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PHENTOLAMINE ACTIVATION OF GLYCOGENOLYSIS IN RAT SKELETAL MUSCLE*

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Abstract—Administration of phentolamine to the rat or dog increases significantly the concentration of blood lactic acid, and reduces blood glucose. In the rat, the lacticacidemia appears due to an activation of glycogenolysis in skeletal muscle, since phentolamine administration results in an increase in phosphorylase *a* and a reduction of the glycogen content of this tissue. The plasma concentration of catecholamines also increases suggesting that phentolamine elicits these metabolic effects through their release. The phentolamine-induced changes in the blood lactic acid, phosphorylase *a* and glycogen content of skeletal muscle can be inhibited by pretreating the rat with the adrenergic blocking drugs, butoxamine or 4-(2-isopropyl-1-hydroxyethyl)methanesulfonanilide (MJ1999). The phentolamine-induced lacticacidemia is inhibited partially if the rat is pretreated with guanethidine or reserpine; it is reduced markedly in the adrenal demedullated rat, and is eliminated if guanethidine is administered to the adrenal demedullated rat prior to phentolamine. The results suggest that phentolamine activates glycogenolysis in rat skeletal muscle, and this activation is mediated by the catecholamines.

THE PLASMA free fatty acid (FFA) concentration increases significantly after the administration of the alpha adrenergic blocking drug, phentolamine, to the rat, dog or man.[1, 2] In the rat, the release of catecholamines from peripheral sympathetic nerve endings appears to account for this increase, and for the ability of phentolamine to reduce the weight gain of depot fat.[2, 3] Little is known regarding the effect of phentolamine on other metabolic parameters *in vivo* except that it can cause hypoglycemia in the rabbit, dog or man.[4] This report shows that phentolamine can activate glycogenolysis in rat skeletal muscle and presents evidence that this activation occurs through the action of catecholamines which are released by phentolamine.

METHODS

General procedure. Unanesthetized, male mongrel dogs were used after an overnight fast. Butoxamine or MJ1999 (15 mg/kg) was administered to the dog orally, by capsule, 1 hr prior to the slow, i.v. infusion of 8 mg/kg of phentolamine. In other experiments each dog was treated with phentolamine only, and thereby served as his own control. Blood (5 ml) was taken at various times from the jugular vein. Two control blood samples were taken 30 min apart just prior to the administration of butoxamine or MJ1999; another sample of blood was taken 1 hr later. At this time,

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phentolamine was administered and blood samples were taken at various intervals afterwards. The blood samples were assayed for lactic acid by using lactic dehydrogenase* and for glucose by the Glucostat† method. The same assays were done on the blood of male rats decapitated at various times after the intraperitoneal administration of phentolamine. The rats employed were Sprague–Dawley (CFE) weighing 180–220 g. They were fed a standard laboratory diet. Aqueous solutions of butoxamine, propranolol or MJ1999 were administered s.c. to the rat 30 min prior to phentolamine. In some experiments, rats were pretreated with reserpine (5 mg/kg, i.p.) or guanethidine (35 mg/kg, i.v.) 18 hr prior to the administration of phentolamine.

Phosphorylase *a* was measured in a portion of the rat quadriceps femorii muscle. Rats were anesthetized with dial-urethane (0·6 ml/kg) and 30 min later were injected i.p. with 25 mg/kg of either of the adrenergic blocking drugs, butoxamine or MJ1999. Phentolamine (25 mg/kg, i.p.) was administered 30 min after the blocking drug, and 15 min later a portion of muscle was quickly excised, frozen and then assayed for phosphorylase *a*.[5]

The glycogen content of rat muscle and liver was determined by the method of Seifter *et al.*[6] A portion of tissue was excised 60 min after the i.p. administration of 50 mg/kg of phentolamine. Groups of rats were given phentolamine, without any pretreatment, or were given 50 mg/kg of either MJ1999 or butoxamine 30 min prior to phentolamine.

Adrenal demedullated male, Sprague–Dawley rats‡ were maintained on physiological saline and the usual laboratory diet. The adrenals of some of the demedullated rats were examined by histological methods; the only functional tissue remaining was a portion of the cortex.

The total catecholamine concentration of plasma was determined on the pooled plasma from 10 rats by the alumina extraction method of Anton and Sayre.[7]

Drugs. Butoxamine is *N*-tertiary butyl methoxamine hydrochloride (Burroughs Wellcome & Co.); MJ1999 is 4-(2-isopropyl-1-hydroxyethyl)methanesulfonanilide (Mead Johnson Co.); propranolol is 1-isopropylamino-3-(1-naphthyloxy)-2-propanol hydrochloride (Imperial Chemical Ind.). Phentolamine was obtained as the methane sulfonate from Ciba Pharmaceutical Co.

RESULTS

Effect of phentolamine on the blood glucose and lactic acid of the rat and dog

The change in blood glucose and lactic acid was determined in the rat at various times during a 90-min period after the administration of 10, 25 or 50 mg/kg of phentolamine (Fig. 1). A significant increase in blood lactic acid occurred with all three doses of phentolamine and the response was dose related. Blood glucose was reduced significantly and to about the same extent 30 min after 10 and 25 mg/kg of phentolamine ($P < 0{\cdot}05$), whereas a marked hypoglycemia occurred after the administration of 50 mg/kg. Pretreatment of the rat with butoxamine or the beta adrenergic blocking drugs, propranolol or MJ1999, inhibited the increase in blood lactic acid which

* Sigma Chemical Co., St. Louis, Mo.

† Worthington Biochemical Corp., Freehold, N.J.

‡ Hormone Assay Laboratory, Inc., Chicago, Ill.

occurred 30 min after the administration of 25 mg/kg of phentolamine (Fig. 2). As shown in Fig. 1, the blood lactic acid after this dose is maximally elevated at 30 min. The hypoglycemia induced by the administration of phentolamine was unaffected by pretreating the rat with the adrenergic blocking drugs. In the dog, the effect of phentolamine on the blood lactic acid is qualitatively the same as that observed in

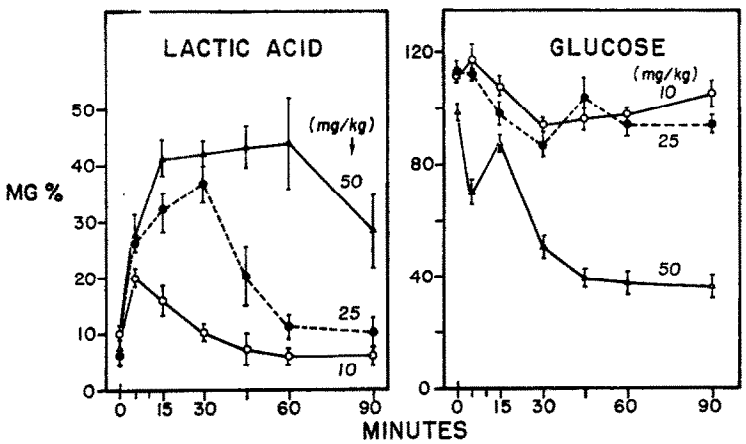

FIG. 1. The blood concentration of lactic acid and glucose at various times after the i.p. administration of phentolamine to the rat. Groups of 8 rats were killed at the times indicated after the administration of 0, 10, 25 or 50 mg/kg of phentolamine. The mean value for each group is plotted and the S.E. is shown by the bar.

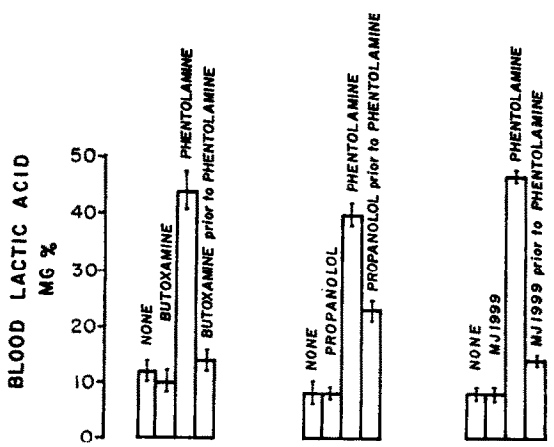

FIG. 2. Effect of pretreatment with butoxamine, MJ1999 or propranolol on the lacticacidemia induced by the administration of phentolamine to the rat. The results obtained in separate experiments with each blocking drug are shown. Groups of 8 rats were given either 25 mg/kg of butoxamine, MJ1999 or propranolol s.c. 30 min prior to administering phentolamine (25 mg/kg, i.p.), and were killed 30 min after phentolamine administration. One group was given either butoxamine, MJ1999 or propranolol and killed 1 hr later. Another group was given only phentolamine and killed 30 min later. The untreated controls are labeled "none". The mean value for each group is plotted and the S.E. is shown by the bar.

the rat (Fig. 3). Blood lactic acid increased significantly in two dogs after the i.v. administration of 8 mg/kg of phentolamine. This increase was blocked by pretreating the dog with 15 mg/kg of butoxamine or MJ1999. A significant lowering of blood glucose also occurred in these dogs, which was not affected by pretreatment with butoxamine or MJ1999.

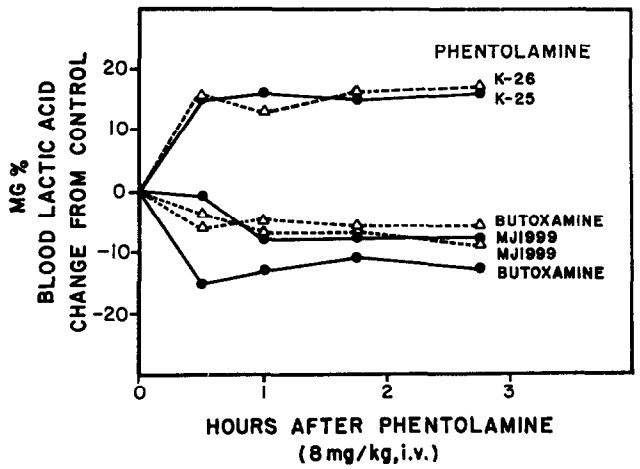

FIG. 3. The hyperlacticacidemia induced by the administration of phentolamine to the dog and inhibition of this response with butoxamine or MJ1999. Two dogs were given phentolamine (8 mg/kg) i.v. The concentration of blood lactic acid was determined at the times indicated after phentolamine administration (upper curves). The results are expressed as the change in blood lactic acid from the mean control blood concentration. In other experiments, the same dogs were given 15 mg/kg of either butoxamine of MJ1999 1 hr prior to phentolamine (lower curves).

Effect of phentolamine on phosphorylase a and the glycogen content of rat skeletal muscle

There was a marked increase in the phosphorylase *a* content of rat skeletal muscle 15 min after the administration of phentolamine (Table 1). This increase was blocked by pretreating the rat with 25 mg/kg of butoxamine or MJ1999. In other experiments, the glycogen content of rat skeletal muscle was determined after the administration

TABLE 1. EFFECT OF PHENTOLAMINE ON THE PHOSPHORYLASE *a* CONTENT OF RAT SKELETAL MUSCLE

Treatment	No. of rats	Phosphorylase *a* (% ± S.E.)
None	6	26 ± 2
Phentolamine (25 mg/kg)	6	68 ± 7
Butoxamine* (25 mg/kg) before phentolamine	6	21 ± 3
MJ1999* (25 mg/kg) before phentolamine	7	31 ± 3

* Phentolamine was administered 30 min after giving butoxamine or MJ1999, and 15 min later a portion of muscle was excised for assay. These drugs inhibited significantly the phentolamine-induced increase in phosphorylase *a* ($P < 0.05$).

of 50 mg/kg of phentolamine (Table 2). The phentolamine-treated group was compared with other groups of rats given 50 mg/kg of either butoxamine or MJ1999 prior to phentolamine. After 60 min, the muscle glycogen of the phentolamine-treated group was significantly lower than that of the groups pretreated with butoxamine or MJ1999, or of the untreated controls, while liver glycogen content was unaffected. In this

TABLE 2. EFFECT OF PHENTOLAMINE ON THE GLYCOGEN CONTENT OF RAT LIVER AND SKELETAL MUSCLE

Treatment*	No. of rats	Glycogen (mg/g ± S.E.)	
		Liver	Skeletal muscle
None (untreated controls)	5	42·0 ± 2	4·5 ± 0·03
Phentolamine (50 mg/kg)	5	41·7 ± 3	3·3 ± 0·03
Butoxamine (50 mg/kg) before phentolamine	5	45·9 ± 3	4·9 ± 0·04
MJ1999 (50 mg/kg) before phentolamine	5	41·3 ± 5	5·4 ± 0·03

* Phentolamine was administered 30 min after giving butoxamine or MJ1999, and 60 min later a portion of muscle was excised for assay. These drugs blocked the phentolamine-induced lowering of muscle glycogen. The glycogen content of skeletal muscle was lowered significantly by giving phentolamine ($P < 0·01$).

experiment, the blood lactic acid was determined 60 min after phentolamine administration; the concentration increased from 14 mg/100 ml in untreated controls to 47 mg/100 ml in rats given phentolamine. This increase did not occur in rats which were pretreated with butoxamine or MJ1999.

Evidence that the hyperlacticacidemia induced by phentolamine in the rat is mediated by the catecholamines

As shown in Fig. 4, the hyperlacticacidemia was reduced markedly in the adrenal demedullated rat given phentolamine (25 mg/kg); these results are compared with the

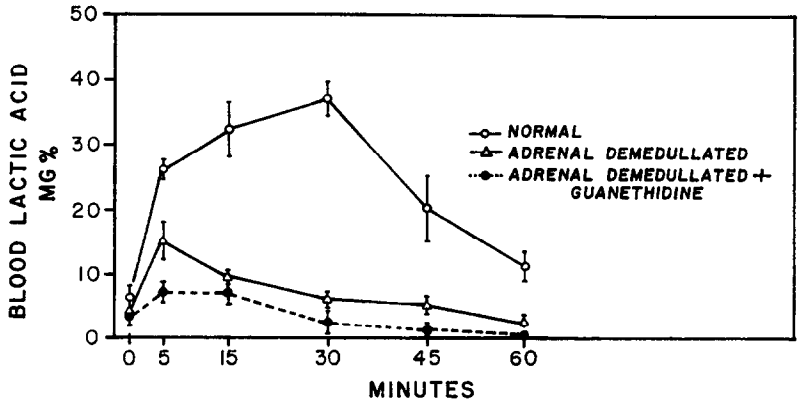

FIG. 4. Effect of phentolamine on the concentration of blood lactic acid of the adrenal demedullated rat. Groups of 8 adrenal demedullated rats were killed at the times indicated after the i.p. administration of 0 or 25 mg/kg of phentolamine and the blood was assayed for lactic acid. Some groups of adrenal demedullated rats were given 35 mg/kg of guanethidine i.v. 18 hr prior to the administration of phentolamine. The mean blood lactic acid for each group is plotted and the S.E. is indicated by the bar.

results obtained with this dose of phentolamine in the normal rat. However, a small but significant elevation of blood lactic acid still occurred in the demedullated rat 5 min after the administration of phentolamine ($P < 0.05$). A significant increase in lactic acid did not occur if the adrenal demedullated rat was given guanethidine 18 hr prior to phentolamine. Pretreatment of the normal rat with reserpine or guanethidine also reduced significantly the hyperlacticacidemia caused by phentolamine (Table 3). As shown in Table 4, there is a significant increase in the catecholamine

TABLE 3. EFFECT OF PRETREATMENT WITH RESERPINE AND GUANETHIDINE ON THE HYPERLACTICACIDEMIA INDUCED BY PHENTOLAMINE IN THE RAT

Treatment	No. of rats	Blood lactic acid (mg/100 ml ± S.E.)
None	10	6 ± 0·7
Reserpine	10	6 ± 3·0
Guanethidine	10	5 ± 0·5
Phentolamine	10	54 ± 2·0
Reserpine* before phentolamine	10	21 ± 2·0
Guanethidine* before phentolamine	10	37 ± 4·0

* Rats were pretreated with reserpine (5 mg/kg, i.p.) or guanethidine (35 mg/kg, i.v.) 18 hr prior to the administration of phentolamine (25 mg/kg, i.p.). The blood lactic acid was determined 30 min after phentolamine. Pretreatment with reserpine or guanethidine inhibited significantly the lacticacidemia induced by phentolamine ($P < 0.05$).

TABLE 4. EFFECT OF PHENTOLAMINE ON THE CONCENTRATION OF CATECHOLAMINES IN THE PLASMA OF THE RAT

Treatment*	No. of assays	Plasma catecholamines (μg/l. ± S.E.)
Saline	5	10·0 ± 0·72
Phentolamine (25 mg/kg)	6	14·5 ± 1·67
Epinephrine (0·3 mg/kg)	2	28·0 ± 1·00

* Rats were killed 15 min after the administration of either saline, phentolamine or epinephrine. The pooled plasma of 10 rats was used for each catecholamine assay. The plasma catecholamine content of phentolamine- and epinephrine-treated rats was significantly higher than that of rats given saline ($P < 0.05$).

content of rat plasma 15 min after the administration of 25 mg/kg of phentolamine. The plasma catecholamine content found in the saline-treated rat (10 ± 0·72 3μ/l.) is approximately the same as that found by Anton and Sayre in the plasma of untreated rats.[7]

DISCUSSION

An increase in the concentration of blood lactic acid occurs after the administration of phentolamine to the rat or dog. In the rat, this response has been shown to be rapid in onset and dose dependent. This response can be inhibited in both species by

administering butoxamine or either of the beta adrenergic blocking drugs, MJ1999 or propranolol, prior to phentolamine. These compounds will also inhibit the epinephrine- and isoproterenol-induced increase in blood glucose and lactic acid in the rat,[8] as well as certain other metabolic effects of the catecholamines in animals and man.[9–13] The ability of these blocking drugs to inhibit the phentolamine-induced hyperlacticacidemia suggested that this response is mediated at the adrenergic receptor sites of skeletal muscle and led to the experiments discussed below.

In addition to an increase in blood lactic acid, there is a significant reduction of blood glucose 15–30 min after the administration of 10, 25 or 50 mg/kg of phentolamine to the rat. The hypoglycemia occurs approximately 15 min after the administration of phentolamine, while lactic acid is elevated significantly after 5 min. The hypoglycemia is not affected by pretreating the rat with butoxamine, MJ1999 or propranolol. The hypoglycemic action of phentolamine was observed previously in the rabbit, dog and man.[4]

After the administration of phentolamine to the rat, the phosphorylase *a* content of skeletal muscle is elevated significantly while the glycogen content is reduced significantly. These changes are prevented by pretreating the rat with butoxamine or MJ1999. These results are consistent with the ability of these drugs to inhibit the increase in blood lactic acid which occurs after phentolamine administration. Both butoxamine and MJ1999 have been shown to inhibit the isoproterenol-induced activation of phosphorylase of rat skeltal muscle.[11] In contrast to its stimulatory effect in skeletal muscle, phentolamine blocks the epinephrine-induced activation of phosphorylase in rat liver slices, and has little effect on the activation of phosphorylase of rat diaphragm by epinephrine.[14, 15] The ability of phentolamine to block the activation of phosphorylase in rat liver probably accounts for its hypoglycemic action.

Experiments were conducted to determine if the activation of glycogenolysis in rat skeletal muscle by phentolamine occurs indirectly through the release of catecholamines. The magnitude of the phentolamine-induced increase in blood lactic acid is reduced significantly by pretreating the rat with reserpine or guanethidine. The major portion of this response is eliminated in the adrenal demedullated rat, and disappears completely if the demedullated rat is pretreated with a dose of guanethidine which depletes the peripheral stores of catecholamines. These results indicate that the hyperlacticacidemia induced by the administration of phentolamine to the rat occurs primarily through the release of catecholamines from the adrenal medulla and to a small extent, through their release from peripheral nerve endings. This proposal is supported by the observation that the catecholamine content of rat plasma increases significantly after phentolamine administration. Other workers have shown that the administration of phentolamine increases the plasma norepinephrine concentration in man, and increases the urinary excretion of norepinephrine in the cat.[16, 17] The phentolamine-induced activation of glycogenolysis in rat skeletal muscle could also be due, in part, to a potentiation of the action of the catecholamines, since the alpha adrenergic blocking drugs are known to be able to sensitize certain tissues to these substances.

The possible mechanisms by which phentolamine could release catecholamines should be considered. Two of the more obvious possibilities are: (1) a direct releasing effect of phentolamine on stored catecholamines and (2) an indirect releasing effect through a reflex stimulation of the sympatho-adrenal system. There is some basis for

the first possibility, since phentolamine has been reported to cause the release of catecholamines and ATP *in vitro* from ox adrenal chromaffine granules.[18] A reflex release of catecholamines through stimulation of the sympatho-adrenal system could be triggered by such changes as the reduction of blood pressure or blood sugar caused by phentolamine. In man, hypoglycemia has been shown to cause a sympatho-adrenal discharge resulting in the release of epinephrine from the adrenal medulla.[19] It has been suggested that a reflex mechanism of this type mediates the phentolamine-induced increase in plasma FFA in man.[20]

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