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Age-related changes in the chronotropic effect and the enzymic decarboxylation of L-*threo*-3,4-dihydroxyphenylserine in the rat heart

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The cardiac effect of L-*threo*-3,4-dihydroxyphenylserine (L-*threo*-DOPS) and the enzymatic decarboxylation of the drug by L-aromatic amino acid decarboxylase (AADC) were studied in atria isolated from rats ranging in age from newborn to adults and the findings compared with the cardiac effect of noradrenaline (NA). L-*threo*-DOPS produced dose-dependent, slow-onset and positive chronotropic effects in atria from rats of different ages. Its effect was inhibited in atria from benserazide-treated rats, suggesting that the effects are due to NA formed from L-*threo*-DOPS by enzymic decarboxylation rather than to the compound itself. Chronotropic sensitivity to L-*threo*-DOPS was highest in the newborn and decreased with age during the first 3 weeks of life. The development of cardiac response to it correlated well with the development of enzymic decarboxylation of the drug but did not correlate with the developments of chronotropic sensitivity to NA and of NA concentrations in the heart. These findings suggest that in newborn rats, L-*threo*-DOPS is effectively converted by AADC to NA which in turn acts on β-receptors in the pacemaker cell membrane.

The formation of noradrenaline (NA) from 3,4-dihydroxyphenylserine (DOPS) by L-aromatic amino acid decarboxylase (AADC) from various tissues in mammals has been demonstrated (Blaschko et al 1950; Schmiterlöw 1951; Creveling et al 1968). Recently, the isomers, L-*threo*-, D-*threo*-, L-*erythro*- and D-*erythro*-DOPS, have been separated and purified, and their enzymic decarboxylations have been also demonstrated in vivo in the rat brain and heart (Puig et al 1974; Bartholini et al 1975) and in vitro in the hog kidney (Inagaki et al 1976) and in the rat heart (Ohmura et al 1978). These findings suggested that L-*threo*-DOPS is the most effective precursor converted to NA by decarboxylase. We have also reported that L-*threo*-DOPS produces a positive chronotropic effect in rat atrial preparations (Araki et al 1978) and a slow-onset and long-lasting hypertensive effect in rats (Araki et al 1981). Thus, L-*threo*-DOPS may be clinically applicable for treating certain disorders related to hypotension (Araki et al 1981).

We have determined the effects of L-*threo*-DOPS in atria isolated from rats at various ages and its enzymic decarboxylation by AADC from the hearts of rats of different ages.

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MATERIALS AND METHODS

Adult and infant Wistar rats of either sex were used. The rats were allowed to suckle for 21 postnatal days after which laboratory rat chow was provided. The groups included in the study were as follows: newborn (within 1 h of birth) (body weight 5·0 ± 0·2 g, N = 45), 3 days (6·8 ± 0·1 g, N = 62) 7 days (12·6 ± 0·4 g, N = 64), 14 days (22·0 ± 1·1 g, N = 50), 21 days (37·1 ± 1·6 g, N = 44), 28 days (63·0 ± 2·6 g, N = 45), 42 days (131·0 ± 3·0 g, N = 43) and 84 days (adult) (362·7 ± 9·4 g, N = 41), after birth.

Mechanical recording

The rats were decapitated, the hearts rapidly isolated and the atrial preparations fixed in an organ bath of 20 ml Locke solution maintained at 30 ± 1 °C and bubbled with a mixture of 95% O_2 and 5% CO_2. A passive tension of 100, 150, 200, 300, 500, 700 and 700 mg was loaded on the atria at days 3, 7, 14, 21, 28, 42 and 84, respectively. Mechanical activity was recorded isometrically with a force-displacement transducer on an ink-writing oscillograph.

L-*threo*-DOPS dissolved in 0·9% NaCl (saline) was added directly to the bath medium. The effects of a single dose of L-*threo*-DOPS were observed for 40 min. Experiments using concentrations over 2×10^{-4} M could not be done because of the relative insolubility of this drug. (−)-NA dissolved in saline

was also added cumulatively to the bath medium and the effect of each dose was observed for 2 min. A ED50 value, defined as the negative logarithm of the dose of agonist causing 50% of maximal response, was calculated for each dose-response curve, by the method of van Rossum (1963).

Decarboxylation of L-threo-*DOPS*

Heart tissues were homogenized in 7 volumes of distilled water and were centrifuged at 8000*g* for 10 min at 4 °C. The supernatant fractions were used as a source of AADC. Protein concentration of the enzyme was determined by the method of Lowry et al (1951). Decarboxylation of L-*threo*-DOPS was carried out under the typical conditions reported by Ohmura et al (1978). After decarboxylation the mixture was centrifuged at 5000*g* for 10 min at 4 °C. The amount of the reaction product, NA, was determined by the method of Bertler et al (1958).

Determination of NA contents

Heart tissues were weighed, homogenized in 4 ml of 0·4 M perchloric acid with 10 mg sodium metabisulphite and 200 mg ethylenediaminetetraacetic acid disodium salt and centrifuged at 8000*g* for 5 min at 4 °C. The catecholamine in the supernatant was adsorbed and concentrated using activated aluminium oxide, and then eluted by 0·1 M HCl. NA contents in the elute were determined using a high performance liquid chromatograph (Yanaco, L-2000) with an electrochemical detector (Yanaco, VMD-101).

Reagents

L-*threo*-3,4-Dihydroxyphenylserine ($[\alpha]_D^{20} = -42{\cdot}6$ (c = 1: M HCl), purity: 99·5%) (Kyowa Hakko Kogyo Co. Ltd., Japan), sotalol hydrochloride (Regis), (−)-noradrenaline bitartrate (Sigma), benserazide hydrochloride (Hoffman-La Roche) were used. Other chemicals used were of reagent grade.

Statistical analysis

The statistical significance of differences between means was determined by unpaired Student's *t*-test and considered significant at $P < 0{\cdot}05$.

RESULTS

Basic rate of spontaneously beating atria from rats of different ages

The systolic tension development of spontaneously beating atria from rats of different ages increased with increase in the passive tension and reached a plateau with a passive tension of 100, 150, 200, 300, 500, 700 and 700 mg at 3, 7, 14, 21, 28, 42 and 84 days after birth, respectively (Fig. 1). However, the atrial rate in each of the age groups was not affected by increases in the passive tension. For the following experiments on atria from animals of different ages, passive tensions which showed the maximal beating tension were used. As shown in Fig. 2, the spontaneous rates of atria from rats of different ages

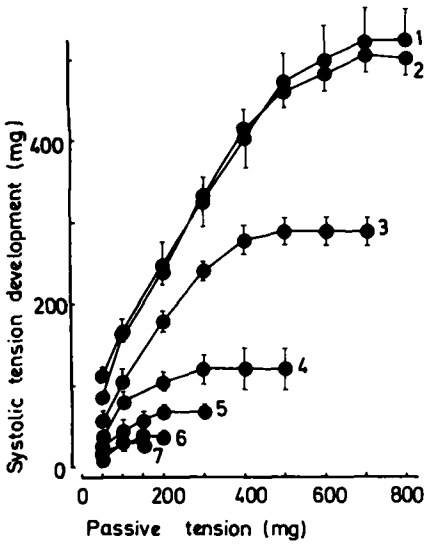

FIG. 1. Relationship between passive tension and systolic tension development in atria from infant and adult rats. Each point represents the mean ± s.e. of the systolic tension development in atria from rats at days 3 (7) (N = 6), 7 (6) (N = 9), 14 (5) (N = 8), 21 (4) (N = 7), 28 (3) (N = 8), 42 (2) (N = 8) and 84 (1) (N = 7) after birth.

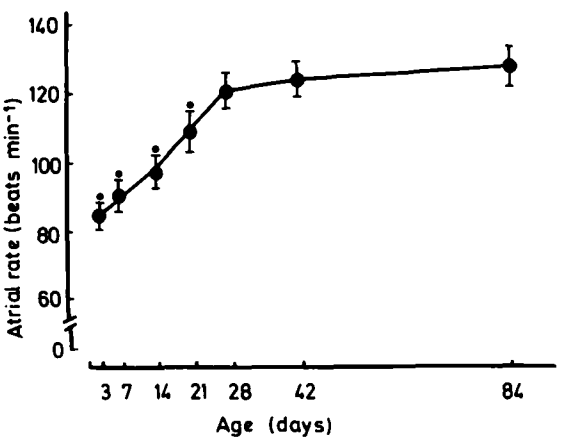

FIG. 2. Basic rates in atria from infant and adult rats. Each point represents the means ± s.e. of 24 to 27 preparations. * Significantly different from the rate of atria from adult rats ($P < 0{\cdot}05$).

increased with age up to 28 days after birth. The atrial rates at 3, 7, 14 and 21 days were significantly lower than rates of atria from adult rats. In addition, the spontaneous rate in any group was not affected by treatment with sotalol 10^{-6} M.

Effect of L-threo-*DOPS on rate of atria isolated from rats at different ages*

The rate of atria from rats of different ages gradually increased over 10 min after addition of L-*threo*-DOPS and a steady state was reached 30 to 40 min later. No significant changes in atrial rates at different ages were obtained during 40 min after the addition of saline (control). This slow-onset, positive chronotropic effect was concentration-dependent and was removed by washout of the drug. The chronotropic responses at 30 min after addition of L-*threo*-DOPS are shown in Fig. 3. In concentrations ranging from 2×10^{-5} to 2×10^{-4} M, the positive chronotropic responses to L-*threo*-DOPS in atria from rats at age up to 28 days were significantly

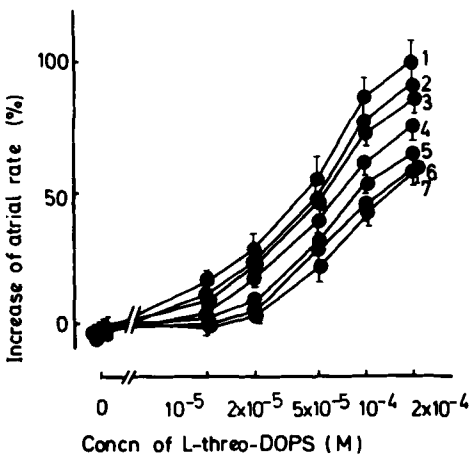

FIG. 3. Increase in rates of atria from infant and adult rats by L-*threo*-DOPS. The curves are expressed as the percentage increase from the basic rates in atria from rats at days 3 (1), 7 (2), 14 (3), 21 (4), 28 (5), 42 (6) and 84 (7) after birth. Each point represents the mean ± s.e. of 6 to 9 preparations.

greater than responses in adult atria. There were no significant differences in increased atrial rates with application of L-*threo*-DOPS in rats over 28 days of age. The ED50s for the chronotropic effect of L-*threo*-DOPS in younger rats tended to be lower than those in adults, but the differences were not significant. Furthermore, the positive chronotropic effect of L-*threo*-DOPS was significantly reduced in atria isolated from rats treated with benserazide (50 mg kg^{-1} i.p.) or in atria pretreated with 10^{-6} M sotalol.

Effect of NA on atrial rate at different ages

Increases of atrial rate were observed with the addition of NA to bath media in concentrations ranging from 10^{-9} to 10^{-5} M, and this effect reached a maximum 1 to 2 min later. The positive chronotropic effect of NA 2 min after addition of the drug is shown in Fig. 4. Increases of the atrial rate with application of NA were related to age in young rats: in concentrations ranging from 10^{-9} to 10^{-5} M, the percentage increases of atrial rate at ages of up to 21 days were significantly smaller than that in adults.

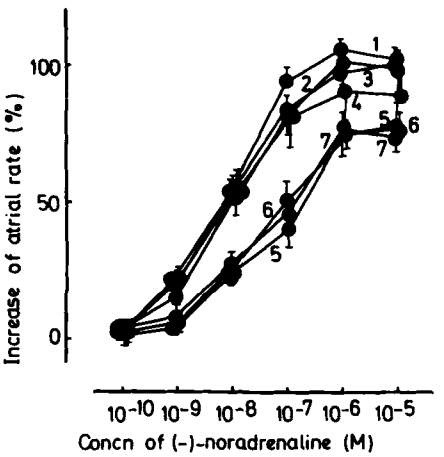

FIG. 4. Increase in rates of atria from infant and adult rats by (−)-NA. The curves are expressed as the percentage increase from the basic rates in atria from rats at days 3 (7), 7 (6), 14 (5), 21 (4), 28 (3), 42 (2) and 84 (1) after birth.

No significant differences in the increase of atrial rate with NA were obtained in atria from rats over 21 days old. Moreover, as shown in Table 1, the ED50s of NA on the increase of atrial rate of younger rats (at day 3–14) were significantly higher than those in adult rats. The positive chronotropic effect of NA on atria was significantly decreased by the pretreatment with 10^{-6} M sotalol.

NA contents and specific activity of AADC in rat heart at different ages

NA contents in the rat heart at up to 7 postnatal days were low and the concentrations markedly increased during the next 2 weeks. NA contents at days up to 21 were significantly lower in comparison with

Table 1. NA contents, AADC activity and median effective concentrations of the chronotropic effects of L-*threo*-DOPS and (−)-noradrenaline in rats at different ages

Age (days)	NA contents (μg g^{-1} wet wt)	AADC activity (n mol NA mg^{-1} protein/30 min)	ED50 L-*threo*-DOPS (× 10^{-5} M)	ED50 (−)-NA (× 10^{-9} M)
0	0·14 ± 0·05* (8)	8·78 ± 0·27* (9)	—	—
3	0·12 ± 0·02* (8)	7·21 ± 0·55* (6)	4·2 ± 0·6 (6)	47·0 ± 8·9* (8)
7	0·16 ± 0·01* (9)	6·61 ± 0·23* (6)	4·3 ± 0·5 (9)	43·2 ± 7·0* (7)
14	0·34 ± 0·06* (10)	2·62 ± 0·10* (8)	4·3 ± 0·5 (7)	47·4 ± 6·2* (6)
21	0·47 ± 0·04 (10)	1·33 ± 0·08 (9)	4·3 ± 0·5 (7)	8·3 ± 1·0 (6)
28	0·46 ± 0·04 (10)	1·29 ± 0·08 (9)	5·0 ± 0·4 (8)	8·0 ± 0·8 (7)
42	0·50 ± 0·03 (9)	1·14 ± 0·08 (6)	5·1 ± 0·7 (8)	8·0 ± 0·8 (7)
84	0·59 ± 0·06 (9)	1·06 ± 0·17 (6)	5·8 ± 0·5 (7)	8·0 ± 0·7 (7)

Each value represents the mean ± s.e. Figures in parentheses indicate the number of preparations.
* Significantly different from the value at 84 days ($P < 0·05$).

findings in adults, although NA contents at days from 21 to 42 did not differ from the concentrations in adult rats (Table 1).

The specific activity of AADC in rat heart was highest at birth and decreased with advancing age. The activity of this enzyme at days up to 21 was significantly higher in comparison with findings in adults, although the activities from 21 to 42 days did not differ from those in adults (Table 1).

DISCUSSION

L-*threo*-DOPS produced dose-dependent positive chronotropic responses in isolated atria from postnatal developing rats, as previously reported in atria from adult rats (Araki et al 1978). The spontaneous rates of atria increased with the age of rats up to 28 days after birth, but at 28 and 42 days did not differ significantly from those in atria of adult rats. This is in agreement with the data of Vlk & Vincenzi (1977) and Standen (1978), but not with the data of Nukari-Siltovuori (1977) who found the basic rate of atria to be similar at different ages. The discrepancy in the spontaneous rates may be due to the difference in experimental conditions.

The positive chronotropic response produced by L-*threo*-DOPS was reduced in atria from developing rats treated with the amino acid decarboxylase inhibitor, benserazide and the onset was slow compared with that of NA, suggesting that the effect of L-*threo*-DOPS was produced by NA formed from L-*threo*-DOPS, as in atria of adult rats (Araki et al 1978). Therefore, the most effective positive chronotropic response of the newborn rat atria to L-*threo*-DOPS is considered to be due to the high activity of AADC in the atria. This concept is supported by the finding of enzymic decarboxylation of L-*threo*-DOPS to NA in the postnatal developing heart. In the present study, the fast-onset, positive chronotropic response to NA was observed in atria from rats at different ages. This chronotropic sensitivity to exogenous NA was significantly lower in the younger rats. The development of chronotropic sensitivity to L-*threo*-DOPS is, at least, not correlated with that to exogenous NA.

We also demonstrated an age-related increase of NA concentration in the heart of postnatal developing rats, as was described by Iversen et al (1967). This is well correlated to the development of adrenergic innervation in postnatal developing rat hearts (De Champlain et al 1970). The development of most of the adrenergic functions is considered to be counter to the development of chronotropic sensitivity to L-*threo*-DOPS. Hence, the decrease in positive chronotropic effect of L-*threo*-DOPS with age may reflect the age-related decrease in the activity of AADC, although the participation of the development of adrenergic innervation (De Champlain et al 1970) and NA uptake mechanism (Glowinski et al 1964; Iversen et al 1967; Sachs et al 1970; Atwood & Kirshner 1976) in the age-related decrease of the positive chronotropic effect cannot be excluded. We have also demonstrated the regional distribution of L-*threo*-DOPS decarboxylase activity in the adult rat heart (Ohmura et al 1978), that is, NA formation from L-*threo*-DOPS was more extensive in the atrial body including the S-A node and the right auricle than in the left auricle and the ventricle. These results suggest that in the atrium of newborn rats, L-*threo*-DOPS is effectively converted by AADC to NA which in turn acts on the β-adrenoceptor of the S-A nodal pacemaker cell membrane. In the previous paper (Araki et al 1981),

we reported the slow-onset and long-lasting pressor effect of L-*threo*-DOPS and suggested the usefulness of this drug for the treatment of hypotension. However, L-*threo*-DOPS may produce an intensive hypertensive effect, in young animals.

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