MOLECULAR PHARMACOLOGY, 8, 780-785

SHORT COMMUNICATION

Adrenal Corticosteroids and the Response to Epinephrine or Electrical Stimulation of the Enzymes of Glycogen Metabolism in Rat Skeletal Muscle

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(Received June 19, 1972)

SUMMARY

Previous studies have shown that epinephrine administration to adrenalectomized rats does not elicit conversion from the *b* to the *a* form of skeletal muscle glycogen phosphorylase (α-1,4-glucan:orthophosphate glucosyltransferase, EC 2.4.1.1) to the same extent as in normal animals. Extension of these results has shown that while conversion from the *I* to the *D* form of glycogen synthetase (UDP-glucose:glycogen α-4-glucosyltransferase, EC 2.4.1.11) is also impaired in adrenalectomized rats, conversion of phosphorylase *b* kinase (ATP:phosphorylase phosphotransferase, EC 2.7.1.37) from the nonactivated to the activated form (pH 6.8:8.2 ratio of activities) is unaffected by the deficiency of adrenal corticoid hormones. Administration of hydrocortisone or deoxycorticosterone restores the normal response of both glycogen phosphorylase and synthetase to epinephrine. Tetanic electrical stimulation *in vivo* brings about conversion of phosphorylase *b* to *a* and of synthetase *I* to *D*, even in adrenalectomized rats. These findings suggest that the defect due to adrenalectomy does not lie in a decreased amount of one of the converting enzymes, but rather in some factor(s) governing the activity of one (or more) of them.

The sequence of events in the pathway leading to glycogenolysis and inhibition of glycogen synthesis in response to epinephrine are relatively well understood (see ref. 1 for a recent review). A specific site for the permissive action of adrenal corticoids on the response of glycogen phosphorylase to epinephrine has been suggested on the basis of investigations on liver (2) and cardiac muscle (3), but not for sekeletal muscle. Schaeffer *et al.* (4) did find that the response of muscle glycogen phosphorylase to epinephrin injection (*b* to *a* conversion) was impaired in adrenalectomized rats. The deficient epinephrine response was found not

This investigation was supported in part by Grant 3078 from the Consejo Nacional de Investigaciones Científicas y Técnicas and a grant from the Instituto Nacional de Farmacología y Bromatología.

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to occur at the adenylate cyclase level. Therefore the adrenalectomy-induced defect(s) may lie somewhere in the "cascade" (5) between the site of cyclic AMP action and glycogen phosphorylase activation.

We previously showed that 4 min after tetanic stimulation (10 sec) of rat skeletal muscle *in situ*, glycogen synthetase was mainly in the glucose-6-P-independent form, and glycogen phosphorylase in the *b* form (6). This condition is suited to the detection of epinephrine-induced conversion of glycogen synthetase from the *I* to the *D* form, and of phosphorylase from the *b* to the *a* form. We have therefore investigated the degrees of conversion of phosphorylase *b* kinase, glycogen phosphorylase, and glycogen synthetase, produced either by epinephrine or by electrical stimulation in normal and adrenalectomized rats. Epinephrine and electrical stimulation act at different levels in the glycogenolytic pathway (7). Their effect on these enzymes should give additional information characterizing the site(s) of corticoid action in skeletal muscle. A preliminary report of these findings has been presented (8).

The results obtained by epinephrine injection to normal and adrenalectomized rats, without treatment or after administration of hydrocortisone or deoxycorticosterone acetate, are shown in Table 1. In normal intact rats glycogen synthetase *I* activity decreased appreciably upon epinephrine injection. In contrast, in adrenalectomized rats the change after epinephrine injection was only one-third of that observed in normal animals. Control muscles from normal and adrenalectomized rats had a similar glycogen synthetase *I:D* ratio (0.45–0.49). Removal of the muscle from one leg (control) did not affect the basal enzymatic activities or responses to epinephrine of the second leg; glycogen synthetase activity was constant during the sampling period for control and epinephrine-stimulated muscles (65 sec). The synthetase response to tetanic stimulation was identical in normal and adrenalectomized rats, at least during the 1-min period used in the present study (Table 2). Administration of hydrocortisone (a glucocorticoid) or deoxycorticosterone acetate (a mineralcorticoid) restored the normal response to epinephrine. This restoration could not be achieved by acute treatment (1–2 hr), but required administration of the corticosteroids for at least 2–3 days. Total glycogen synthetase activity remained constant throughout (0.9 μmole of glucose incorporated into glycogen per minute per gram of muscle).

Epinephrine injection to normal, adrenalectomized, and hydrocortisone-treated adrenalectomized rats produced changes in the phosphorylase *a* content similar to those reported by Schaeffer *et al.* (4). Deoxycorticosterone acetate or hydrocortisone restored the response to epinephrine of adrenalectomized rats. Phosphorylase *b* kinase, however, showed similar changes in the pH 6.8:8.2 ratios in all four groups, indicating that adrenalectomy did not affect the response of this enzyme to epinephrine stimulation. In addition, the phosphorylase *b* kinase activity of adrenalectomized rats at pH 8.2 was similar to that of control animals (13,000 units/of muscle). The relatively high pH 6.8:8.2 ratios observed in control muscles were not due to the previous tetanic stimulation, since similar ratios were obtained with unstimulated muscles. Moreover, these values are of the same order as those found by Posner *et al.* (11) in a similar system.

The results of tetanic electrical stimulation are shown in Table 2. Electrical stimulation produced similar changes in the three groups for both glycogen synthetase and glycogen phosphorylase. No effects of adenalectomy or adrenal steroids were observed.

The activity at pH 6.8 attained by phosphorylase *b* kinase upon epinephrine stimulation is similar in normal and adrenalectomized rats (Table 1), indicating that the impairment present in muscles from adrenalectomized animals does not occur at the level of protein kinase (acting as phosphorylase *b* kinase kinase) but rather involves some factor(s) necessary for the normal catalysis of the conversion of phosphorylase *b* to *a*. Namm *et al.* (12) have shown that Ca^{2+} is necessary for the epinephrine-induced conversion of heart phosphorylase *b* to *a*. On the other hand, Miller *et al.* (3) found that per-

TABLE 1

Effect of epinephrine treatment on glycogen synthetase, glycogen phosphorylase, and phosphorylase b kinase of rat skeletal muscle

Sprague-Dawley rats (200 g) were adrenalectomized under pentobarbital anesthesia 10–14 days before the experiment, and received food and 0.9% NaCl ad libitum thereafter. Adrenalectomized rats were divided into three groups: (a) adrenalectomy; (b) adrenalectomy plus hydrocortisone (1 mg/100 g subcutaneously, twice a day for 3 consecutive days prior to the experiment); and (c) adrenalectomy plus deoxycorticosterone acetate (2 mg/100 g subcutaneously once a day for 3 days). Rats were anesthetized with sodium pentobarbital (4 mg/100 g) and subjected to tracheotomy, cannulation of the jugular vein, and isolation of the thigh muscles (9). In order to obtain low initial values of glycogen phosphorylase *a* and high levels of glycogen synthetase *I*, a 10-sec tetanic electrical stimulation was first applied to the sciatic nerves of both hind legs. Four minutes after the end of this stimulation the muscle from one leg was removed and instantly frozen to serve as a control. The principal blood vessels were clamped to avoid bleeding, and epinephrine was then injected (1.5 μg/100 g) through the jugular vein; after 1 min the other muscle was removed. Isolation of thigh muscles *in situ*, tetanic stimulation, sampling, preparation of homogenates, and assays for glycogen synthetase and phosphorylase were performed as previously described (9). Phosphorylase *b* kinase was measured at pH 6.8 and 8.2 by the method of Krebs *et al.* (10). The levels of the three enzymes obtained from the muscles of normal and adrenalectomized rats which received 0.9% NaCl instead of epinephrine were similar to those of control muscles. The number of experiments is indicated in parentheses, and the values are averages ± standard errors of the mean. Statistical significance has been calculated for the difference (Δ) between paired determinations.

Treatment	Glycogen synthetase (−glucose-6-P:+glucose-6-P) × 100				Glycogen phosphorylase (−AMP:+ AMP) × 100				Phosphorylase *b* kinase (pH 6.8:8.2) × 100			
	Control	Epinephrine	Δ	*p*	Control	Epinephrine	Δ	*p*	Control	Epinephrine	Δ	*p*
None	(9) 45.1 ± 4.3	30.7 ± 2.9	14.4 ± 2.5	<0.01	(8) 5.1 ± 0.8	37.5 ± 4.1	32.4 ± 3.4	<0.001	(6) 19.2 ± 2.4	33.8 ± 3.6	14.6 ± 2.0	<0.02
Adrenalectomy	(16) 49.0 ± 1.6	43.1 ± 1.4	5.9 ± 1.5	<0.05	(15) 5.3 ± 1.3	10.8 ± 2.1	5.5 ± 1.4	<0.05	(10) 19.0 ± 4.2	37.1 ± 4.6	18.1 ± 2.4	<0.02
Adrenalectomy + hydrocortisone	(9) 46.4 ± 1.1	29.1 ± 2.4	17.3 ± 1.9	<0.01	(9) 6.3 ± 1.3	41.1 ± 3.4	34.8 ± 3.4	<0.001	(5) 16.6 ± 4.5	38.6 ± 3.8	22.0 ± 3.5	<0.02
Adrenalectomy + Deoxycorticosterone acetate	(11) 47.6 ± 2.2	30.3 ± 2.0	17.3 ± 1.9	<0.01	(11) 3.5 ± 1.3	34.9 ± 3.4	31.4 ± 2.3	<0.001	(7) 15.7 ± 4.3	38.3 ± 4.3	22.6 ± 1.5	<0.01

TABLE 2

Effect of electrical stimulation on glycogen synthetase and glycogen phosphorylase of skeletal muscles from normal and adrenalectomized rats

Experimental conditions are the same as those described in Table 1. After removal of the control muscle, a second tetanic stimulation (10 sec) was applied and the other muscle was removed. Similar results were obtained with normal and adrenalectomized rats if the control muscle was removed after the one which received the stimulation.

Treatment	Glycogen synthetase (−glucose-6-P:+glucose-6-P) × 100				Glycogen phosphorylase (−AMP:+AMP) × 100			
	Control	Stimulated	Δ	*p*	Control	Stimulated	Δ	*p*
None	(7) 45.7 ± 4.4	33.8 ± 3.4	11.9 ± 2.6	<0.02	(7) 8.4 ± 1.7	39.9 ± 3.2	31.5 ± 3.4	<0.001
Adrenalectomy	(8) 49.0 ± 1.8	37.4 ± 1.9	11.6 ± 2.0	<0.02	(8) 9.5 ± 1.8	38.3 ± 2.9	28.8 ± 2.3	<0.001
Adrenalectomy + hydrocortisone	(7) 41.7 ± 3.4	29.9 ± 3.2	11.8 ± 2.0	<0.02	(8) 4.9 ± 1.3	34.5 ± 5.5	29.6 ± 4.6	<0.001

fusion of hearts from adrenalectomized rats with Ringer's solution containing elevated levels of Ca^{2+} could restore the normal response of phosphorylase to epinephrine. These results *in vivo* agree with the observations *in vitro* of Brostrom *et al.* (13), who have shown that both activated and nonactivated phosphorylase *b* kinases require Ca^{2+} for activity, the latter at a somewhat higher concentration. Therefore it is plausible to ascribe the impairment in skeletal muscles of adrenalectomized rats to a deficiency of Ca^{2+} at the site of enzymatic conversions. In this regard Stull and Mayer (14) have recently hypothesized that catecholamines affect Ca^{2+} translocation in skeletal muscle, thus explaining the stimulation by isoproterenol they observed for phosphorylase *a* formation, without changes in phosphorylase *b* kinase pH 6.8:8.2 activity ratios. If catecholamines indeed act through this mechanism, one can further speculate that corticosteroid hormones play a "permissive" role in the catecholamine-promoted translocation of Ca^{2+}, either across the plasma membrane or within the muscular fiber. Whether through this mechanism, by directly maintaining the proper Ca^{2+} levels, or some other means, corticosteroids do play a role in allowing certain enzymatic conversions to take place, at least under conditions of *limiting* Ca^{2+} concentration.[5] In fact, both a glucocorticoid (hydrocortisone) and a mineralcorticoid (deoxycorticosterone acetate) reversed the effect of adrenalectomy on the epinephrine-induced conversions of glycogen synthetase and glycogen phosphorylase. On the other hand, the deficient conversions of these enzymes found in the absence of corticoid hormones were overcome by the rather energetic stimulus of tetanic contraction. In this situation intracellular Ca^{2+} rises to 1–10 μM (15, 16), thereby perhaps overcoming any ionic defect that might be present in the muscles of adrenalectomized rats. The possibility has to be considered, however, that with more moderate electrical stimulation the enzymatic response might be attenuated in adrenalectomized animals.

The responses of glycogen synthetase and phosphorylase to both electrical stimulation and epinephrine are reciprocal, regardless of the experimental condition of the animals used. This indicates that the two systems are interlocked at a common site and/or by a common factor. The first possibility follows from the finding of Soderling and coworkers (17) that protein kinase has the activities of both synthetase *I* kinase as well as of phosphorylase *b* Kinase kinase. However, conversion of phosphorylase *b* kinase to the activated state is normal in adrenalectomized animals (Table 1), indicating that protein kinase activity is not affected. The question then arises why conversion of synthetase *I* to *D* is impaired in adrenalectomized rats. Perhaps phosphorylase *b* kinase kinase and glycogen synthetase kinase are not the same enzyme, or the requirements for action of protein kinase as phosphorylase *b* kinase kinase and as glycogen synthetase kinase are not identical.

Present evidence seems to be rather heavily against the first possibility (17–19). On the other hand, protein kinase does not seem to require Ca^{2+} (20), but conversion of glycogen synthetase by electrical stimulation is as rapid as that of phosphorylase (6) (Table 2), suggesting that Ca^{2+} might also play a role in the synthetase *I* to *D* conversion. These observations, together with the dissociated response of phosphorylase *b* kinase and glycogen synthetase found in this study, indicate that more work is necessary to understand the nature of the interaction, under various physiological conditions, between the enzymes involved in the synthesis and degradation of glycogen.

[5] Intracellular free Ca^{2+} of resting muscles (15, 16) is already in the lower range for phosphorylase *b* kinase activity (13), and therefore any further decrease would seriously impair the activity of this enzyme.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Marta M. Hernandez for skillful help in the course of this work, and to the members of the Instituto de Investigaciones Bioquímicas for advice and criticism.

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