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Endothelin-induced contractions of rat pulmonary artery are not affected by drugs acting on potassium channels*

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Abstract—The effects of the potassium channel opening drug, pinacidil, and the potassium channel closing drug, tetraethylammonium (TEA), on concentration-response (contraction) curves to spasmogens on rat pulmonary artery were examined. Pinacidil (3 μM) decreased, and TEA (2 mM) increased contractions to 5-hydroxytryptamine (5-HT) more than it did to noradrenaline but contractions to endothelin-1 were only minimally affected. It is concluded that the mechanism whereby endothelin-1 contracts rat pulmonary artery differs from that of noradrenaline or 5-HT in that it does not involve membrane depolarization or calcium entry through voltage operated calcium channels.

In a recent study, the spasmolytic effects of vasodilator drugs (i.e. reversal of contractions to spasmogens) on isolated pulmonary arteries from rats were examined (O'Donnell et al 1991). It was noted that all the vasodilator drugs tested, irrespective of their mechanism of action, were less effective as spasmolytic agents against endothelin-1 (ET-1) than against the other spasmogens tested, noradrenaline and $PGF_{2\alpha}$ (O'Donnell et al 1991). This difference was particularly noticeable for the vasodilator drug pinacidil. The first objective of the present study was to examine whether pinacidil was also a less effective anti-spasmogenic agent for rat pulmonary artery when contractions were induced by ET-1 as compared with noradrenaline or 5-hydroxytryptamine (5-HT).

Pinacidil relaxes smooth muscle by opening potassium channels, causing hyperpolarization (Southerton et al 1988). Tetraethylammonium bromide (TEA) closes potassium channels and causes depolarization. Hence TEA has the potential to contract smooth muscle and to increase the contractile response to spasmogens (Haeusler & Thorens 1980). Thus, the second objective of the present study was to examine the effects of TEA on responses to spasmogens on rat pulmonary artery and to see whether, like pinacidil, TEA was less effective against ET-1 than against the other spasmogens in potentiating contractile responses.

Materials and methods

Drugs and solutions. The drugs used were: acetylcholine chloride (Sigma), endothelin-1 (porcine ET-1, Peptide Institute), 5-hydroxytryptamine creatinine sulphate (5-HT, Sigma), (−)-noradrenaline acid tartrate (Sigma), pinacidil (gift from Leo Pharmaceuticals, Denmark) and tetraethylammonium bromide (TEA, Sigma). Stock solutions of drugs were prepared as follows: noradrenaline (100 mM) and pinacidil (10 mM) in 10 mM HCl; acetylcholine (10 mM), ET-1 (10 μM), 5-HT (10 mM) and TEA (1 M) in deionized water. Dilutions of all drugs were made in physiological salt solution (PSS), kept on ice during the experiment and then discarded. The composition of the PSS was (mM): NaCl 118, KCl 5·9, $CaCl_2$ 1·5, $MgSO_4$ 0·72, $NaHCO_3$ 25, glucose 11·7, ascorbic acid 1·14 (95% O_2 − 5% CO_2, pH 7·4).

Methods. Male Wistar rats (2–3 months; 300–450 g) were used. Isolated single rings of main pulmonary artery (2 or 3 mm) were set up in PSS at 37°C at a resting force corresponding to 10 mm Hg transmural pressure, i.e. at 7 and 10 mN for 2 or 3 mm preparations, respectively, as described by Wanstall & O'Donnell (1990). Changes in force in the circular muscle were recorded isometrically using a Statham Universal Transducer (UC3 and UL5). The endothelium was not removed from the preparations.

All preparations were allowed to equilibrate for 1 h and were then contracted with noradrenaline (0·1 μM). At equilibrium, acetylcholine (1 μM) was added and a relaxation response indicated the presence of endothelium. After washing the preparations with PSS, a reference contraction to potassium depolarizing PSS (replacement of 80 mM Na^+ with 80 mM K^+) was obtained before obtaining concentration-response curves to the spasmogens.

In preliminary experiments, 6 out of 9 preparations contracted to TEA (concentration range 1–30 mM). The highest concentration of TEA that was consistently without effect on all these preparations was 2 mM. Pinacidil (3 μM) has been shown to have a near maximal effect on potassium channels (^{86}Rb efflux experiments (Cook et al 1988)). Thus the effects of 2 mM TEA (10 min contact time) and of 3 μM pinacidil (30 min contact time) on responses to spasmogens were studied.

Concentration-response (contraction) curves to noradrenaline and 5-HT in the absence (control) and then in the presence of either TEA or pinacidil were obtained in the same preparations and responses to spasmogens were expressed as a percentage of the maximum response to spasmogen in the control curve (100%). Since responses to ET-1 cannot be repeated on the same preparation, control curves, and curves in the presence of TEA or pinacidil, had to be obtained on separate preparations for this spasmogen. Hence all responses to ET-1 were expressed as % K^+ 80 mM, i.e. as a percentage of the reference contraction to potassium depolarizing PSS in the same preparation (100%). Data for contractions to spasmogens in control curves are presented as mN mm^{-2}. Mean values are quoted with their standard errors (s.e.).

Results and discussion

Pinacidil (3 μM) decreased contractions of rat pulmonary artery to spasmogens and its effectiveness against the spasmogens was 5-HT > noradrenaline > > ET-1 (Fig. 1). This order (for the anti-spasmogenic effects of pinacidil) agrees with that previously reported for the spasmolytic effect of pinacidil (noradrenaline > > ET-1 (O'Donnell et al 1991)). The effects of TEA were examined in a second series of experiments and, for each spasmogen, the control curves were superimposed on the control curves from the pinacidil experiments (Fig. 1). TEA (2 mM) increased contractions of rat pulmonary artery to spasmogens and the order of its effectiveness was 5-HT > noradrenaline > > > ET-1 (Fig. 1).

Pinacidil and TEA both act on membrane potassium channels. Irrespective of whether these drugs act on the same or different postulated types of potassium channel (Quast & Cook 1989), the net effect for both drugs is an alteration of membrane

*A preliminary account of these data was presented to the 23rd meeting of the Australasian Society of Clinical and Experimental Pharmacologists, Sydney, December 1989 (O'Donnell & Wanstall 1990).

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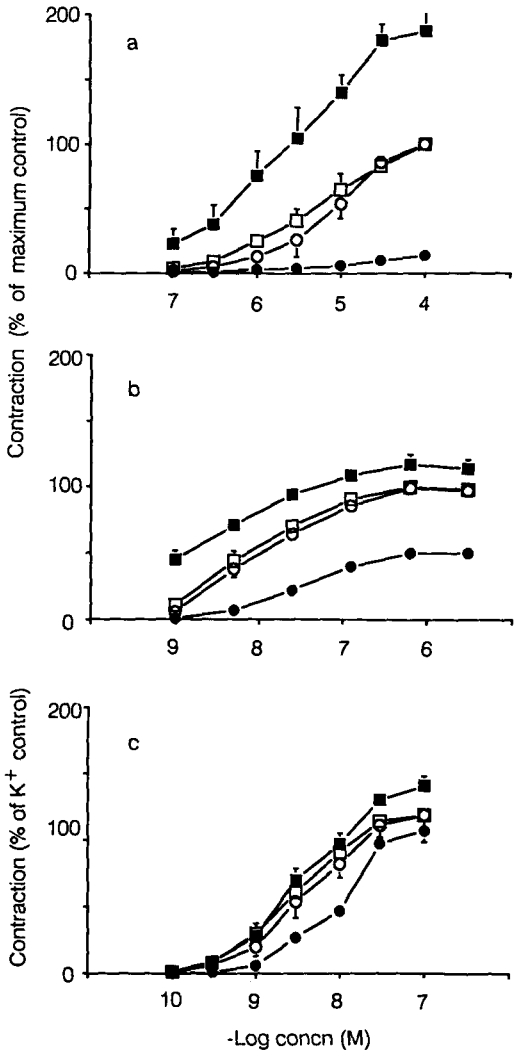

FIG. 1. Mean concentration-response curves to spasmogens on rat pulmonary artery preparations. The spasmogens were (a) 5-HT (b) noradrenaline and (c) ET-1. Curves were obtained in the absence (controls, open symbols) and presence (closed symbols) of 3 μM pinacidil (○, ●) or 2 mM tetra ethylammonium (TEA) (□, ■). Since responses to ET-1 could not be repeated on the same tissue, data in (c) are expressed as a % of the contraction in 80 mM K^+ in the same preparation. The s.e. values are shown by vertical bars except where smaller than the symbols. The maximum contractions to spasmogens in the control curves (mN mm^{-2}) for the pinacidil and TEA series respectively were: a) 5-HT; 5·5±0·81 (4), 5·7±0·82 (5): (b) noradrenaline; 10·6±1·49 (4), 14·8±2·39 (4); (c) ET-1; 29·6±2·25 (4), 22·0±4·05 (4).

potential which influences voltage-operated calcium channels (VOCs) and hence contractile responses. Pinacidil acts by opening potassium channels thus causing hyperpolarization whereas TEA closes potassium channels causing depolarization. Hence the observation that pinacidil and TEA caused attenuation and potentiation, respectively, of contractile responses to 5-HT and noradrenaline suggest that responses of rat pulmonary artery to these spasmogens depend, at least in part, on the influx of calcium through VOCs.

Pinacidil and TEA had little influence on contractile responses to ET-1, except for a small decrease in responses to 3 and 10 nM ET-1 by pinacidil. The inability of TEA to increase, and of pinacidil to decrease, contractions to ET-1 indicates that membrane depolarization and influx of calcium through VOCs are of little importance in the functional contractile response of rat pulmonary artery to ET-1. A similar argument was used to explain the inability of TEA to potentiate contractions to histamine and acetylcholine in guinea-pig trachea (Boyle et al 1988). Our previous finding that the calcium entry blocking drug, felodipine, did not inhibit ET-1 contractions in rat pulmonary artery (Wanstall et al 1991) supports a lack of involvement of VOCs in contractile responses to ET-1 in rat pulmonary artery.

The findings with TEA in this study are of additional interest in light of some previous findings for pulmonary artery preparations taken from rats with monocrotaline-induced pulmonary hypertension (Wanstall & O'Donnell 1990). Pulmonary hypertension increased the potencies of 5-HT more than noradrenaline but had no effect on that of ET-1. Since pulmonary arteries from rats with pulmonary hypertension have been suggested to be partially depolarized (Suzuki & Twarog 1982), we postulated that depolarization might be responsible for the increased potencies of 5-HT and noradrenaline in these preparations (Wanstall & O'Donnell 1990). The observation made in the present study that the depolarizing drug, TEA, increased contractile responses to 5-HT and noradrenaline but not to ET-1 could support this postulate.

In summary, the main finding from this study is that contractions to ET-1 differ from those to noradrenaline or 5-HT in that they are not affected by either of two drugs which alter membrane potential by an action on potassium channels, pinacidil and TEA. If the effectiveness of potassium channel drugs against different spasmogens reflects the mechanisms of contraction of the spasmogens, then it can be concluded that the mechanism for ET-1 differs from that for noradrenaline or 5-HT. This supports our conclusions from two previous studies with vasodilator drugs on rat pulmonary artery, in which we suggested that the mechanism of action of ET-1 differed not only from that of noradrenaline or 5-HT (Wanstall et al 1991) but also from that of $PGF_{2\alpha}$ (O'Donnell et al 1991). If ET-1 is of pathophysiological importance as a vasoconstrictor, then there may be a need to find new vasodilator drugs which are effective against this spasmogen.

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Effects of nifedipine on renal responses to several diuretic agents in rats

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Abstract—The influence of the dihydropyridine calcium antagonist nifedipine has been studied on the diuretic response to frusemide, acetazolamide and hydrochlorothiazide in water-loaded (25 mL kg^{-1}) conscious rats. Oral administration of nifedipine (10 mg kg^{-1}) markedly inhibited frusemide- and hydrochlorothiazide-induced diuresis as evidenced by a reduction in 5 h urine volume and urinary sodium and potassium elimination. However, it neither significantly enhanced nor limited urine and electrolyte excretion promoted by acetazolamide. Nifedipine, 5 and 10 mg kg^{-1} but not 1 mg kg^{-1}, significantly ($P < 0.05$) inhibited the diuretic response of hydrochlorothiazide. At doses which affect hydrochlorothiazide diuresis (5 and 10 mg kg^{-1}), nifedipine was found to depress the mean arterial pressure by 32% in normotensive rats. These results are of interest in view of the often reported clinical side effect of nifedipine in promoting peripheral oedema in hypertensive patients and its use in combination with a thiazide or loop diuretic.

The antihypertensive efficacy of the slow calcium channel blocker nifedipine has been well documented in recent years (MacGregor et al 1982, 1983; Schnapp et al 1987). Controlled clinical studies have shown that nifedipine is well suited for use by itself as well as in combination with β-adrenoceptor blockers or diuretics (Duffy & MacDonald 1987). Its usefulness in combination with diuretics, however, is not very clear. Clinical experience suggests that in some patients at least, a diuretic can cause a further decrease in blood pressure when added to a nifedipine therapy (Robinson 1985) while in others no such additive effect was observed (Rosenthal 1982). Acute administration of nifedipine to normotensive and hypertensive subjects produces increased renal blood flow (Yokoyama & Kaburagi 1983), natriuresis and diuresis (Leonetti et al 1982; Schnapp 1989), but the mechanisms of nifedipine-induced natriuresis are incompletely understood and do not show any relationship to fall in systemic blood pressure (Schnapp 1989). Short or long term use of nifedipine is associated with a moderate increase in plasma renin activity and will also promote the development of peripheral oedema which is sometimes severe and resistant to diuretic therapy (Aoki et al 1978; Guazzi et al 1983; Duffy & MacDonald 1987). Experimental studies showed nifedipine to cause overt urine and electrolyte retention in saline-loaded rats (Barret et al 1988). A similar observation has been made by Rao et al (1988) in water-loaded normotensive rats. Since a combination of nifedipine and diuretics is sometimes preferred clinically for the treatment of hypertension, it was of interest to study the effects of nifedipine on systemic blood pressure and on diuretic responses to frusemide, acetazolamide and hydrochlorothiazide in water-loaded, conscious rats.

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Materials and methods

Animals

Wistar female rats, 200–350 g, were maintained under a 12/12 h light/dark cycle at 23 ± 1 °C with free access to a standard pellet diet (Purina rat chow) and water. All experiments were performed between 0900 and 1400 h.

Diuresis in rats

Rats were deprived of food and water for 16 h and received a priming dose of 0·9% NaCl (saline) (25 mL kg^{-1}) by the oral route. The rats were divided into two groups of 10 in each cage and the test drugs nifedipine (10 mg kg^{-1}), hydrochlorothiazide (10 mg kg^{-1}) and acetazolamide (20 mg kg^{-1}), suspended in distilled water containing 0·5% gum arabic, were administered orally in a volume of 25 mL kg^{-1}; frusemide (2 mg kg^{-1}) was given intraperitoneally. The control animals received the same volume of vehicle. The animals were placed in metabolic cages (2 rats/cage) immediately after administration of the drugs. A pooled 5 h urine sample was obtained from each treatment group. Excreted urine volume, pH, urinary sodium and urinary potassium were measured. In another set of experiments, hydrochlorothiazide-induced diuresis was evaluated at doses of 1, 5 and 10 mg kg^{-1} nifedipine in a time course study.

Blood pressure in rats

Rats were anaesthetized with pentobarbitone sodium (40 mg kg^{-1}, i.p.) and the mean arterial pressure was continuously monitored from the right carotid artery through a Statham transducer. Mean blood pressure changes following intraduodenal application of nifedipine (5 and 10 mg kg^{-1}) were measured. Five animals were used for each dose of nifedipine.

Estimation of pH and urinary electrolytes

Urine pH was measured with a pH blood gas analyser (Instrumentation Laboratory Inc. Type 213) and sodium and potassium were measured by a flame photometer (Instrumentation Laboratory Inc. Type 443).

Statistical analysis

Values were expressed as mean ± s.e.m. Analysis of variance and Student's *t*-test were used to evaluate the results.