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Polyamine biosynthetic decarboxylases in muscles of rats with different experimental myopathies

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SUMMARY

The activities of the two polyamine biosynthetic decarboxylases (PBD), L-ornithine decarboxylase (ODC) and *S*-adenosyl-L-methionine decarboxylase (SAMD), have been measured in quadriceps femoris of rats killed at different times after the induction of calciphylaxis- or serotonin(5-HT)-induced myopathy. Decreases in both PBD levels were observed at early times after both myotoxic treatments. Subsequent progressive increases in both enzyme levels were observed to nearly control values by 4 days after 5-HT administration. In the 5-HT-treated rats, the effects on the myocardial PBD activities were different from those in skeletal muscle, with no effect on ODC but much on SAMD, when rats were killed shortly after 5-HT injection. These results demonstrate that the time-course of the changes in PBD activities in quadriceps femoris mirrors quite well the successive occurrence of degenerative and regenerative processes during the calciphylaxis-induced myopathy and the 5-HT-induced myopathy; it is 5-HT that is mainly responsible for the decreases in PBD levels observed in both experimental myopathies, since dihydrotachysterol alone was without any effect on PBD activity levels and 5-HT alone was effective; myocardial ODC reacts more slowly to 5-HT than quadriceps femoris ODC.

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INTRODUCTION

A possible causal relationship between biogenic amines and the pathogenesis of some experimental myopathies of skeletal muscles in mammals is still being debated. It is well known that the administration of serotonin (5-HT), alone or in combination with other drugs (e.g., dihydrotachysterol (DHT)) or with aortic ligation, causes diffuse damage in the skeletal muscles of rats and mice (Canal et al. 1969, 1983; Parker and Mendell 1974; Munsat et al. 1977; Takamori 1977; Kelts and Kaiser 1979; Fowler et al. 1985). In more detail, a peculiar muscular injury can be produced by administering 5-HT to rats previously treated with DHT (Canal et al. 1969; Pelosi et al. 1972). From the histological point of view, the ensuing experimental myopathy is very similar to human polymyositis (Pelosi et al. 1972). From the biochemical point of view, marked decreases in some muscular extramitochondrial enzymes have been previously reported (Pelosi et al. 1972). In the experimentally induced myopathies, noticeably in those induced by 5-HT, prominent regenerative processes follow the muscular damage (O'Steen et al. 1967; Pelosi et al. 1970, 1972).

Polyamines are polybasic molecules ubiquitously present in living cells and they have many functions, the most important of which are those connected with the synthesis of nucleic acids and proteins (Williams-Ashman and Canellakis 1979; Scalabrino and Ferioli 1981; Tabor and Tabor 1984). It is not surprising, then, that polyamine biosynthesis has been widely studied in relation to growth and differentiation of mammalian tissues, including muscle cells of different types. Although polyamine biosynthesis in muscles has received relatively little attention, studies of muscle polyamine content have dealt with experimental myocardial hypertrophy, denervated muscles of the rat and with the differentiation of the muscle cells in vitro (Russell et al. 1971; Feldman and Russell 1972; Kremzner et al. 1978a,b; Pegg and Hibasami 1980; Hopkins and Manchester 1981; Kaminska et al. 1981a, 1982; Ewton et al. 1984; Flamigni et al. 1986). Furthermore, it is well known that the two polyamine biosynthetic decarboxylases (PBD) L-ornithine decarboxylase (EC 4.1.1.17) (ODC) and *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) (SAMD), are the key points of the regulation of the polyamine biosynthetic pathway in eukaryotic cells (Williams-Ashman and Canellakis 1979; Scalabrino and Ferioli 1981; Tabor and Tabor 1984). ODC catalyzes the conversion of ornithine to putrescine and SAMD provides decarboxylated *S*-adenosyl-L-methionine, which is the sole aminopropyl donor substrate for converting putrescine into spermidine and this latter into spermine (Williams-Ashman and Canellakis 1979; Scalabrino and Ferioli 1981; Tabor and Tabor 1984). A large body of experimental evidence also indicates that both these decarboxylases, especially ODC, are critical for the regulation of cell proliferation and differentiation (Williams-Ashman and Canellakis 1979; Scalabrino and Ferioli 1981, 1982; Tabor and Tabor 1984). In

general, in conditions that favor cell proliferation there are large increases in PBD activities and, conversely, in conditions in which cell growth ceases, there are decreases in PBD activities (Williams-Ashman and Canellakis 1979; Scalabrino and Ferioli 1981, 1982; Tabor and Tabor 1984). As for PBD activities in muscles, previous studies have suggested that both PBD, especially ODC, are central in the control of the onset of skeletal muscle differentiation (Kremzner et al. 1978b; Stoscheck et al. 1980; Olson and Spizz 1986).

To get further information about the biochemical derangements in rat skeletal muscles injured by treatment(s) that induce experimental myopathies, we have determined the activities of both the PBD in the quadriceps femoris (as a paradigm for the skeletal muscles) of rats killed at different times after the induction of a diffuse myopathy by calciphylaxis. Furthermore, since in a preliminary study we found that 5-HT alone significantly modified the PBD levels in rat quadriceps femoris, we also determined PBD activities at different times after 5-HT administration. Finally, we also determined PBD activities in the myocardium of rats treated with 5-HT alone to see whether or not the trend of the changes in these enzymes resembles those observed in quadriceps femoris.

MATERIALS AND METHODS

Chemicals

L-[1-^{14}C]Ornithine monohydrochloride (S.A. 58 mCi/mmol) and *S*-adenosyl-L-[*carboxyl*-^{14}C]methionine (S.A. 60 mCi/mmol) were purchased from the Radiochemical Centre (Amersham, Buckinghamshire, U.K.). 5-HT (creatinine sulfate) and DHT were purchased from Sigma Chemical Co. (St. Louis, MO). 5-HT was dissolved in isotonic saline raised to neutral pH, DHT in oil. 5-HT solutions were calculated as the base.

Animals

Adult male non-inbred Sprague–Dawley rats obtained from Charles River Italia (Calco, Italy) were used. All rats weighed between 240–260 g at the time of treatment and they were housed as previously described (Scalabrino et al. 1979).

Treatment of animals

Calciphylaxis was induced in rats with DHT and 5-HT, following the experimental schema in Table 1. DHT was administered p.o. in a single dose and 5-HT was injected i.p. in a single dose. Control animals for DHT-treatment were given isovolumetric solvent oil, p.o.. Control animals for 5-HT-treatment were given isovolumetric, isotonic and neutralized saline, i.p., while those for calciphylaxis-inducing treatment were given first isovolumetric solvent oil p.o. and then isovolumetric, isotonic and neutralized saline i.p. Injections were always less than 1.0 ml. In order to minimize the effects of the well known diurnal changes in PBD activity in rat organs (Scalabrino et al. 1979), rats were always killed between 10.00 a.m. and 02.00 p.m. and the injections were always given between 08.00 a.m. and 10.00 a.m. The amounts of drugs for the different treatments and the times of killing are reported in Tables 1–4.

TABLE 1

TIME-COURSE OF THE CHANGES IN ORNITHINE DECARBOXYLASE (ODC) AND *S*-ADENOSYL-L-METHIONINE DECARBOXYLASE (SAMD) ACTIVITIES IN QUADRICEPS FEMORIS OF RATS AFTER INDUCTION OF CALCIPHYLAXIS (CP) AND AFTER DIHYDROTACHYSTEROL (DHT) ADMINISTRATION

DHT, 10 mg/kg b.w., whether or not followed by serotonin (5-HT), and 5-HT 20 mg/kg b.w. For CP-induction, DHT was given p.o. at 0 time and 5-HT i.p. 72 h later. The times of killing indicated for calciphylactic rats and for sham-calciphylactic rats were computed from the time of 5-HT injection or of saline injection. In sham-CP-induction, rats were given solvent oil instead of DHT and saline instead of 5-HT. Mean values ± SEM. Number of animals in parentheses.

Treatment	Time of killing after treatment (h)	Polyamine biosynthetic decarboxylases (PBD)	
		ODC activity (pmol CO_2/mg protein/30 min)	SAMD activity (pmol CO_2/mg protein/30 min)
Solvent	6	45.4 ± 4.89 (12)	62.7 ± 4.88 (12)
DHT	6	43.7 ± 3.37 (6)	65.1 ± 5.46 (6)
DHT	78	44.8 ± 3.52 (5)	66.3 ± 5.22 (5)
Sham-CP-inducing	6	46.8 ± 4.50 (6)	60.9 ± 5.08 (6)
CP-inducing	6	25.4 ± 2.79 (8)*	13.9 ± 2.56 (9)*,†
CP-inducing	24	23.2 ± 3.10 (6)*	29.2 ± 1.90 (6)*,†
CP-inducing	48	25.2 ± 3.90 (6)*	37.8 ± 3.60 (6)*,†

* $P < 0.01$ vs. sham-calciphylactic rats (Duncan's multiple range test (1955)).

† In the calciphylactic rats, the mean SAMD value for the rats killed after 24 h after CP treatment was significantly ($P < 0.01$) higher than that for the rats killed 6 h after the CP-inducing treatment and significantly ($P < 0.01$) lower than that for the rats killed 48 h after the CP-inducing treatment; the mean SAMD value for this last group is significantly ($P < 0.01$) higher than that for rats killed 6 h after the CP-inducing treatment (Duncan's multiple range test (1955)).

Mean ODC and SAMD values in quadriceps femoris of untreated rats are not given, because they do not significantly differ from those observed in solvent- and sham-CP-treated rats.

Preparation of the tissue extracts

After decapitation of the rats, quadriceps femoris and myocardium were rapidly removed, frozen in liquid nitrogen and stored at − 80 °C until the enzyme assays were performed. All the muscles were homogenized with an Ultra-Turrax homogenizer for 15 s in 3 vols of a medium whose composition has been previously described (Scalabrino et al. 1979). An aliquot of the 20 000 × *g* supernatant was used to assay the activities of both the PBD. The quadriceps femoris and the myocardia were processed separately for each animal.

Assays for PBD activities

The composition of the mixtures and the experimental conditions for the enzyme assays were those routinely used in our laboratory (Scalabrino et al. 1979). Decarboxylase activity is expressed as pmoles of carbon dioxide liberated from the appropriate carboxyl-labeled substrate/mg protein/30 min of incubation. Non-collagen protein of tissue supernatants was measured as previously described (Scalabrino et al. 1979).

Statistical analysis

The data are reported as means ± SEM. The significances of the differences between the experimental and the control means and of the differences between the experimental means at the different times were evaluated by Duncan's multiple range test (Duncan 1955). Differences for which the *P*-values were greater than 0.05 were not considered to be significant.

RESULTS

The effect of administration of DHT only and of DHT followed by 5-HT (calciphylactic myopathy) on PBD activities in rat quadriceps femoris are shown in Table 1. These data demonstrate that the treatment with DHT alone had no effect at either early times, i.e., 6 h, or later times, i.e., 78 h (which time coincides with that of killing of the first group of rats given DHT and 5-HT). Table 1 also shows the temporal patterns of PBD activities in the same muscle of rats killed at different times after the induction of calciphylaxis. The decrease in ODC activity initially (at 6 h) brought about by the calciphylaxis-inducing treatment was essentially the same in all the different groups of animals killed at the various subsequent times. On the contrary, there was a biphasic change in SAMD activity, with an initial (at 6 h) decrease followed by progressive and significant increases between both 6 h and 24 h and between 24 h and 48 h.

The data in Table 2 demonstrate that treatment with 5-HT only, administered at different dosages, always inhibited the PBD activities in quadriceps femoris of rats 6 h after 5-HT administration. This inhibition was dose-dependent. The levels of PBD activities in quadriceps femoris were monitored in rats killed at different times after the injection of a fixed dose of 5-HT and they are reported in Table 3. The temporal trend was the same for both ODC and SAMD. It was biphasic, with an initial decrease at 6 h after 5-HT administration followed by significant increases between 6 h and 24 h, between 24 h and 48 h and between 48 h and 96 h after 5-HT administration. At the end, the levels of the PBD activities were very close to the control values 4 days after 5-HT injection (see again Table 3).

Table 4 shows both the effects of different dosages of 5-HT on myocardial PBD activities of rats killed at a fixed time (part A) as well as the time-course of the changes in the same enzyme levels in the same muscle of rat killed at different times after administration of one fixed dose of 5-HT (part B). Surprisingly enough, 5-HT, even when injected at the highest dose we used, did not inhibit ODC activity to any significant extent at 6 h after its administration, while, at the same time, myocardial SAMD activity was inhibited by all the doses of 5-HT (Table 4A). As for the temporal trend of myocardial PBD, it was the same for both ODC and SAMD and it showed a biphasic trend. Both the ODC and SAMD mean values were significantly decreased on the first and the second days after 5-HT administration. Thereafter, the mean values of the PBD levels increased, being nearly those of normal controls by 4 days after 5-HT injection (Table 4B).

TABLE 2

ORNITHINE DECARBOXYLASE (ODC) AND *S*-ADENOSYL-L-METHIONINE DECARBOXYLASE (SAMD) ACTIVITIES IN QUADRICEPS FEMORIS OF RATS WITH EXPERIMENTAL MYOPATHY INDUCED BY DIFFERENT DOSES OF SEROTONIN (5-HT)

Doses of 5-HT and number of rats in parentheses. Mean values ± SEM.

Treatment	Time of killing after treatment (h)	Polyamine biosynthetic decarboxylases (PBD)	
		ODC activity	SAMD activity
		(pmol CO_2/mg protein/30 min)	
Saline	6	50.6 ± 8.22 (6)	67.2 ± 3.61 (6)
5-HT (5 mg/kg b.w.)	6	31.8 ± 4.39 (6)*,†	57.8 ± 4.72 (6)*,†
5-HT (10 mg/kg b.w.)	6	21.4 ± 3.95 (6)*,†	43.4 ± 5.45 (6)*,†
5-HT (20 mg/kg b.w.)	6	15.3 ± 2.87 (6)*,†	24.7 ± 3.18 (6)*,†

* Mean ODC and SAMD values for all 3 experimental groups were significantly ($P < 0.01$) lower than the mean ODC and SAMD values for the control group (Duncan's multiple range test (1955)).

† For the 3 experimental groups, the mean ODC and SAMD values for the first group were both significantly ($P < 0.01$) higher than those for the two other groups; the mean ODC and SAMD values for the second group were both significantly ($P < 0.01$) higher than those for the third groups (Duncan's multiple range test (1955)).

Mean ODC and SAMD values in quadriceps femoris of untreated rats are not given, because they do not significantly differ from those observed in saline-treated rats.

TABLE 3

TIME-COURSE OF CHANGES IN ORNITHINE DECARBOXYLASE (ODC) AND *S*-ADENOSYL-L-METHIONINE DECARBOXYLASE (SAMD) ACTIVITIES IN QUADRICEPS FEMORIS OF RATS WITH EXPERIMENTAL MYOPATHY INDUCED BY SEROTONIN (5-HT)

5-HT 20 mg/kg b.w. i.p.. Number of animals in parentheses. Mean values ± SEM.

Treatment	Time of killing after treatment (h)	Polyamine biosynthetic decarboxylases (PBD)	
		ODC activity	SAMD activity
		(pmol CO_2/mg protein/30 min)	
Saline	24	50.3 ± 9.54 (6)	67.2 ± 3.61 (6)
5-HT	6	15.3 ± 2.87 (6)*,†	24.7 ± 3.18 (6)*,†
5-HT	24	23.4 ± 6.65 (6)*,†	49.3 ± 3.29 (6)*,†
5-HT	48	40.1 ± 4.19 (6)*,†	59.1 ± 6.56 (6)*,†
5-HT	96	43.2 ± 3.91 (6)	65.2 ± 5.94 (6)

* The mean values for both ODC and SAMD for the first 3 experimental groups are significantly ($P < 0.01$) lower than those for the control groups (Duncan's multiple range test (1955)).

† For the experimental groups, mean ODC and SAMD values for the rats killed 24 h after treatment were both significantly ($P < 0.01$) higher than those for the rats killed 6 h after treatment, but significantly ($P < 0.01$) lower than those for the rats killed 48 h after treatment; mean ODC and SAMD values for this last group were both significantly ($P < 0.01$) higher than those for the rats killed 6 h after treatment (Duncan's multiple range test (1955)).

Mean ODC and SAMD values in quadriceps femoris of untreated rats are not given, because they do not significantly differ from those observed in saline-treated rats.

TABLE 4

(*A*) MYOCARDIAL ORNITHINE DECARBOXYLASE (ODC) AND *S*-ADENOSYL-L-METHIONINE DECARBOXYLASE (SAMD) ACTIVITIES OF RATS TREATED WITH DIFFERENT DOSES OF SEROTONIN (5-HT)
(*B*) TIME-COURSE OF CHANGES IN MYOCARDIAL ODC AND SAMD ACTIVITIES OF RATS KILLED AT DIFFERENT TIMES AFTER THE INJECTION OF A FIXED DOSE OF 5-HT

Doses of 5-HT and number of animals in parentheses. Mean values ± SEM.

Treatment	Time of killing after treatment (h)	Polyamine biosynthetic decarboxylases (PBD)	
		ODC activity (pmol CO_2/mg protein/30 min)	SAMD activity (pmol CO_2/mg protein/30 min)
(*A*)			
Saline	6	126.3 ± 11.76 (5)	76.1 ± 4.88 (5)
5-HT (5 mg/kg b.w.)	6	123.3 ± 12.66 (6)	44.2 ± 3.70 (6)*
5-HT (10 mg/kg b.w.)	6	138.2 ± 13.09 (5)	58.4 ± 3.32 (5)*
5-HT (20 mg/kg b.w.)	6	133.9 ± 11.54 (6)	55.7 ± 4.15 (6)*
(*B*)			
Saline	24	130.5 ± 10.44 (6)	72.4 ± 3.18 (6)
5-HT (20 mg/kg b.w.)	24	96.2 ± 10.60 (6)†	39.9 ± 2.75 (6)†
5-HT (20 mg/kg b.w.)	48	72.2 ± 5.62 (6)†	30.7 ± 2.66 (6)†
5-HT (20 mg/kg b.w.)	96	115.1 ± 9.84 (6)	68.9 ± 5.41 (6)

* Mean SAMD values of all 3 experimental groups injected with different 5-HT doses were significantly ($P < 0.01$) lower than the mean SAMD value for the controls (Duncan's multiple range test (1955)).

† Mean ODC and SAMD values for the rats killed 24 h and 48 h after 5-HT injection (with the former significantly ($P < 0.01$) higher than the latter) were significantly ($P < 0.01$) lower than the mean ODC and SAMD values of both the controls and the rats killed 96 h after 5-HT injection (Duncan's multiple range test (1955)).

Mean ODC and SAMD values in myocardium of untreated rats are not given, because they do not significantly differ from those observed in saline-treated rats.

DISCUSSION

One major result of this study is the demonstration of definite changes in the levels of PBD activities in quadriceps femoris of rats killed at different times after the induction of different types of experimental myopathy. The prominent feature of these changes is the characteristic time-course, with ODC and SAMD activities both greatly reduced at first and then progressively increasing. The only exception to this statement was the time-course of ODC activity after the calciphylaxis-inducing treatment, because the phase of increase in ODC activity was not observed in this 48-h time period after the myotoxic treatment, which may be too short. One of the conceivable explanations for these increases in muscular ODC and SAMD levels is that regenerative processes occur in both calciphylaxis-induced myopathy and in 5-HT-induced myopathy (O'Steen et al. 1967; Pelosi et al. 1970, 1972). Similar results were obtained by Sadech et al. (1984) for another model of experimental myopathy and they put forth the same explanation (Sadech et al. 1984). All these results strongly suggest that muscular PBD activities

might mirror well the changes in gene expression in skeletal muscle in the different models of experimental myopathies, in which there is a temporal succession of degenerative and regenerative processes. The increases observed in intracellular muscle polyamines in biopsies from patients with Duchenne muscular dystrophy or polymyositis and in urinary polyamines of patients with Duchenne muscular dystrophy have also been suggested to be related to the regenerative processes well known to occur in those neuromuscular diseases (Kremzner et al. 1978a; Rudman et al. 1980; Kaminska et al. 1981b; Russell and Stern 1981).

It has been emphasized many times that 5-HT induces muscle ischemia (Mendell et al. 1971). It is unclear at this time whether the muscle lesions caused by 5-HT are the result of functional ischemia in skeletal muscle or of direct myotoxicity. However, previous studies on the time-courses of PBD activities in rat organs, such as liver and brain, recovering function after a period of ischemia, have demonstrated changes in ODC and SAMD activities that are temporally quite similar to those observed in rat quadriceps femoris after 5-HT-treatment and these organs also show induction of the ODC and SAMD activities during the post-ischemic period (Kleihues et al. 1975; Ferioli et al. 1980). Thus, post-ischemic repair in rat muscle after the administration of 5-HT alone may be another cause of the increase of PBD activities in skeletal muscle at late times after 5-HT-treatment.

The last but not least result presented here is the slower reaction of rat myocardial ODC activity to 5-HT than ODC of rat quadriceps femoris. At the present we have no explanation for this. It is yet another important difference in the biochemistry and metabolism of mammalian skeletal muscle and myocardium.

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