EPINEPHRINE INDUCED FORMATION OF ADENOSINE 3' ,5' -MONOPHOSPHATE IN MOUSE SKELETAL MUSCLE

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Most investigators agree that muscle glycogenolysis, stimulated by epinephrine, proceeds through a series of reactions that begin with the acceleration of adenyl cyclase and an increased formation of adenosine 3' ,5' -monophosphate (cyclic AMP). Cyclic AMP apparently binds to phosphorylase kinase kinase (1), and this enzyme converts phosphorylase kinase from a form that is nearly inactive at pH 7 to an active form. The active kinase converts phosphorylase b to phosphorylase a, the active form, and glycogen breakdown obtains. The evidence has been comprehensively reviewed (2,3).

In adult mice of the I/FnLn strain, neither phosphorylase kinase nor phosphorylase a can be detected, yet glycogen breakdown in skeletal muscle occurs following either epinephrine (4) or electrical (5) stimulation. Hybrid mice derived from crosses of the I and the C_{57} strains also lack these two enzymes (6). The most likely explanation for glycogenolysis in I strain mice is that phosphorylase b, which is present, is activated by AMP.

This study was initiated to examine the first phase of stimulation by epinephrine in I strain mice, the production of cyclic AMP. Further, more information on cyclic AMP formation in skeletal muscle would be of some interest. Only one study has been published (7); a concomitant increase in cyclic AMP, and a conversion of phosphorylase kinase and phosphorylase to the active forms were demonstrated, but only one time point after epinephrine was examined.

EXPERIMENTAL PROCEDURE

Nonfasted male mice of the I/FnLn and the C_{57}BL/FnLn strains were anesthetized (4) between 9 and 10 a.m. One gastrocnemius muscle was removed and frozen (4,6), and the contralateral muscle was exposed. A single dose of epinephrine (5 μg/kg body weight in a volume of 0.05 ml/10 g body weight) was immediately injected into the tail vein. Within 20 seconds the second muscle was removed and frozen. The time was recorded from the completion of the injection to the moment that the muscle was frozen.

The epinephrine effect is defined as the difference in cyclic AMP found in the two muscles from each animal. An increase in cyclic AMP was found in all mice given epinephrine. Injections of 0.85% NaCl into the tail vein of C_{57}-mice produced no change; the average cyclic AMP content in the muscle of four mice before injection, and 11 to 15 seconds after injection, was 2.04 ± 0.27 and 2.05 ± 0.53 (S.E.M.) μmoles/kg muscle respectively.

Cyclic AMP was assayed by a modification (8) of the procedure described by Posner et al (9) following extraction and treatment with trypsin (9). When these extracts were incubated with nucleotide 3' ,5'-phosphodiesterase[1] some residual stimulation of the assay system, equivalent to cyclic AMP, could be detected; values of 0.83 ± 0.12 and 0.78 ± 0.06 μmoles/kg muscle were found for I and C_{57} strain mice respectively. Since the same values were found in muscle before and after epinephrine a correction was not made.

The conversion of phosphorylase kinase and phosphorylase to the active forms following the tail vein injection of epinephrine (described above) was determined by described procedures (4,6) in male C_{57} strain mice and in F_1 females derived from crosses of the I and the C_{57} strains. The data were similar for the two groups and were averaged together.

RESULTS AND DISCUSSION

The results, shown in Table 1, demonstrate that cyclic AMP increases

1. A gift from Drs. R. E. Butcher and J. G. Hardman, Vanderbilt University.

rapidly after epinephrine and declines sharply, but the time course of the response was different in the two strains of mice. In the I strain a maximal response was evident within 10 seconds, but a response of the same magnitude did not occur in the C_{57} strain until 14 seconds after epinephrine. Further, the peak response was maintained longer in the I-mice. It is conceivable that hereditary differences in the rate of activation of adenyl cyclase, or in the

TABLE 1

CYCLIC AMP IN MOUSE SKELETAL MUSCLE AFTER EPINEPHRINE

Seconds Post Epinephrine		No.of Mice	Increase Cyclic AMP	P-value[1]
Range	Mean		μmoles/kg	
		I STRAIN MICE		
Initial Concentration[2]		25	2.51 ± 0.28[3]	
6.6 - 8.8	7.5	4	1.70 ± 0.56	
9.2 - 13.3	11.5	12	6.10 ± 0.58	< 0.01
14.0 - 15.0	14.7	4	3.22 ± 1.49	= 0.04
15.8 - 18.4	16.6	5	3.53 ± 1.18	> 0.10
		C_{57} STRAIN MICE		
Initial Concentration		30	1.95 ± 0.21	
4.0 - 8.6	5.9	10	0.67 ± 0.12	
9.8 - 13.2	10.8	10	2.23 ± 0.46	= 0.02
14.0 - 15.2	14.8	7	5.02 ± 0.66	< 0.01
16.2 - 17.0	16.5	3	1.91 ± 0.53	< 0.01

1. P-value for the difference between averages of the cited value and the one directly above it within each strain.

2. Average for all muscle removed before epinephrine administration.

3. Standard error of the mean.

activity of nucleotide 3' ,5'-phosphodiesterase are responsible.

The observation that cyclic AMP increases rapidly in I-mice indicates that the first phase of epinephrine action proceeds in these mutant animals according to the currently accepted theory. Moreover, the rate coincides nicely with the rate of epinephrine response in the isolated perfused heart (10,11,12). However, the mechanism whereby the increase in cyclic AMP activates phosphorylase in these mice, assuming that it does, must remain speculative for the present time.

Phosphorylase in mouse skeletal muscle was activated maximally within 10 seconds by epinephrine (6). The data in Table 2 confirm this observation, and, in addition, show that phosphorylase kinase, measured at pH 6.8, increases simultaneously. Thus, the slower rate of cyclic AMP formation in C_{57}-mice was unexpected. Clearly, the maximum concentration of cyclic AMP (Table 1) did not occur until 5 to 6 seconds after the maximal activation of both phosphorylase kinase and phosphorylase had been attained (Table 2).

However, in spite of this lag period, it seems reasonable to assume at the present time that the phosphorylase system is activated by cyclic AMP in C_{57}-mice. Several observations support this conclusion: (i) Cyclic AMP did increase between 4 and 13 seconds, and one-half the maximum concentration was attained within 10 seconds (Table 1). (ii) The concentration of cyclic AMP found in mouse skeletal muscle at rest, and after stimulation by epinephrine, is several times greater than that found in other species (7,8). (iii) The dose of epinephrine was large and consequently cyclic AMP could have been produced in excess, and perhaps because epinephrine was not rapidly removed from the receptor site. (iv) Even small amounts of cyclic AMP should be adequate to activate the phosphorylase system since it has been shown that the activation of phosphorylase kinase is autocatalytic (13).

On the other hand, alternative mechanisms should be considered. For example, electrical stimulation does not increase the formation of cyclic AMP in skeletal muscle, yet both phosphorylase kinase and phosphorylase are

TABLE 2

EPINEPHRINE ACTIVATION OF PHOSPHORYLASE AND PHOSPHORYLASE KINASE IN MOUSE SKELETAL MUSCLE[1]

Seconds Post Epinephrine		No.of Mice	Phosphorylase[2]	Phosphorylase[3] Kinase
Range	Mean		% A	6.8/8.5
4.4 - 5.0	4.8	3	17.0 ± 4.9[4]	0.38 ± 0.05
8.0 - 11.6	10.2	6	52.5 ± 4.5	0.67 ± 0.04
16.4 - 18.0	17.4	4	51.0 ± 7.4	0.69 ± 0.03
20.0 - 29.0	24.2	3	41.9 ± 4.9	0.57 ± 0.02

1. Averages of values from male C_{57} strain mice and F_1 females derived from crosses of C_{57} and I strain mice.
2. Phosphorylase (-AMP) / Phosphorylase (+ AMP) X 100.
3. Ratio of phosphorylase kinase measured at pH 6.8 to that at pH 8.5.
4. Standard error of the mean.

activated (7). Moreover, the activity of phosphorylase kinase is dependent upon calcium (12). Thus, mechanisms of activation of these two enzymes other than through the mediation of cyclic AMP are possible.

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