

PHOSPHORYLASE ACTIVITY IN RAT LIVER AND SKELETAL MUSCLE AFTER CATECHOLAMINES*

K. R. HORN BROOK† and T. M. BRODY

Department of Pharmacology, University of Michigan, Ann Arbor, Mich., U.S.A.

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Abstract—The time course of liver phosphorylase activation after catecholamine administration and the relative abilities of several adrenergic blocking agents to block catecholamine-induced phosphorylase activation in liver and skeletal muscle was studied in intact anesthetized rats. Subcutaneously injected epinephrine and norepinephrine increased the liver phosphorylase activity, the peak effect occurring 10 min after injection. The liver phosphorylase levels were somewhat reduced 30 min after injection. Isoproterenol administration did not increase the level of rat liver phosphorylase. Blood glucose changes after catecholamine treatment did not mirror the changes in liver phosphorylase activity.

Complete blockade of the liver phosphorylase response to epinephrine was observed with the α -adrenergic blocking agent ergotamine. Phenoxybenzamine and two β -adrenergic blocking agents, dichloroisoproterenol and nethalide, gave partial blockade. These data indicate the difficulty in the classification of the liver metabolic receptor. The activation by epinephrine of skeletal muscle phosphorylase appears to be affected only by β -adrenergic blocking agents.

THE blood glucose elevation, liver phosphorylase activation, and glycogenolysis induced by the catecholamines have been extensively investigated.^{1,2} Although the phosphorylase response has been reported consistently for liver slices *in vitro*, an effect *in vivo* after catecholamine administration has been both confirmed and denied.³⁻⁵ The effect of adrenergic blocking agents on liver phosphorylase activation after catecholamine administration has not been clarified.⁶

Phosphorylase activation in skeletal muscle after catecholamine administration is well documented;^{7,8} however, no data could be found demonstrating the nature of this response after adrenergic blocking agents.

The present investigation will be concerned with the time-response relationships of phosphorylase activation and blood glucose levels in rat liver after the administration of L-epinephrine, L-norepinephrine, and DL-isoproterenol and the susceptibility of epinephrine-induced activation of liver and skeletal muscle phosphorylase to adrenergic blockade.

METHODS

White female rats (180 to 200 g, Holtzman strain) were used throughout the investigation. Rats were maintained on food at all times. Anesthesia was produced by the intraperitoneal injection of hexobarbital sodium (150 to 180 mg/kg) 10 min before sacrifice or catecholamine infusion.

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† Present address: Dept. of Pharmacology, Washington Univ., School of Medicine, St. Louis, Mo., U.S.A.

Commercial solutions of L-epinephrine (Adrenalin, Parke-Davis and Co), or crystalline L-epinephrine, L-norepinephrine (Levophed, Winthrop Lab.), and DL-isoproterenol (Isuprel, Winthrop Lab.) were diluted in a 0.9% NaCl-0.1% NaHSO₃ solution to obtain the proper concentration for injection. The time-response curves were determined after subcutaneous injection of the catecholamine (500 µg/kg). The intravenous infusion of L-epinephrine (0.25 µg/kg·min⁻¹ for 10 min) was accomplished as described previously.⁹

The tissues were excised rapidly from anesthetized animals and frozen in beakers of isopentane cooled in alcohol-dry ice. The liver was excised before the skeletal muscle in all experiments. Phosphorylase and glycogen were assayed as previously reported.⁹ The liver sample was diluted such that an equivalent of 5 mg wet weight of liver (1:200 dilution) was assayed. The incubation time for the liver assay was 15 min. All liver phosphorylase determinations were carried out in the presence of 1 mM adenosine monophosphate. The data are expressed as micromoles inorganic phosphate liberated per gram wet weight of liver·minute⁻¹ incubation time. The skeletal muscle phosphorylase was assayed both with and without the addition of 1 mM adenosine monophosphate and the data are expressed as per cent phosphorylase *a*.⁹

Blood glucose was determined by the method of Nelson.¹⁰ Blood was obtained from a tail vein by means of a Unopette* (generously supplied by Dr. Horace W. Gerarde, Esso Research Lab. and Mr. J. Lucker, Becton, Dickinson and Co.). This allowed the accurate sampling of 13 µl of blood which was then diluted and deproteinized. Blood glucose is expressed as a per cent increase over the daily control level. Over-all mean blood glucose control levels were 92.7 ± 3.7 g/100 ml (mean \pm standard error, N = 27).

Dichloroisoproterenol (DCI), obtained from the Aldrich Chemical Co., and nethalide (ICI-38, 174), generously supplied by Dr. J. W. Black, were injected subcutaneously 30 min before catecholamine administration. Ergotamine tartrate (Gynergen, Sandoz) was injected subcutaneously (1.25 mg/kg) 30 min before catecholamine infusion. Phenoxybenzamine (Dibenzylamine, Smith Kline & French Labs.) was injected intraperitoneally (5 mg/kg and 20 mg/kg) 2 to 3 hr before catecholamine administration. Hexamethonium chloride (10 mg/kg intraperitoneally) was administered 30 min before catecholamine infusion.

RESULTS

The influence of sacrifice under anesthesia and/or decapitation on control liver phosphorylase levels

Sacrifice by decapitation markedly activated liver phosphorylase (Table 1) even if the animal was previously anesthetized. For this reason decapitation was avoided, and tissue samples were obtained under surgical anesthesia.

Liver phosphorylase and blood glucose levels after catecholamine administration

Table 2 gives the liver phosphorylase and blood glucose responses after subcutaneous L-epinephrine, L-norepinephrine, and DL-isoproterenol administration. L-Epinephrine injection caused an activation of liver phosphorylase which was maximal at 10 min after injection. In contrast to the phosphorylase response, the blood glucose levels

* Disposable blood-diluting pipet (Becton, Dickinson and Co., Rutherford, N. J.).

after epinephrine administration showed a steady rise throughout the sampling period. Blood glucose determinations at 60 min after epinephrine injection were somewhat higher than those found at 30 min (unpublished observations). L-Norepinephrine injection produced liver phosphorylase activation similar to that observed after epinephrine administration; however the blood glucose elevation was neither so rapid

TABLE 1. LIVER PHOSPHORYLASE VALUES AFTER DECAPITATION AND/OR ANESTHESIA

Group no.	Treatment	N*	Phosphorylase, $\mu\text{moles P}_i/\text{g} \cdot \text{min}^{-1} \pm \text{S.E.}$
1	Hexobarbital (180 mg/kg, 10 min)	6	4.5 ± 0.9
2	Hexobarbital + decapitation	5	$10.1 \pm 1.6^\dagger$
3	Decapitation	6	$11.8 \pm 1.1^\ddagger$

* N, number of animals per treatment.

† 1 vs. 2; $P < 0.01$.

‡ 1 vs. 3; $P < 0.01$.

TABLE 2. TIME-RESPONSE RELATIONSHIPS OF LIVER PHOSPHORYLASE AND BLOOD GLUCOSE LEVELS AFTER SUBCUTANEOUS CATECHOLAMINE*

Treatment	Time after injection				
	Control	5 min	10 min	20 min	30 min
Phosphorylase ($\mu\text{moles P}_i/\text{g} \cdot \text{min}^{-1}$)					
L-Epinephrine	4.8 ± 0.7 (8)	$7.8 \pm 1.1^\dagger$ (8)	$13.3 \pm 2.2^\dagger$ (8)	$10.4 \pm 2.0^\dagger$ (8)	7.1 ± 1.3 (8)
L-Norepinephrine	3.9 ± 0.4 (6)	5.3 ± 0.6 (6)	$10.2 \pm 2.0^\dagger$ (6)	$7.2 \pm 1.1^\dagger$ (6)	3.6 ± 1.2 (6)
DL-Isoproterenol	3.6 ± 0.4 (7)	4.8 ± 0.9 (6)	5.1 ± 0.8 (7)	4.6 ± 0.6 (7)	5.5 ± 0.9 (5)
Blood glucose (% over control)					
L-Epinephrine		23 ± 2 (9)	33 ± 9 (9)	58 ± 12 (9)	90 ± 9 (13)
L-Norepinephrine		8 ± 2 (3)	6 ± 17 (3)	48 ± 12 (3)	47 ± 34 (3)
DL-Isoproterenol		1 ± 5 (7)	9 ± 10 (7)	22 ± 12 (7)	49 ± 13 (7)

* All catecholamines given subcutaneously (500 $\mu\text{g}/\text{kg}$).

Values are means \pm S.E. Numbers of animals per treatment are in parentheses.

† Significantly different from control ($P < 0.05$).

nor so marked as that observed after epinephrine injection. DL-Isoproterenol elicited no statistically significant increase in liver phosphorylase levels at all times measured, and only the 30-min blood glucose sample was significantly higher than control levels,

The influence of adrenergic blockade on liver and skeletal muscle phosphorylase activation by catecholamines

The data in Table 3 show that L-epinephrine infusion, $0.25 \mu\text{g}/\text{kg} \cdot \text{min}^{-1}$ intravenously, for 10 min, elicited an increase in active phosphorylase levels in both liver and skeletal muscle. No significant decrease in glycogen was observed with this dose and time of catecholamine treatment. (Skeletal muscle: control 4.71 ± 0.28 mg/g, $N = 8$; epinephrine 4.15 ± 0.35 mg/g, $N = 12$. Liver: control 49.2 ± 1.9 mg/g; epinephrine 43.6 ± 4.8 . All, mean \pm standard error.)

TABLE 3. LIVER AND SKELETAL MUSCLE PHOSPHORYLASE RESPONSES TO EPINEPHRINE AFTER ADRENERGIC BLOCKING AGENTS

Group no.	Treatment	N	Skeletal muscle \pm S.E. (% phosphorylase a)	N	Liver, μ moles P _i /g \cdot min ⁻¹ \pm S.E.
1	Control	8	11.0 \pm 1.5	8	2.8 \pm 0.4
	Epinephrine, 10 min 0.25 μ g/kg \cdot min ⁻¹	12	67.3 \pm 3.1*	12	10.4 \pm 0.4*
	Dichloroisoproterenol 10 mg/kg s.c., 30 min	5	36.2 \pm 5.4*	5	1.7 \pm 0.1
	Dichloroisoproterenol + epinephrine	9	42.0 \pm 2.5†	9	6.5 \pm 1.1* †
	Nethalide 10 mg/kg s.c., 30 min	6	12.2 \pm 2.2	6	1.8 \pm 0.2
	Nethalide \pm epinephrine	9	15.3 \pm 2.4†	9	6.7 \pm 0.8* †
	Ergotamine 1.25 mg/kg s.c., 30 min	3	17.0 \pm 4.1	3	2.1 \pm 0.5
	Ergotamine + epinephrine	6	58.3 \pm 5.8* †	6	3.7 \pm 0.4†
	Phenoxybenzamine 5 mg/kg i.p., 2-3 hr	4	11.7 \pm 5.8	4	3.5 \pm 0.5
	Phenoxybenzamine + epinephrine	8	62.6 \pm 3.7* †	9	7.9 \pm 0.7* †
2	Control	6	10.3 \pm 3.4	6	4.1 \pm 0.4
	Epinephrine	7	45.8 \pm 3.1*	11	11.2 \pm 1.1*
	Phenoxybenzamine 20 mg/kg i.p., 2-3 hr	5	15.8 \pm 4.3	5	4.7 \pm 1.1
	Phenoxybenzamine + epinephrine	9	42.6 \pm 3.2* †	9	6.9 \pm 0.4* †
	Phenoxybenzamine + dichloroisoproterenol	3		3	5.3 \pm 1.0
	Phenoxybenzamine + dichloroisoproterenol + epinephrine	7	32.4 \pm 2.36	12	4.5 \pm 0.7†
3	Control	4		6	3.5 \pm 0.2
	Epinephrine	4	68.2 \pm 7.4*	9	12.2 \pm 0.6*
	Hexamethonium 10 mg/kg i.p., 30 min	5		5	5.6 \pm 1.0
	Hexamethonium + epinephrine	8		8	11.9 \pm 0.7* †
	Dichloroisoproterenol 25 mg/kg, 30 min	2	64.7 \pm 5.0*	2	3.0 \pm 4.3
	Dichloroisoproterenol + epinephrine	5		5	5.4 \pm 0.9† §

* Significantly different from controls ($P < 0.01$).† Significantly different from epinephrine ($P < 0.05$).‡ Significantly different from animals which received only blocking agent ($P < 0.05$).§ Significantly different from controls ($P < 0.05$).

Dichloroisoproterenol (10 or 25 mg/kg) caused a moderate activation of skeletal muscle phosphorylase and decreased the epinephrine-induced activation of skeletal muscle phosphorylase to the level of DCI treatment alone (Table 3). DCI treatment produced no activation of liver phosphorylase with the dose of either 10 or 25 mg/kg. At both DCI dose levels, partial to complete blockade of the liver response to epinephrine infusion was observed.

Treatment with nethalide (ICI-38, 174), a β -adrenergic blocking agent with no demonstrable sympathomimetic action,¹¹ produced a complete blockade of the epinephrine-induced phosphorylase activation in skeletal muscle and a partial inhibition of the epinephrine effect on liver phosphorylase.

Ergotamine did not significantly affect the skeletal muscle phosphorylase response to epinephrine but did effectively prevent the liver phosphorylase response to epinephrine infusion.

Phenoxybenzamine treatment (5 or 20 mg/kg) did not influence the skeletal muscle phosphorylase response to epinephrine but partially reduced the increase in liver phosphorylase produced by epinephrine. In contrast to DCI and nethalide, only a moderate blockade of the epinephrine response was observed in all animals at both dose levels of phenoxybenzamine.

Treatment of the animal with both phenoxybenzamine and DCI prior to epinephrine infusion produced more blockade of the epinephrine-induced liver phosphorylase response than that observed after phenoxybenzamine treatment but was not significantly different from the blockade observed after DCI treatment. The skeletal muscle phosphorylase response to epinephrine after the combination blockade was not significantly different from that obtained with DCI treatment alone.

Prior treatment of the rat with hexamethonium (10 mg/kg) produced no significant change in either the skeletal muscle or liver phosphorylase response to epinephrine infusion.

The skeletal muscle phosphorylase response to epinephrine in the second group of Table 3 is somewhat lower than the epinephrine responses observed in the other two groups, whereas the liver phosphorylase responses to epinephrine in all groups are similar. An explanation for this difference is not apparent.

DISCUSSION

The mechanism of the catecholamine-induced activation of glycogen phosphorylase has been extensively investigated by Sutherland and his co-workers. In recent reports they have indicated that the catecholamine effect is an indirect one mediated through an accumulation of cyclic 3', 5' adenosine monophosphate, which then stimulates the formation of active phosphorylase kinase. This enzyme catalyses the conversion of inactive to active phosphorylase.^{1,12-14} The applicability of this system to liver and skeletal muscle has been demonstrated.^{13,15}

The activation of liver phosphorylase by the catecholamines has been demonstrated conclusively in the liver slice of several species.^{3,16,17} Liver phosphorylase activation after catecholamine administration *in vivo* has been observed in the rabbit³ but not in the rat.^{4,5,18} The present data indicate that, with an adequate dose of catecholamine, rat liver phosphorylase activation can be obtained *in vivo*. Although the dose of catecholamine was larger than that used by other investigators, the method of sacrifice

of the animal and the time of sampling appear to be as important as the dose of catecholamine employed to demonstrate liver phosphorylase activation.

In the investigations mentioned above in which no liver phosphorylase activation was observed after epinephrine administration, the control phosphorylase levels reported were in the range of 9 to 10 μM inorganic phosphate/g·min⁻¹,^{4, 5, 18}. This is somewhat higher than the control values (2.8 to 4.9 μM inorganic phosphate/g·min⁻¹) reported in the present investigation and may be sufficiently high so that an epinephrine-induced activation would be masked. Also, in the investigations in which phosphorylase activation could not be demonstrated after epinephrine administration, tissue samples were not usually taken before 30 min after epinephrine injection. As may be seen in Table 2, at this time a considerable portion of the catecholamine-induced phosphorylase activation was dissipated.

The problem of what constitutes a "reasonable" control phosphorylase value for liver has not been extensively discussed. It has been generally found that liver slices have lower phosphorylase levels than those values obtained after tissue sampling *in vivo*.⁵ An exact level of active liver phosphorylase as a percentage of the total enzyme present is difficult to determine because inactive liver phosphorylase is not stimulated to activity by the addition of adenosine monophosphate.¹⁶ From our data with epinephrine stimulation of rat liver phosphorylase, it appears that the total liver phosphorylase activity is in the range of 15 to 20 μM inorganic phosphate/g·min⁻¹ assayed at 30°. If it is further assumed that the active liver phosphorylase is present *in vivo* in the same relationship to total enzyme content as occurs in heart and skeletal muscle (0 to 12% active phosphorylase),^{15, 19, 20} then the control liver phosphorylase levels *in vivo* would be approximately 0.0 to 2.4 μM inorganic phosphate/g·min⁻¹. Our control values for liver phosphorylase *in vivo* approach this range. Cornblath¹⁶ has reported liver phosphorylase values of this magnitude for rabbit liver slices *in vitro*. Other investigators using both *in-vitro*¹⁷ and *in-vivo* sampling^{4, 5, 18} have reported higher control phosphorylase levels for rat liver. A possible explanation for some of these differences in control phosphorylase levels may be that a conversion of inactive phosphorylase to active phosphorylase occurs after excision of the liver even though the tissue is maintained at 0°. A similar conversion of inactive to active phosphorylase during homogenization has been reported for skeletal muscle¹⁹ and heart⁶ samples.

The relative potencies of the catecholamines for rat liver phosphorylase activation *in vivo* appear to be L-epinephrine > L-norepinephrine > OL-isoproterenol. This order of potency has also been observed for the hyperglycemic response to catecholamines in the intact rat²¹ and glucose production by rat liver slices.²² A different order of potency for glycogen mobilization has been previously determined for rat heart⁹ (I > E = NE) and rat skeletal muscle²³ (E > I > NE). Mayer *et al.*⁶ determined that catecholamine potency ratios for hyperglycemia in the intact dog are I = E > NE. A similar order of catecholamine potency was reported by Sutherland and Rall¹ for phosphorylase activation in dog liver homogenate and by Murad *et al.*¹³ for cyclic 3', 5'-adenosine monophosphate production in the washed particulate fraction of dog liver. It would therefore appear, on the basis of sensitivity of the liver phosphorylase activation to catecholamines, that a considerable species difference exists between the adrenergic receptor of the rat and dog liver. The insensitivity of rat liver phosphorylase to isoproterenol administration is further demonstrated by the fact that the dose used in this

investigation is ten times higher than that necessary to produce a marked activation of heart and skeletal muscle phosphorylase.⁹

The time course of the liver phosphorylase activation after subcutaneous catecholamine administration is different from that observed in rat heart and skeletal muscle.⁹ In the latter tissues, peak phosphorylase activation occurred at 5 min or earlier after catecholamine injection. In the liver, peak phosphorylase activation did not occur until 10 min after injection and was decreased 30 min after injection. In contrast to heart and skeletal muscle phosphorylase, the liver phosphorylase time-response curve did not parallel the plasma epinephrine level which we have previously reported to be maintained at a relatively constant concentration (approximately 9 $\mu\text{g/l}$) from 5 to 60 min after a subcutaneous epinephrine injection of 500 $\mu\text{g/kg}$.⁹ An explanation for this delayed response in the liver is not apparent. It is possible that catecholamine-induced vascular changes in the liver altered the drug distribution pattern leading to a gradual rise in the concentration of the catecholamine at the receptor site.

The relationship of the blood glucose changes to the alteration in liver phosphorylase after catecholamine administration suggest that a change in blood glucose is not indicative of the degree or duration of a liver phosphorylase response. In fact, the blood glucose level after epinephrine injection was still increasing at 1 hr after epinephrine, even though the phosphorylase activity had declined somewhat at 30 min. In addition, the blood glucose responses to norepinephrine and isoproterenol did not mirror the responses of liver phosphorylase. Cahill *et al.*²⁴ have reported a similar lack of correlation between liver phosphorylase activation and blood glucose changes after glucagon infusion in the dog.

The classification of the liver phosphorylase response to epinephrine as either an α - or β - or both an α - and β -adrenergic receptor appears to be somewhat difficult. The inability of phenoxybenzamine to cause more than a partial reduction in the epinephrine-induced increase in liver phosphorylase, whereas ergotamine completely abolished the response, suggests that the usual α -adrenergic receptor is not involved in the epinephrine action. Harvey *et al.*²⁵ reached a similar conclusion based upon the dissociation between the relative abilities of phenoxybenzamine and ergotamine to block the vasoconstrictor response and the hyperglycemia observed after epinephrine administration.

In addition, the liver phosphorylase response was reduced by the β -adrenergic blocking agents, DCI and nethalide. Both of these compounds caused a partial, and in several animals a complete, blockade of the rat liver phosphorylase response to epinephrine. Treatment with both α - and β -receptor blocking agents did not aid in defining the nature of the liver adrenergic receptor. Although the catecholamine potency ratio for rat liver phosphorylase activation *in vivo* ($\text{E} > \text{NE} > \text{I}$) fits that order postulated by Furchgott²⁶ necessary to define an α -adrenergic receptor, we do not feel that a definite classification can be determined at this time.

The experiments with hexamethonium treatment demonstrate that depression of the autonomic nervous system, at a site other than the postganglionic adrenergic receptor, does not alter the epinephrine-induced liver phosphorylase activation.

The phosphorylase data presented above for the interaction of adrenergic blocking agents with epinephrine is in general agreement with the data of Kennedy and Ellis,²⁷ who measured tissue glycogen levels after epinephrine administration and α - and β -adrenergic blocking agents.

The skeletal muscle receptor for phosphorylase activation by catecholamines appears to be classified as a β -adrenergic receptor both with respect to relative potencies of the catecholamines and the comparative ability of the various adrenergic blocking agents to block the epinephrine response. The blockade of skeletal muscle phosphorylase activation produced by DCI and methalide is dependent on the dose of catecholamine employed. The phosphorylase activation produced by the subcutaneous injection of 50 mg of isoproterenol/kg was not blocked in skeletal muscle by 5 mg of DCI/kg, whereas in the same animal the isoproterenol-induced cardiac phosphorylase activation was abolished (unpublished observations). This dependence of DCI blockade in skeletal muscle on the test dose of catecholamine employed may help explain why it has been difficult to demonstrate consistent blockade of catecholamine-induced lactic acidemia.⁶

The dosage and time schedule of epinephrine infusion used in this investigation did not cause a significant reduction in the glycogen level of either liver or skeletal muscle. We have previously observed decreased glycogen levels in skeletal muscle at this time after epinephrine administration, using a different dose and route of administration.⁹

It is possible to estimate the glycogen mobilization from the published values for phosphorylase activities in liver and skeletal muscle (assayed in the direction of glycogen breakdown).²⁸ In liver, phosphorylase activity of $5 \mu\text{moles/g}\cdot\text{min}^{-1}$ (which corresponds to a phosphorylase activity of approximately 10 to $15 \mu\text{moles/g}\cdot\text{min}^{-1}$ assayed in the direction of glycogen synthesis) would mobilize 9 mg glycogen/g wet weight liver in 10 min. This glycogen loss would be difficult to detect in fed rats and may explain why significant glycogen loss is more easily detected in fasted rats which have lower initial glycogen levels. In skeletal muscle, however, the level of phosphorylase activity after epinephrine administration (assuming 65% activation) is $18 \mu\text{moles/g}\cdot\text{min}^{-1}$. This should mobilize glycogen at the rate of $3.6 \text{ mg glycogen/g wet weight}\cdot\text{min}^{-1}$. That this rate of glycogen loss is not approached, in this or a previous report on catecholamine-induced skeletal muscle glycogenolysis,⁹ further emphasizes that factors other than the level of tissue phosphorylase activation control the degree and rate of glycogen loss in skeletal muscle.

One possibility is that glycogen synthesis by the uridinediphosphoglucose-transglycolase pathway is sufficient to compensate for the glycogen loss. However, the maximal tissue levels of skeletal muscle glycogen synthetase activity (assayed in the presence of glucose-6-phosphate, $2.0 \mu\text{moles/g}\cdot\text{min}^{-1}$)^{29,30} would appear to be considerably lower than those necessary to compensate for glycogen breakdown by phosphorylase activation.

The importance of factors other than the level of tissue phosphorylase in regulating glycogen metabolism has been noted recently by us⁹ and by others.³¹⁻³⁴

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