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Inhibition by butoxamine, propranolol and MJ1999 of the glycogenolytic action of the catecholamines in the rat

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THE USE of either the fed or fasted rat provides a convenient method of preparing a rat sensitive to the glycogenolytic action of either alpha- or beta-stimulant drugs. Norepinephrine elevates blood glucose only in the fed rat, whereas isoproterenol does so only in the fasted animal. Epinephrine elevates blood glucose and lactic acid in either the fed or fasted rat.[1] It was our purpose to determine the effect of pretreatment with either butoxamine,* propranolol or MJ1999 on the activation of glycogenolysis by these catecholamines in the fed or fasted rat. Propranolol and MJ1999[2, 3] are beta-adrenergic blocking drugs. Butoxamine has similar metabolic blocking properties, but is not considered a true beta-adrenergic drug.[4–6]

METHODS

The rats were male Sprague–Dawley (CFE) and weighed 180–200 g. Food was withdrawn from fasting rats 20 hr prior to each experiment, but free access to water was allowed. Butoxamine, MJ1999 or propranolol was administered 30 min prior to either epinephrine, norepinephrine or isoproterenol. All drugs were injected subcutaneously in water. The rats were decapitated and blood was collected for assay 30 min after the injection of the catecholamine or 1 hr after the blocking drug. This time interval was selected because of the short half-life of butoxamine in the rat (45 min). Aliquots of blood were assayed for glucose by the Glucostat† method, and for lactic acid by using lactic dehydrogenase.‡

RESULTS

Activation of glycogenolysis by isoproterenol. The concentration of blood glucose and lactic acid increases significantly after the administration of 0·01 mg/kg of isoproterenol to the fasted rat. These increases can be prevented by pretreating the rat with either butoxamine or one of the beta-adrenergic blocking drugs, propranolol or MJ1999 (Table 1). An increase in blood lactic acid but not in glucose occurs after the administration of 0·1 mg/kg of isoproterenol to the fed rat (Table 2). This increase can also be blocked by pretreating the rat with either butoxamine, MJ1999 or propranolol.

Activation of glycogenolysis by norepinephrine. A significant increase in the concentration of blood glucose occurs in the fed rat but not in the fasted rat after administration of a large dose of norepinephrine (2 mg/kg). The blood lactic acid does not change. The hyperglycemia induced by norepinephrine in the fed rat is inhibited significantly by pretreating with either butoxamine, MJ1999 or propranolol (Table 3).

Activation of glycogenolysis by epinephrine. The concentration of blood glucose and lactic acid increases significantly after the administration of 0·3 mg/kg of epinephrine to the fed rat (Table 4) or 0·1 mg/kg epinephrine to the fasted rat (Table 5). The hyperglycermia induced by epinephrine administration is inhibited in the fed or fasted rat by pretreatment with either butoxamine, MJ1999 or propranolol (Tables 4, 5). The hyperlacticacidemia induced by epinephrine in these experiments is blocked completely by these drugs.

* Butoxamine is *N*-tertiary butyl methoxamine HCl (Burroughs Wellcome & Co.); MJ1999 is 4-(2-isopropylamino-1-hydroxyethyl) methanesulfonalide (Mead Johnson Co.); propranolol is 1-isopropylamino-3-(1-naphthyloxy)-2-propanol HCl (Imperial Chem. Ind.).

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

‡ Sigma Chemical Co., St. Louis, Mo.

TABLE 1. EFFECT OF PRETREATMENT WITH EITHER BUTOXAMINE, MJ1999 OR PROPRANOLOL ON THE ISOPROTERENOL-INDUCED INCREASE IN BLOOD GLUCOSE AND LACTIC ACID IN THE FASTED RAT*

Treatment	Blood glucose (mg/100 ml)			Blood lactic acid (mg/100 ml)		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
None	68 ± 1·5	80 ± 2·0	71 ± 2·0	7 ± 0·6	9 ± 0·6	5 ± 0·6
Butoxamine	58 ± 1·7			7 ± 0·6		
MJ1999		78 ± 2·4			7 ± 0·4	
Propranolol			88 ± 5·0			3 ± 0·3
Isoproterenol	111 ± 2·5	105 ± 4·7	110 ± 2·0	64 ± 4·0	39 ± 3·4	37 ± 3·0
Isoproterenol after:						
Butoxamine	56 ± 1·0			9 ± 0·6		
MJ1999		70 ± 1·7			8 ± 0·4	
Propranolol			85 ± 2·0			6 ± 1·0

* Each value is the mean ± S.E. for a group of 10 rats. The results of three experiments are shown In each, one group was untreated (None). Another group was given 50 mg/kg (s.c.) of either butoxamine, MJ1999 or propranolol and killed 1 hr later. One group was administered 0·01 mg/kg isoproterenol (s.c.) and killed 30 min later; other rats were given either butoxamine, MJ1999 or propranolol 30 min prior to isoproterenol and killed 30 min later. In each experiment, the catecholamine effects were significantly less in pretreated animals ($P < 0·05$).

TABLE 2. EFFECT OF PRETREATMENT WITH EITHER BUTOXAMINE, MJ1999 OR PROPRANOLOL ON THE ISOPROTERENOL-INDUCED INCREASE IN BLOOD LACTIC ACID IN THE FED RAT*

Treatment	Blood lactic acid (mg/100 ml)		
	Expt. 1	Expt. 2	Expt. 3
None	14 ± 1	6 ± 1	9 ± 1
Butoxamine	10 ± 1		
MJ1999		8 ± 0·9	
Propranolol			10 ± 0·5
Isoproterenol	78 ± 4	75 ± 3	58 ± 2
Isoproterenol after:			
Butoxamine	37 ± 3		
MJ1999		5 ± 0·8	
Propranolol			7 ± 0·6

* The experimental procedure was the same as that described in Table 1, except that the dose of isoproterenol was 0·1 mg/kg. Blood glucose was determined but did not increase after isoproterenol. In each experiment, the catecholamine effect was significantly less in pretreated animals ($P < 0·05$).

DISCUSSION

The activation of glycogenolysis by norepinephrine or isoproterenol is dependent in the rat upon the prandial state.[1] Norepinephrine elevates blood glucose only in the fed rat, but isoproterenol does so only in the fasted animal. According to their pharmacological properties, norepinephrine is considered to be primarily an alpha-stimulant drug, whereas isoproterenol is a beta-stimulant drug.[7] Epinephrine has properties of both and elevates blood glucose and lactic acid in both the fed and fasted rat. The use of norepinephrine in the fed rat or isoproterenol in the fasted rat therefore provides a means to test the effect of adrenergic blocking drugs under conditions in which the rat is sensitive to activation of glycogenolysis by either an alpha- or beta-stimulant drug. In the present study, only drugs with beta-adrenergic blocking properties were examined. It would be of interest to examine the effect of the alpha-adrenergic blocking drugs under these conditions as well.

An increase in blood glucose and lactic acid occurs after the administration of a small dose of isoproterenol to the fasted rat. This response is accompanied by a reduction of the glycogen content

TABLE 3. EFFECT OF PRETREATMENT WITH EITHER BUTOXAMINE, MJ1999 OR PROPRANOLOL ON THE NOREPINEPHRINE-INDUCED INCREASE IN BLOOD GLUCOSE IN THE FED RAT*

Treatment	Blood glucose (mg/100 ml)		
	Expt. 1	Expt. 2	Expt. 3
None	99 ± 1·2	117 ± 3·9	100 ± 2·0
Butoxamine	83 ± 3·7		
MJ1999		131 ± 2·9	
Propranolol			100 ± 1·0
Norepinephrine	182 ± 6·8	198 ± 6·0	168 ± 6·0
Norepinephrine after:			
Butoxamine	126 ± 3·6		
MJ1999		164 ± 3·5	
Propranolol			148 ± 3·0

* The experimental procedure was the same as that described in Table 1. The dose of norepinephrine was 2 mg/kg. Blood lactic acid was determined but did not increase after norepinephrine. In each experiment, the catecholamine effect was significantly less in pretreated animals ($P < 0.05$).

TABLE 4. EFFECT OF PRETREATMENT WITH EITHER BUTOXAMINE, MJ1999 OR PROPRANOLOL ON THE EPINEPHRINE-INDUCED INCREASE IN BLOOD GLUCOSE AND LACTIC ACID IN THE FED RAT*

Treatment	Blood glucose (mg/100 ml)			Blood lactic acid (mg/100 ml)		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
None	123 ± 3·6	121 ± 2·0	100 ± 2·0	4 ± 0·3	4 ± 0·5	14 ± 0·8
Butoxamine	100 ± 2·0			5 ± 0·3		
MJ1999		117 ± 2·0			5 ± 0·3	
Propranolol			100 ± 1·0			12 ± 0·8
Epinephrine	198 ± 10	219 ± 3·6	168 ± 6·0	39 ± 2·3	30 ± 1·0	38 ± 3·0
Epinephrine after:						
Butoxamine	125 ± 4·1			5 ± 0·3		
MJ1999		174 ± 5·0			3 ± 0·3	
Propranolol			131 ± 5·0			8 ± 1·0

* The experimental procedure was the same as that described in Table 1, except that the dose of epinephrine was 0·3 mg/kg. In each experiment, the catecholamine effects were significantly less in pretreated animals ($P < 0.05$).

TABLE 5. EFFECT OF PRETREATMENT WITH EITHER BUTOXAMINE, MJ1999 OR PROPRANOLOL ON THE EPINEPHRINE-INDUCED INCREASE IN BLOOD GLUCOSE AND LACTIC ACID IN THE FASTED RAT*

Treatment	Blood glucose (mg/100 ml)			Blood lactic acid (mg/100 ml)		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
None	88 ± 3·5	80 ± 2	74 ± 2	4 ± 0·4	9 ± 0·5	10 ± 2
Butoxamine	75 ± 3			5 ± 0·4		
MJ1999		78 ± 2			7 ± 0·4	
Propranolol			89 ± 3			11 ± 0·6
Epinephrine	148 ± 15	125 ± 5	118 ± 4	44 ± 5·3	39 ± 3	30 ± 02·2
Epinephrine after:						
Butoxamine	95 ± 2			3 ± 1·1		
MJ1999		91 ± 3			10 ± 0·5	
Propranolol			94 ± 4			10 ± 0·9

* The experimental procedure is the same as that described in Table 1, except that the dose of epinephrine was 0·1 mg/kg. In each experiment, the catecholamine effects were significantly less in pretreated animals ($P < 0.05$).

of skeletal muscle while the glycogen content of liver is either unchanged or increases.[1, 8] The increase in blood glucose is thought to occur through the conversion in liver of lactic acid to glycogen.[9] The increase in blood glucose and lactic acid that occurs after the administration of isoproterenol to the fasted rat can be inhibited by pretreating the rat with propranolol, MJ1999 or butoxamine. These results are in accord with the generally accepted theory that beta receptors mediate the activation of glycogenolysis in skeletal muscle.[10, 11]

Isoproterenol appears to activate glycogenolysis in the skeletal muscle of the fed rat also, since it causes an increase in blood lactic acid. However, blood glucose does not increase. Pretreatment of the fed rat with either butoxamine, MJ1999 or propranolol prevents the elevation of blood lactic acid that follows the administration of isoproterenol. These results suggest that isoproterenol can activate glycogenolysis in skeletal muscle of either the fed or fasted rat.

The concentration of blood glucose increases significantly in the fed rat only after the administration of a large dose of norepinephrine, and it should be noted that cardiovascular changes might contribute to the glucose response.[12] Blood lactic acid does not increase after norepinephrine administration nor is the concentration of muscle glycogen reduced,[1] suggesting that glycogenolysis is activated only in liver. Others have shown that a reduction of liver glycogen by fasting decreases the hyperglycemic response to norepinephrine.[13] The norepinephrine-induced increase in blood glucose in the fed rat can be inhibited by pretreating with butoxamine or the beta-adrenergic blocking drugs. These results are in accord with other observations which indicate that the receptor involved in the activation of glycogenolysis in liver cannot be clearly defined as an alpha receptor.[11] This does not imply that these receptors are beta. It is apparent from the observations of others that both alpha- and beta-adrenergic blocking drugs inhibit to some degree the effect of catecholamines on blood glucose, lactic acid, and liver phosphorylase. The nature of the receptor in liver that mediates glycogenolysis remains undefined.

The hyperglycemia and hyperlacticacidemia that occur after the administration of epinephrine to either the fed or fasted rat are greater and more sustained than those obtained after the administration of norepinephrine or isoproterenol.[1] The hyperglycemia caused by epinephrine can be inhibited by pretreating with both alpha-[14] and beta-adrenergic[15] blocking drugs. Our results show that propranolol, MJ1999 and butoxamine can inhibit the increase in blood glucose and lactic acid that follows the administration of epinephrine to either the fed or fasted rat.

There is considerable evidence that the activation of glycogenolysis by the catecholamines is mediated in a variety of tissues through the formation of adenosine-3′-5′-phosphate, which leads to the activation of phosphorylase. Inhibition of any of the steps involved in this process would probably inhibit glycogenolysis. Dichloroisoproterenol, for example, appears to inhibit the synthesis of adenosine-3′-5′-phosphate in liver cells through an effect on adenyl cyclase.[16, 17] It would be of interest to determine if other drugs that inhibit glycogenolysis interfere at some point in this activation process.

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The effect of ethchlorvynol on the drug-metabolizing enzymes of rats and dogs

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THERE HAS been much concern recently about drug interactions, especially with respect to drugs that induce the enzymes which metabolize other drugs. For example, phenobarbital and chloral hydrate have been implicated in an increased rate of metabolism of bishydroxycoumarin.[1, 2] The enzymes that are induced by such drugs as phenobarbital have been shown to metabolize (and thus control the serum concentration and pharmacological effect of) such varied drugs as barbiturates, coumarins, codeine and morphine, erythromycin, phenothiazine tranquilizers, zoxazolamine, imipramine, and several steroids.[3–5]

Since two widely used sedatives, phenobarbital and chloral hydrate, have been shown to induce these liver microsomal enzymes, it was of interest to investigate the effect of another sedative, ethchlorvynol. (Ethchlorvynol has recently been reported to perhaps show such a property in man.[6]) Experiments in which ethchlorvynol was administered to dogs and rats are reported below.

MATERIALS AND METHODS

Rats. Phenobarbital, ethchlorvynol, glutethimide or methyprylon* was administered orally or i.p. to 160–200 g male Sprague–Dawley rats daily. All doses were 100 mg/kg. After the fifth dose the rats were starved for 18–24 hr. Liver microsomes from individual rats were then isolated.[7] The final suspension in 0·25 M sucrose was stored at −15° until analysis (less than a week of storage).

Dogs. The half-life of bishydroxycoumarin was determined after an oral dose of 40 mg/kg. Serum samples were taken periodically 1–7 days after dosing. A minimum of four determinations of measurable bishydroxycoumarin was used to determine the half-life. The dogs were then fed a 500 mg capsule of ethchlorvynol (50–100 mg/kg) daily for 24 days. The determination of the half-life of bishydroxycoumarin was then repeated. To serve as a measure of the sensitivity of the dogs to enzyme induction, they were then fed 16 mg/kg phenobarbital for 20 days, and the half-life of bishydroxycoumarin was determined again.

Analytical determinations

The protein content of the rat liver microsome preparations was analyzed by the biuret procedure;[8] the bishydroxycoumarin concentration in plasma was determined by extraction and u.v. analysis.[9]

Chemicals

NADPH, enzymatically reduced, was purchased from Sigma Chemical Company. Drugs were commercial samples. All other chemicals were reagent grade.

* Luminal, Placidyl, Doriden, and Noludar, respectively.