Effects of bunaphtide on contractions induced by different agonists in the rat isolated aorta

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Bunaphtide is a new antiarrhythmic drug, with properties between those of quinidine and lidocaine, which has been shown to be a membrane stabilizer (Ferroni & Monticelli, 1973). Our previous evidence (Aleixandre, Tamargo & others, 1977) indicates that, like other antiarrhythmics, bunaphtide can produce a relaxation of vascular smooth muscle. Therefore, we have examined the influence of bunaphtide on the contractions induced by noradrenaline, 5-hydroxytryptamine (5-HT), potassium (K^+) and barium (Ba^{2+}) on the rat aorta and also the role of calcium ions (Ca^{2+}) on the effects exhibited by bunaphtide.

Helically cut strips of rat thoracic aorta were prepared as described by Furchgott & Bhadrakom (1953) and suspended in chambers containing 20 ml of Krebs-bicarbonate solution (pH 7·4) maintained at 37° and bubbled with 5% CO_2 in oxygen. Contractions were measured isometrically with a force-displacement transducer and recorded on a Grass polygraph. A resting tension of 2 g was applied and 2 h was allowed to elapse before the drugs were added. Complete concentration-response curves for the different agonists were obtained by cumulative addition of drugs to the bath fluid. This procedure was repeated at 90 min intervals until two successive curves were obtained in which the maximum tissue response was identical in height. Then the muscles were incubated with bunaphtide for 5 min and the agonist was re-added to the bath. Drugs used were: noradrenaline bitartrate, 5-hydroxytryptamine sulphate and bunaphtide hydrochloride. All concentrations refer to the salts.

Bunaphtide (5×10^{-6} — 5×10^{-4} M) did not modify the resting tension of the aortic strips. However, treatment for 5 min with concentrations higher than 5×10^{-6} M shifted the concentration-response curves for the different agonists to the right and produced a downward displacement of the maximum response. At 5×10^{-4} M, the contractile response to all four agonists was almost completely abolished. The ID50 (i.e. the concentration of bunaphtide required for 50% inhibition of the maximal tissue response) for the antagonist against each agonist is shown in Table 1. No significant differences in the effect of bunaphtide were observed for the different agonists. In other experiments, aortic strips were suspended in Ca^{2+}-free solution containing NaEDTA (0·1 mM) for 2 h. During the last 5 min of the incubation period, bunaphtide (5×10^{-5} M) was added to the medium. Five min later, K^+ (80 mM) was added to the bath and then Ca^{2+} (1–5 mM) was added in stepwise fashion over 45 min. Bunaphtide shifted the concentration-response curve of Ca^{2+} to the right (Fig. 1) and the maximal contraction induced by addition of 5 mM of Ca^{2+} was significantly reduced ($P < 0.001$).

Table 1. *Relative potency of bunaphtide in inhibiting contractions to different agonists.*

Agonist	n	ID50 (spasmolytic) ± s.e.m.
5-HT	6	$4·3 \pm 2·9 \times 10^{-5}$ M
K^+	6	$4·6 \pm 2·8 \times 10^{-5}$ M
Ba^{2+}	5	$6·8 \pm 1·8 \times 10^{-5}$ M
Noradrenaline	8	$7·9 \pm 3·1 \times 10^{-5}$ M

The fact that bunaphtide induced qualitatively similar inhibition in the contractions elicited by all four agonists indicates that some common step in the contractile process distal to the receptor level was influenced. It has been postulated (Somlyo & Somlyo, 1970) that stimulating agents initiate contraction by directly increasing the permeability of the membrane to calcium, thus increasing the myoplasmic Ca^{2+} concentration. Noradrenaline appears to act primarily by releasing calcium from a firmly bound fraction,

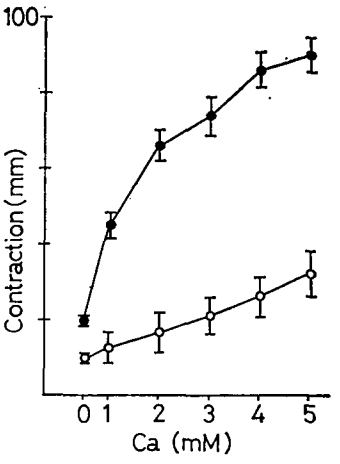

FIG. 1. Effects of bunaphtide (5×10^{-5} M) on the restoration of the isometric contractile responses of strips of the rat thoracic aorta to potassium (80 mM) by addition of calcium to calcium-free medium. Bunaphtide was added to the treated strips 5 min before the addition of potassium to both control (●) and treated (○) strips. Each point is a mean from six different muscles. Vertical bars indicate s.e.

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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-

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