J. Pharm. Pharmacol. 1983, 35: 46–48
Communicated June 10, 1982

0022–3573/83/010046–03 $02.50/0

Differential inhibitory effect of taurine on contractile responses to potassium and noradrenaline in rabbit ear artery

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In the rabbit ear artery, taurine reduces in a reversible and dose-dependent manner the increase in vascular tone induced by a depolarizing medium (54×10^3 μM KCl). On the other hand, the contraction induced by a supramaximal dose of noradrenaline (5 μM) is hardly affected by taurine (Franconi et al 1982). To establish that this apparently specific antagonism of contraction was not limited to the test dose of the stimulatory agent used, cumulative dose-response curves to noradrenaline and KCl were determined in the presence or absence of 40×10^3 μM taurine. The vasodilator action of taurine could be ascribed to an interaction with calcium handling (Franconi et al 1982). An interaction between calcium and taurine has been described in many tissues (Dolara et al 1973; Chovan et al 1980; Azari & Huxtable 1980).

Furthermore, recent findings show that the inotropic effect of taurine in cardiac tissue (Bandinelli et al 1981; Khatter et al 1981) and $^{45}Ca^{2+}$ uptake in retina (Lopez-Colomè & Pasantes-Morales 1981) is strictly dependent on extracellular calcium concentrations. In view of these reports we have examined the influence of different calcium concentrations on the vasodilator action of taurine on KCl-induced contractions in the rabbit ear artery.

Methods

A 3-cm segment of the central ear arterty was removed from male albino New Zealand rabbits, 2500–3000 g, previously anaesthetized with urethane (1·5 g kg^{-1} i.p.) and heparinized (1000 I.U. i.v.). After being carefully cleaned of its periarterial connective tissue, the artery was cannulated at both ends with polyethylene tubing and placed in a 15 ml organ bath at 37 °C. The artery was internally perfused by means of a De Saga 13100 six-channel peristaltic pump at a constant rate of 5 ml min^{-1} while extraluminal perfusion at a rate of 8 ml min^{-1} was obtained by means of a Mariotte bottle. Both intraluminal and extraluminal perfusion fluids were gassed with 95% O_2 and 5% CO_2 and heated to 37 °C. Intraluminal perfusion pressure was measured with a Statham T/1/A pressure transducer connected with a preamplifier and a Hewlett Packard recorder (7402 A).

Solutions. The standard solution was a Krebs solution of the following composition (mM): NaCl 119, KCl 4·7, $MgSO_4$ 1·5, KH_2PO_4 1·2, $NaHCO_3$ 25, $CaCl_2$ 2·5, glucose 11 and sucrose or taurine 40. Isotonic high potassium solutions were obtained by replacing NaCl with equimolar amounts of KCl. Ascorbic acid (0·05 mg ml^{-1}) was added to the solution containing noradrenaline.

Experimental procedure. The segment of central ear artery was perfused with normal Krebs solution for 60 min. The cumulative dose-response curves both for noradrenaline (5×10^{-2}–1·0 μM) and KCl ($24–72 \times 10^3$ μM) were constructed step by step by substituting the perfusion fluid with media containing progressively higher concentrations of the vasoconstrictor agents; the concentration was increased when a steady-state increase in vascular tone to the previous concentration was reached. While noradrenaline was only administered intraluminally, KCl concentrations were simultaneously increased in both the extraluminal and intraluminal media. After 20 min the dose-response curve was repeated in the presence of 40×10^3 μM taurine, added intraluminally.

In another set of experiments the action of taurine (40×10^3 μM) was determined on the tonic component of the contraction induced by high-potassium medium (54×10^3 μM) in the presence of different $CaCl_2$ concentrations (0·9; 2·5; $3·6 \times 10^3$ μM). The artery was perfused with depolarizing medium intra (and extra) luminally. When the tonic component of contraction was stable (usually after less than 10 min), taurine was administered intraluminally, until a new steady-state was reached. In this group of experiments each preparation was used for only one type of high-potassium (54×10^3 μM) medium.

The drugs used were (−)-noradrenaline bitartrate (Fluka A. G. Buchs S. G., Switzerland), taurine (E. Merck, Darmstadt, Germany) and prazosin which was kindly donated by Pfizer.

Results

Fig. 1 shows a typical recording of cumulative increase in vascular tone induced by progressively higher concentrations of KCl and noradrenaline.

Taurine 40×10^3 μM produced a shift to the right of the cumulative dose-response curve to KCl (Fig. 2B). Points from this study were used to construct a

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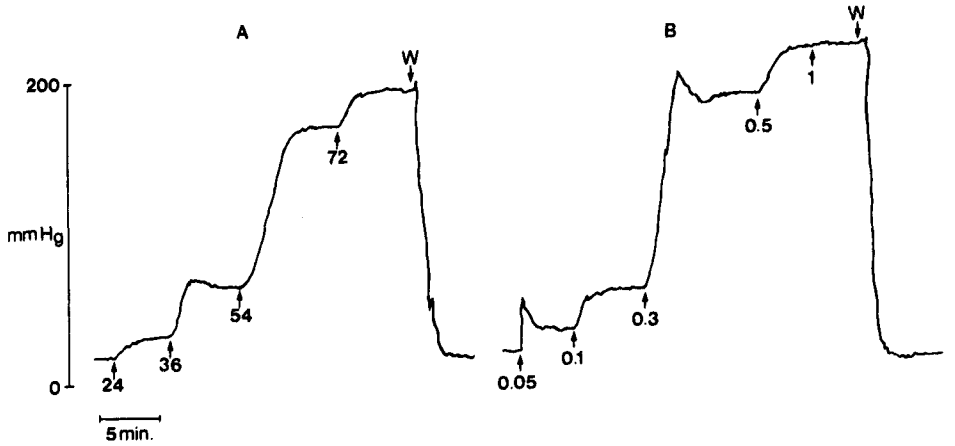

FIG. 1. Vasoconstrictor mechanical responses of rabbit central ear artery to (A) increasing concentrations of KCl (24–72 × 10^3 μM), (B) increasing concentrations of noradrenaline (0·05–1 μM).

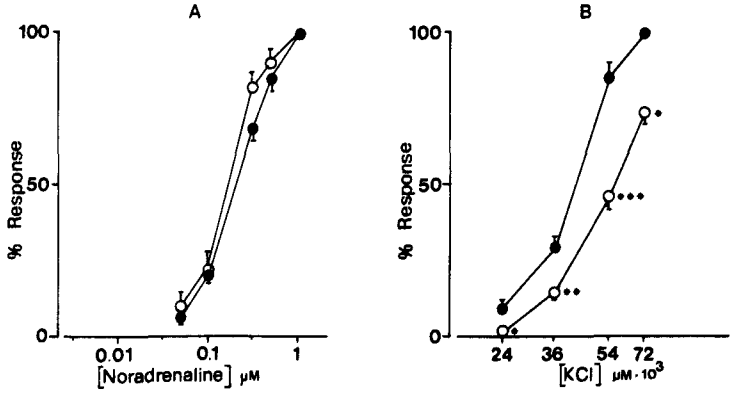

FIG. 2A. The effect of taurine (○) on the cumulative dose-response curve to noradrenaline (●). Each point is the mean ± s.e. of 6 experiments. B. The effect of 40 × 10^3 μM taurine (○) on the cumulative dose-response curve to KCl (●). Each point is the mean ± s.e. of 6 experiments. Significance of difference from control values: *0·05 ≥ P ≥ 0·01 ** 0·01 ≥ P ≥ 0·001 ***P ≥ 0·001.

Lineweaver-Burk Plot which shows that taurine antagonizes the KCl response in a non-competitive way. On the other hand taurine did not significantly modify the cumulative dose-response curve to noradrenaline (Fig. 2A). In the same experimental conditions the selectivity of α_1-adrenoceptor blocker, prazosin (0·1 μM), shifted the cumulative dose-response of noradrenaline to the right (pA_2 7·76 ± 0·06; n = 4).

The influence of calcium ions on the relaxing action of taurine on the tonic component of contraction induced by 54 × 10^3 μM KCl shows a positive log-linear relationship (y = 27·02 × + 73·62; r = 0·99) between the vasodilator potency of the taurine and the log of extracellular calcium ion concentrations.

Discussion

These data confirm that taurine is a specific antagonist of KCl-induced contractions in the rabbit ear artery, whereas it is without effect on noradrenaline-induced contraction. This selectivity is present in the whole dose-response range of both vasoconstrictor agents. The lack of effect of taurine on noradrenaline-mediated increases in perfusion pressure is high-lighted by the blocking activity of prazosin under the same experimental conditions. Some organic calcium blockers (Kondo et al 1980; Berner et al 1980) preferentially block KCl-induced contractions. It is well established that both noradrenaline and high potassium depolarization-induced contractions utilize an influx of calcium from extracellular space, moreover noradrenaline contraction also mobilizes an intracellular pool of calcium (Bolton 1979; Deth & Van Breemen 1977; Manzini et al 1982). Consequently, it seems unlikely that the effects of taurine are due to interference with the mobilization of intracellular calcium. There is evidence for separate pathways in the plasmalemma for calcium influx stimulated by high potassium and by noradrenaline (Meisheri et al 1981) and these could explain the selective action of taurine. The suggestion that the vasodilator activity of taurine is correlated with modulation of calcium kinetics is prompted also by the fact that under our experimental

conditions the vasodilator action of taurine was enhanced by increasing the calcium concentrations.

We thank Prof. A. Meli, for his interest and advice. We are grateful to Mrs Silvana Romanelli for technical assistance. The study was supported by MPI and CNR grant 81.00137.04.

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J. Pharm. Pharmacol. 1983, 35: 48–49
Communicated June 29, 1982

0022–3573/83/010048–02 $02.50/0

Identification of arachidonate metabolites in normal, benign and malignant human mammary tissues

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Most malignant tumours, including human mammary carcinomas, produce more prostaglandin-like material (PG-lm) than do the tissues in which they arise (see Bennett 1979, 1982). This finding contributes to the conclusion that PGs may play important roles in tumour growth and spread. Breast tumour prostaglandins have been tentatively characterized using paper chromatography (Bennett et al 1977). We now report formal identification of various arachidonate metabolites, and quantitation of PGD_2, PGE_2, $PGF_{2\alpha}$ and the PGI_2 hydrolysis product 6-keto-$PGF_{1\alpha}$, using gas chromatography-mass spectrometry.

Materials and methods

Tissue from 12 cancers, 3 benign lesions (fibroadenomas) and 4 specimens of macroscopically normal tissue were collected from patients undergoing breast surgery. Each sample were trimmed of fat, cut into small pieces and washed in Krebs solution. The methods were as described by Hensby et al (1982). In brief, weighed samples were homogenized in Krebs solution, extracted into chloroform, and purified using chromatography on LH20 and Amberlite XAD-2 columns and silica gel thin layer plates. The chloroform extraction method gives recoveries of about 70% with PGE, PGF and PGA compounds. Recoveries on subsequent purification were about 60–80%.

Chemical derivatization of the prostaglandin residues produced *O*-methyloximes which were converted into the corresponding methyl esters and then into trimethylsilyl ethers.

Samples were analysed by gas chromatography mass-spectrometry (g.c.–m.s.) using a Riber 10-10C mass spectrometer, and a Jirdel 31 gas chromatograph equipped with a 12·5 metre fused-silica capillary column (Hewlett Packard, SE30). Helium was used as the carrier gas at a flow rate of 2 ml min^{-1}, with a column temperature of 210–260 °C. The mass spectrometer was operated at 70 eV electron energy and an electron multiplier setting of 2200 V.

The samples were assessed qualitatively by full spectral scans for various eicosanoids. Quantitative g.c.-m.s. of PGD_2, PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ by selective ion monitoring was carried out on 18 extracted samples with added deuterated standards.

Results and discussion

The results of the qualitative g.c.-m.s. on 4 tumours and 2 normal tissues are shown in Table 1. Only compounds formed from arachidonate metabolism were detected. If metabolites of eicosatrienoic acid or eicosapentaenoic acid were present, their recovered amounts were less than the limit of detection which was approximately 20 ng g^{-1}. All extracts contained arachidonic acid, TxB_2, 6-keto-$PGF_{1\alpha}$, 6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$, 6,15-diketo-$PGF_{1\alpha}$ and 15-keto-13,14-dihydro-TxB_2. Using a less sensitive m.s. (Finnigan 9600) 12-HETE was detected in all breast tissues studied (6

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