IN VIVO RESPONSE OF SKELETAL MUSCLE GLYCOGEN PHOSPHORYLASE, PHOSPHORYLASE b KINASE AND CYCLIC AMP TO EPINEPHRINE ADMINISTRATION[1]

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Introduction

Present hypothesis suggests that epinephrine stimulates glycogenolysis by the following reaction sequence:

(a) Epinephrine stimulates adenosine-3',5'-phosphate (cyclic AMP) production from ATP (Rall and Sutherland, 1958, Sutherland and Rall, 1960)

(b) Cyclic AMP, with ATP, activates phosphorylase b kinase (Krebs, et al, 1959)

(c) Active phosphorylase b kinase, converts phosphorylase b (inactive) to phosphorylase a (active) (Krebs and Fischer, 1956)

(d) Phosphorylase a catalyzes glycogen breakdown to glucose-1-phosphate. (Cori, et al, 1943)

This paper tests the above hypothesis in skeletal muscle in vivo and demonstrates that in the intact animal epinephrine injection is followed by increased cyclic AMP levels, phosphorylase b kinase activation and phosphorylase a formation.

Methods

Adult Sprague-Dawley rats were anesthetized and the gastrocnemius muscles exposed bilaterally. One muscle was quickly removed and frozen

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in isopentane precooled to -160°C. Epinephrine, 10 µg./kg., was then injected into the heart and 60 seconds later the second gastrocnemius muscle was removed and frozen. Control animals received the same treatment except that Ringer's solution replaced the epinephrine.

A 200-300 mgm. portion of each muscle was powdered in a precooled tissue pulverizer and then extracted at -30°C (Danforth, et al., 1962) in 1cc of a 60% glycerin solution containing .02 M NaF, .005M EDTA, .03 M cysteine and .04 M Na glycerophosphate, pH 6.8. The suspension was then diluted to appropriate volume (7-8 cc) in an aqueous solution of the same salts, centrifuged, and the supernatant fluid assayed for phosphorylase and phosphorylase b kinase (see below). A second portion of each muscle (approximately 1 gram) was pulverized and extracted in 4 volumes of water at 100°C for 5 minutes. The suspension was then centrifuged and the supernatant fluid heated at 100°C for 5 minutes in 0.1 N. NaOH to destroy a cyclic AMP inhibitor. The alkaline extract was neutralized with HCl and assayed for cyclic AMP (see below).

Glycogen phosphorylase was assayed using Cori's method. (Cori, et al., 1943) The results are expressed as the ratio of phosphorylase units assayed without AMP (phosphorylase a) to those assayed with AMP (total phosphorylase). Phosphorylase b kinase was assayed by the method of Krebs et al., (1959). These results are expressed as the ratio of phosphorylase b kinase units assayed at pH 6.8 to those assayed at pH 8.2. Cyclic AMP in the boiled, alkali treated extract was assayed utilizing the specific accelerating effect of this nucleotide on the activation of purified phosphorylase b kinase. (Krebs, et al., 1959, Krebs and Fischer, 1960) This assay's specificity and reliability were checked by separating cyclic AMP from the boiled muscle extract chromatographically and by using purified cyclic AMP phosphodiesterase kindly supplied by Dr. R. W. Butcher.

Results

Results are shown in the table. Epinephrine injection was followed by a marked rise in the ratio of phosphorylase a to total phosphorylase

and a similarly striking rise in the ratio of kinase units at pH 6.8 to those at pH 8.2. These increases were present in all animals and were accompanied in each instance by an increase in the level of cyclic AMP. All these changes were statistically significant. Control animals showed no significant change in activity of the three substances measured.

TABLE

RESPONSE TO EPINEPHRINE INJECTION

Exp. No.	GLYCOGEN PHOSPHORYLASE ACTIVITY Ratio $\frac{\text{Without AMP}}{\text{With AMP}}$		PHOSPHORYLASE b KINASE ACTIVITY Ratio $\frac{\text{pH 6.8}}{\text{pH 8.2}}$		CYCLIC AMP μ moles kg. muscle.	
	CONTROL	EPINEPHRINE	CONTROL	EPINEPHRINE	CONTROL	EPINEPHRINE
I	.17	.74	.04	.20	0.52	1.3
II	.07	.42	.14	.40	1.8	3.0
III	.13	.66	.04	.31	0.42	2.6
IV	.11	.52	.10	.34	0.38	0.99
V	.07	.40	.06	.30	0.44	1.8
Mean ±S.D	.11±.04	.55±.15	.08±.04	.31±.07	0.71±.54	1.9±.85
	$P<.01$		$P<.01$		$P<.02$	
					(0.44±.05)	(1.7±.61)
					($P<.01$)*	

* Values in parenthesis are calculated omitting experiment No. II

Discussion

The present study provides the first direct evidence that an increase in skeletal muscle phosphorylase a caused by epinephrine administration can be correlated with increased tissue cyclic AMP levels and changes in phosphorylase b kinase activity. That epinephrine raises phosphorylase a levels when administered in vivo has been previously demonstrated. (Cori and

Illingworth, 1956) Whether epinephrine exerts its physiologic effect on phosphorylase through phosphorylase b kinase activation or phosphorylase phosphatase inhibition is unknown although kinetic studies (Danforth, et al., 1962) suggest the former mechanism. The current data clearly demonstrates that epinephrine increases kinase activity measured at pH 6.8 relative to that measured at pH 8.2. This can be interpreted as kinase activation, since previous work in this laboratory (Krebs, et al., 1959) has shown that this enzyme exists in two activity states, the less active characterized by a lower pH 6.8 to 8.2 ratio than the activated kinase. Until now these kinase forms had not been correlated with physiologic function. Although epinephrine is known to stimulate cyclic AMP production in particulate fractions of liver (Sutherland and Rall, 1960) and in other tissue preparations in vitro, no data exists on cyclic AMP response to epinephrine in vivo. The increases in tissue cyclic AMP levels shown in this paper are not surprising in view of the in vitro evidence of Sutherland and his co-workers that this nucleotide constitutes a link in epinephrine induced phosphorylase activation.

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