± 1.8 to a maximal elevation of 52.56 ± 11.6 mg/100 ml ($p<0.02$) at 2.5 hr after injection of the drug. Plasma pyruvate significantly increased from control values of 0.56 ± 0.1 to 1.35 ± 0.18 mg/100 ml ($p<0.02$) 30 min, and from 0.69 ± 0.1 mg/100 ml to a maximal value of 1.74 ± 0.13 mg/100 ml ($p<0.005$) at 2.5 hr after injection of the drug. Plasma FFA increased to a maximal value of 1052 ± 169 μeq/liter in the treated as compared to 448 ± 42 μeq/liter ($p<0.005$) in the control animals 30 min after the injection. Plasma glycerol increased from 77.4 ± 11 μmoles/liter in the control to 150.3 ± 19 μmoles/liter ($p<0.01$) 2.5 hr after injection of tranylcypromine.

4. The effect of tranylcypromine on plasma lactate, FFA, and glycerol in reserpinized rabbits. Plasma lactate increased from 11.1 ± 1 to 19.56 ± 2.9 mg/100 ml ($p<0.025$) 30 min after the injection of the drug. The increase in plasma lactate after the administration of tranylcypromine in this group was significantly lower than that observed in the nonreserpinized rabbits. Plasma FFA and glycerol remained unchanged during the entire experimental period.

5. The effect of nitroglycerin and tranylcypromine on body temperature in normal, reserpine- and propranolol-treated rabbits. The average body temperature in control animals was 39.1 ± 0.2°. Both nitroglycerin and tranylcypromine gradually increased body temperature during the experimental period. Maximal elevation of body temperature was 0.74 ± 0.2° ($p<0.005$) for nitroglycerin (100 μg/kg) and 0.85 ± 0.2° ($p<0.005$) for tranylcypromine.

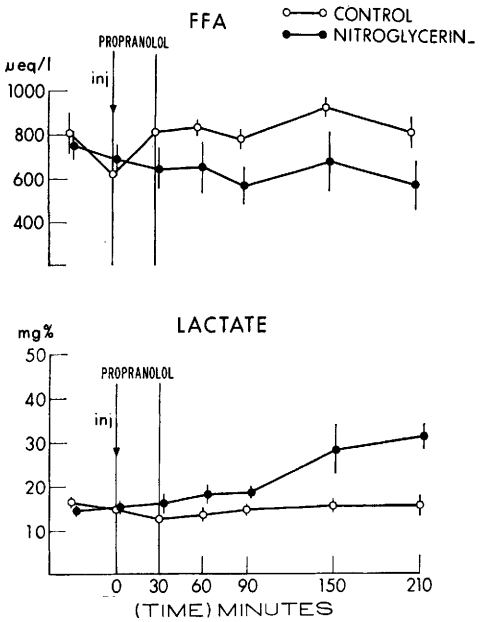

FIG. 3. Effects of nitroglycerin (100 μg/kg) on plasma FFA and lactate in propranolol-treated rabbits. The drug was injected at 0 time. Each point is a mean value ± SEM.

The initial body temperature of the reserpinized animals was 35.54 ± 0.9°. At the end of experiment the temperature declined in all three groups: —1.44 ± 0.1° in the control, —0.70 ± 0.3° in the nitroglycerin and —0.58 ± 0.38° in the tranylcypromine-treated animals.

The average body temperature in propranolol-treated animals was 38.3 ± 0.2° and remained unchanged both in control and nitroglycerin groups.

Discussion. The present study describes metabolic changes in unanesthetized rabbits after ip administration of nitroglycerin and tranylcypromine. Sudden and marked elevations of plama FFA and glycerol were observed in rabbits treated with both drugs. Higher levels of plasma FFA and glycerol occurred when the dose of nitroglycerin was increased. It is recognized that the level of FFA in plasma is controlled mainly by two mechanisms: the release of FFA from lipid depots and removal of FFA from the blood stream. No turnover studies were performed in our experiments to distinguish between these mechanisms. However, from the studies of Steinberg (9), it is clear that increased levels of plasma FFA are usually associated with their accelerated turnover. Furthermore, in view of the simultaneous sudden elevations in both plasma FFA and glycerol, it is most likely that the changes observed in our study were due to increased lipolysis. The possibility that both these drugs could stimulate lipolysis directly was excluded by our *in vitro* studies with isolated adipose tissue (unpublished data).

The possible role of endogenous catecholamines as a mediator in increasing lipolysis was considered for the following two reasons: (i) Administration of exogenous catecholamines *in vivo* is known to cause metabolic effects similar to those observed in our nitroglycerin- or tranylcypromine-treated animals. (ii) Aoyama (11) described an increase in norepinephrine and dopamine contents in infarcted and noninfarcted heart muscles of dogs treated with 15 μg/kg of nitroglycerin. Tranylcypromine was able to cause demonstrable accumulation of catecholamines in different animals in much smaller doses than used in our study (5, 12).

Reduced uptake of catecholamines by tissue and increased excretion of catecholamines in urine are known factors contributing to the catecholamine-depleting effect of reserpine (13, 14). It has been demonstrated that a large dose of reserpine, similar to that used in our study, could deplete almost completely the catecholamine stores from brain, heart, and adrenals of the rabbit within 16 hr (15). Under such a condition of catecholamine depletion both nitroglycerin and tranylcypromine failed to increase plasma FFA and glycerol.

Much of the evidence indicates that the metabolic effects of catecholamines on carbohydrate and lipid metabolism are closely associated with the stimulation of the adenylcyclase system and that these effects are mediated by beta receptors (16). Propranolol, a pure beta adrenergic blocking agent, is able to block the lipolytic action of epinephrine in adipose tissue *in vitro* (17). The rise in plasma FFA following the administration of catecholamines was inhibited by propranolol in dogs (18). In the present study, the elevations of plasma FFA and glycerol in nitroglycerin-treated rabbits were inhibited by the administration of propranolol.

Nitroglycerin and tranylcypromine were also effective in concomitantly increasing plasma lactate and pyruvate, suggesting an increased rate of glycolysis in peripheral tissue, since the proportional elevations of both these carboxylic acids in the bood may serve as an index of increased glycolysis *in vivo* (10). Of interest is the fact that a significant increase of plasma lactate was still present in reserpine or propranolol-pretreated rabbits when nitroglycerin or tranylcypromine was injected. These findings suggest that the increased glycolysis occurred despite depletion of tissue catecholamines or the inhibition of beta receptors. The expected catecholamine-induced increase in plasma glucose concentration was not observed in nitroglycerin- or tranylcypromine-treated rabbits. Tranylcypromine was demonstrated to cause release of insulin and hypoglycemia in mice (19), and it might be possible that both catecholamines and insulin acted simultaneously to cause the unchanged plasma glucose concentration observed in our MAO inhibitor-treated rabbits.

The metabolic alterations observed in our nitroglycerin- and tranylcypromine-treated animals were accompanied by a rise in body temperature. The tranylcypromine-induced rise is attributed to the stimulatory effects of this MAO inhibitor on the central nervous system, which seems to be mediated through elevated levels of catecholamines or serotonin in the brain, and is probably dose related (12). Although the doses of nitroglycerin used in the present study were somewhat higher than those used by others (7, 11), it is probable that this drug, like tranylcypromine, possesses a similar stimulating effect on the central nervous system and thereby causes an elevation of body temperature. Another mechanism which could contribute to the increased body temperature in nitroglycerin- and tranylcypromine-treated animals would be the promotion of oxidation of such metabolities as FFA and pyruvate, as the consequence of their increased supply to the peripheral tissues. In support of such a mechanism is the fact that following administration of these two MAO inhibitors in reserpinized and propranolol-treated animals, a decrease in body temperature occurred simultaneously with inhibited lipolysis.

Summary. Metabolic alterations caused by nitroglycerin were compared with those of tranylcypromine. Following administration of 100 μg/kg of nitroglycerin in unanesthetized rabbits, simultaneous elevations of plasma FFA (+98%), glycerol (+53%), lactate (+178%), and pyruvate (+135%) were observed. The same dose of nitroglycerin caused a significant elevation of plasma lactate but failed to increase plasma FFA and glycerol in reserpinized and propranolol-treated rabbits. Tranylcypromine (30 mg/kg) caused similar alterations: increase in plasma FFA (+135%), glycerol (+85%), lactate (+335%), and pyruvate (+152% Similarly, an increase in plasma lactate without change in plasma FFA and glycerol levels, was observed in reserpinized animals. An increase in body temperature was found in nitroglycerin- and tranylcypromine-treated rabbits. This effect was diminished when the two drugs were administered in reserpine- and propranolol-treated rabbits. These results indicate that the rate of lipolysis and glycolysis is increased in rabbits treated with both MAO inhibitors. Unlike glycolysis, increased lipolysis seems to be mediated by endogenous catecholamines.

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