whereas K^+ acts primarily by facilitating the entry of membranal or extracellular lightly bound Ca^{2+} (Hudgins & Weiss, 1968). The inhibition of noradrenaline and K^+ responses induced by bunaphtide suggests that although the experiments do not clearly identify the sites of bunaphtide action, the results could be interpreted as possible action at three sites: (1) the membrane to inhibit the influx of extracellular Ca^{2+} (the experiments performed in Ca^{2+}-free high-K^+ solution provided additional evidence for a membrane site of action of bunaphtide), (2) the intracellular Ca^{2+} storage sites to block the release of Ca^{2+} from the stores or (3) at both sites, decreasing the avilability of Ca^{2+} at the contractile apparatus. January 17, 1978

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Positive chronotropic effect of *threo*-3,4-dihydroxyphenylserine as a precursor of noradrenaline in rat isolated atria

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The formation of noradrenaline from 3,4-dihydroxyphenylserine (DOPS) by L-aromatic amino acid decarboxylase from various mammalian tissues has been demonstrated *in vitro* (Blaschko, Burn & Langeman, 1950) and *in vivo* (Schmiterlöw, 1951; Creveling, Daly & others, 1968; Puig, Bartholini & Pletscher, 1974; Bartholini, Constantinidis & others, 1975). DOPS has four stereoisomers, L-*threo*-, D-*threo*-, L-*erythro*- and D-*erythro*-DOPS. Until recently, the racemate of threo-DOPS has been used for studies on catecholamine function. Recently, the isomers have been separated and purified and their enzymic decarboxylation investigated *in vivo* (Puig & others, 1974; Bartholini & others, 1975) and *in vitro* (Fujiwara, Inagaki & others, 1976; Inagaki, Fujiwara & Tanaka, 1976). Among the isomers and racemate of *threo*-DOPS, L-*threo*-DOPS is considered to be the most effective precursor of natural noradrenaline (Inagaki, Fujiwara & Tanaka, 1976).

The present work was an attempt to demonstrate the enzymic decarboxylation of L-*threo*-DOPS by L-aromatic amino acid decarboxylase of the rat atria and the cardiac effect of L-*threo*-DOPS in the rat isolated atria.

Male Wistar rats, 250 to 300 g, were divided into, untreated, benserazide-treated and α-methyl-*p*-tyrosine (α-MT)-treated groups. A single dose of benserazide (50 mg kg^{-1}, i.p.) dissolved in saline (0·9% NaCl) or α-MT (200 mg kg^{-1}, i.p.) suspended in saline was given at 8 or 1 h before death by a blow on the neck and exsanguination from the carotid arteries. After the heart had been rapidly isolated, the atria were fixed under a resting tension of 0·7 g in an organ bath of 20 ml Locke solution containing (mM) NaCl 154, KCl 5·6, $CaCl_2$ 2·2, glucose 5·6 and $NaHCO_3$ 4·8 (pH 7·2–7·4) maintained at 29 ± 1° and continuously bubbled with 5% CO_2 in oxygen. Preparations were allowed to equilibrate for 1 h before measurements were taken. During this time the medium was replaced every 30 min. For the untreated rats, atria with beats of 90 to 135 min^{-1} were used. Mechanical activity was recorded isometrically with a force-displacement transducer (Nihonkohden Kogyo Co. Ltd, SB-1T) on an oscillograph. *Threo*-DOPS was dissolved in saline and 0·2 ml of the solution was added directly to the bath.

The rate of beat of *threo*-DOPS-treated atria from untreated rats was compared with that of control atria using an unpaired Student's *t*-test. The changes between untreated and benserazide-treated or α-MT-treated rats effected by L-*threo*-DOPS (20×10^{-5} M) were compared with that before treatment using a paired Student's *t*-test.

In biochemical experiments, the isolated atria were homogenized with 7 volumes of distilled water. The homogenate was centrifuged at 8000 *g* for 15 min and the supernatant used as the enzyme preparation, L-aromatic amino acid decarboxylase. Decarboxylation was started at 37° by addition of 1 mM L-*threo*-DOPS as substrate to the medium (final volume 2 ml) contain-

* Correspondence.

ing (mM) tris-HCl buffer (pH 8·6) 125, pargyline 0·2, pyridoxal-5-phosphate 0·1, pyrogallol 0·001 and 1 to 3 mg of protein of the supernatant fraction, and was stopped by the addition of 4 ml of 0·4 M ice cold perchloric acid. The amount of noradrenaline formed from L-*threo*-DOPS was determined by the method of Bertler, Carlsson & Rosengren (1958).

Optical isomers of *threo*-DOPS, L-*threo*- ($[\alpha]_D^{20} = -42{\cdot}6$ (c = 1; M HCl), purity: 99·5%), D-*threo*- ($[\alpha]_D^{20} = +43{\cdot}2$ (c = 1; MHCl), purity: 99·8%) and DL-*threo*-DOPS (Kyowa Hakko Kogyo Co. Ltd, Japan) were used. The contaminating noradrenaline was removed from *threo*-DOPS solution by passing it through columns of Dowex 50W − X4 in Na^+-form.

The effects of L-, D- and DL-*threo*-DOPS on the rate of spontaneously beating atria from rats are summarized in Table 1. The atrial rate gradually increased over 10 to 30 min after addition of L-*threo*-DOPS. A significant increase of atrial rate was obtained at 20 min after the application of the drug (5×10^{-5} M) and at 10 min (10 and 20×10^{-5} M). The effect was dose-dependent. DL-*threo*-DOPS also produced a significant increase in atrial rate that was dose-dependent from 5×10^{-5} to 20×10^{-5} M and was approximately half that seen with L-*threo*-DOPS. On the other hand, D-*threo*-DOPS even at 20×10^{-5} M produced no changes in atrial rate.

As shown in Table 1, the positive chronotropic effect of 20×10^{-5} M L-*threo*-DOPS was significantly inhibited in atria from rats treated with an intraperitoneal injection of benserazide hydrochloride (Ro 4–4602) (50 mg kg^{-1}) a potent inhibitor of peripheral L-aromatic amino acid decarboxylase (Burkard, Gey & Pletscher, 1962; Pletscher & Gey, 1963), at 1 h before isolation of atria. In addition, an inhibition in spontaneous atrial rate was evident after treatment by benserazide alone. On the other hand, in the atria from rats treated with the tyrosine hydroxylase inhibitor, α-MT (200 mg kg^{-1}) (Nagatsu, Levitt & Udenfriend, 1964; Spector, Sjoerdsma & Udenfriend, 1965; Moore & Dominic, 1971) 8 h before isolation of atria, the positive chronotropic effect of 20×10^{-5} M L-*threo*-DOPS was significantly greater than that in the atria from untreated rats.

Formation of noradrenaline by decarboxylation of L-*threo*-DOPS was examined *in vitro* using atrial homogenate. When atrial homogenate was incubated with L-*threo*-DOPS at 37° for 30 min in the medium, noradrenaline was produced. Its formation from L-*threo*-DOPS by atrial homogenates was greatest in the right auricle 0·582 ± 0·093 nM mg^{-1} protein/30 min followed by the atrial body 0·469 ± 0·067 and the left auricle 0·358 ± 0·008. Non-enzymic decarboxylation during the incubation at 37° was undetectable. Incubation of the enzyme with D-*threo*-DOPS revealed no formation of noradrenaline.

Our results show that L-*threo*-DOPS produced a slow-onset positive chronotropic effect in rat isolated atria. This was inhibited in atria from benserazide-treated rats, suggesting that the effect of L-*threo*-DOPS is due to noradrenaline formed from L-*threo*-DOPS by decarboxylation in the isolated atria but not to L-*threo*-DOPS itself. *In vitro* formation of noradrenaline from L-*threo*-DOPS by atrial decarboxylase was de-

Table 1. *Effect of* threo-3,4-*dihydroxyphenylserine on spontaneous beating in atria isolated from untreated, benserazide-treated and* α-*methyl*-p-*tyrosine-treated rats.*

	Atrial rate (beats min^{-1})			
		After treatment		
Drugs and dose (M)	Before	10 min	20 min	30 min
Untreated rats				
Saline (control) (n = 10)	112·1 ± 4·8	112·6 ± 4·0	113·3 ± 3·9	112·8 ± 4·3
L-*threo*-DOPS				
2×10^{-5} (n = 10)	113·4 ± 3·0	117·5 ± 2·9	119·2 ± 3·1	120·0 ± 3·4
5×10^{-5} (n = 10)	111·6 ± 3·3	119·9 ± 5·0	129·5 ± 6·6[b]	134·7 ± 7·0[b]
10×10^{-5} (n = 10)	113·3 ± 4·1	131·3 ± 6·3[b]	147·3 ± 5·3[b]	155·9 ± 4·8[b]
20×10^{-5} (n = 10)	114·1 ± 2·8	135·0 ± 4·5[b,c]	172·2 ± 5·7[b,d]	191·8 ± 4·0[b,d]
DL-*threo*-DOPS				
5×10^{-5} (n = 10)	111·5 ± 4·2	115·9 ± 4·2	120·8 ± 4·0	122·9 ± 4·1[a]
10×10^{-5} (n = 10)	112·3 ± 3·2	124·2 ± 3·0[a]	134·4 ± 3·2[b]	136·6 ± 3·7[b]
20×10^{-5} (n = 10)	112·6 ± 2·8	138·8 ± 5·0[b]	150·9 ± 4·3[b]	156·6 ± 5·0[b]
D-*threo*-DOPS				
20×10^{-5} (n = 6)	110·8 ± 3·5	109·3 ± 4·1	107·5 ± 4·3	108·8 ± 3·7
Benserazide (50 mg kg^{-1}, i.p.)-treated rats				
L-*threo*-DOPS				
20×10^{-5} (n = 8)	72·7 ± 4·0	78·5 ± 5·7	86·6 ± 5·4[c]	88·8 ± 6·5[c]
α-Methyl-*p*-tyrosine (200 mg kg^{-1}, i.p.)-treated rats				
L-*threo*-DOPS				
20×10^{-5} (n = 8)	105·0 ± 3·5	159·2 ± 8·0[d]	183·7 ± 6·0[d]	202·0 ± 8·8[d]

Each value represents the means ± s.e.
Significantly different from control (unpaired Student's *t*-test, [a]: $P < 0{\cdot}05$, [b]: $P < 0{\cdot}01$).
Significantly different from the value before treatment (paired Student's *t*-test, [c]: $P < 0{\cdot}01$, [d]: $P < 0{\cdot}005$).

monstrated. D-*threo*-DOPS had no effect on the atrial rate and noradrenaline formation.

It has been reported that persistent blockade by α-MT of the synthesis of catecholamines may induce an adaptive change in their receptors thereby increasing sensitivity (Dominic & Moore, 1969; Moore & Dominic, 1971). If this is so, and assuming that the positive chronotropic effect is due to the noradrenaline formed from L-*threo*-DOPS by decarboxylase, the increase of atrial rate induced by L-*threo*-DOPS should be greater in atria from rats treated with α-MT. This was so with 20×10^{-5} M L-*threo*-DOPS and atria from rats treated with α-MT (200 mg kg^{-1}, i.p.) compared with untreated rats. That is, supersensitivity to L-*threo*-DOPS as well as to exogenous noradrenaline was observed in atria after a single administration of α-MT.

The results suggest that L-*threo*-DOPS is converted by decarboxylase to noradrenaline in isolated atrium and this increases the atrial rate.

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Mannitol and delayed hypersensitivity in the rat

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In therapeutics, mannitol, after intravenous administration, is used as an osmotic diuretic (Mudge, 1975) and also to reduce certain cases of cerebral oedema (Krayenbühl & Bühlmann, 1963; Lazorthes & Campan, 1972). It may also be used pharmaceutically as an excipient. We describe here its action when it has been administered for long periods in inflammatory models where delayed hypersensitivity plays an important role.

The experiments were on Sprague Dawley male rats. Mannitol was always injected at a dose of 23 mg kg^{-1} subcutaneously. Control animals received, 0·9% NaCl by the same route.

Mannitol was first studied on polyarthritis produced by Freund's adjuvant (Table 1). The treatment was started on the 10th day of the reaction and was continued for 28 days. While the volume of the injected paw was not significantly modified, mannitol reduced inflammation in the non-injected hind paw and the involvement of other organs affected by secondary arthritis (fore paws, ears, tail and penis) was also reduced significantly. The weight changes were improved by treatment compared with arthritic controls. Furthermore, autopsy revealed restoration of the weight of the thymus and of the adrenal glands.

Mannitol was used in animals with pleurisy due to *Bordetella pertussis* (Dieppe, Willoughby & others, 1976). The technique was described (Tarayre, Delhon & Lauressergues, 1977). The results obtained after chronic treatment, starting 3 weeks before sensitization, are presented in Table 2. Mannitol significantly reduced the volume of the inflammatory exudate. The various white cell counts remained unchanged.

After single and chronic administration, mannitol did not modify acute inflammation induced by carrageenan in the paw and pleural cavity of the rat. After repeated injections, it did not reduce the increase in capillary permeability induced by histamine.

In the doses used subcutaneously, the action of

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