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Choice and concentration of contractile agent influence responses of rat aorta to vascular relaxant drugs

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Concentration-response (relaxation) curves to diltiazem, forskolin, isobutylmethylxanthine (IBMX), procaterol, isoprenaline and sodium nitrite were obtained on isolated ring preparations of rat aorta contracted either submaximally or maximally with noradrenaline or KCl. Diltiazem was more potent on KCl-contracted than on noradrenaline-contracted preparations whether the preparations were submaximally or maximally contracted. The other relaxant drugs were at least 20-fold less potent against KCl than against noradrenaline, but only on maximally contracted preparations. On submaximally-contracted preparations there was no potency difference between preparations contracted with these two contractile agents. Increasing the KCl concentration had a marked influence on the location of the concentration-response (relaxation) curves to all the drugs except diltiazem. This influence was different for drugs that act via cyclic AMP and those that act via cyclic GMP. It is concluded that both the choice of contractile agent (noradrenaline or KCl) and the concentration (especially of KCl) influence relaxant responses of rat aorta to vasodilator drugs.

The examination of the effect of vasodilator drugs on isolated blood vessels requires initial contraction of the preparations. It is apparent from the literature that responses to a variety of vasodilator drugs can be influenced by the choice of the contractile agent (Furchgott & Bhadrakom 1953; Furchgott 1983; Lincoln & Fisher-Simpson 1983; Jones et al 1984). It has even been suggested that the potency difference between depolarized preparations and agonist-contracted preparations could be used as an index for classifying those vasodilator drugs which do not act via specific receptors (Kent et al 1982). In this study, various vasodilator drugs with different, but known, mechanisms of action have been examined on noradrenaline-contracted and KCl-contracted preparations of rat aorta. The drugs were diltiazem (calcium entry blocking drug), forskolin (adenylate cyclase activator), isobutylmethylxanthine (IBMX, phosphodiesterase inhibitor), sodium nitrite (acts via cyclic GMP), isoprenaline and procaterol (β-adrenoceptor agonists). Data have been obtained on both submaximally and maximally contracted preparations so that the influence not only of the choice of contractile agent (noradrenaline or KCl), but also of its concentration, on the potency of the vasodilator drugs could be assessed.

A preliminary account of these data was presented to the 43rd meeting of the Australian Physiological and Pharmacological Society, Sydney, 1985 (Wanstall & O'Donnell 1985).

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Materials and methods

Male, Wistar rats (2 to 2·5 months old; 160 to 280 g) were used. Up to four isolated ring preparations (4 mm in length; endothelium removed by rubbing) were obtained from the descending thoracic aorta and were set up in Krebs solution at 37 °C bubbled with 95% O_2: 5% CO_2. Force in the circular muscle was recorded isometrically as described by O'Donnell & Wanstall (1981). A resting force of 10 mN was maintained throughout the experiments. This was shown, in preliminary experiments, to be the optimal resting force for the development of active tension.

The preparations were allowed to equilibrate for 1 h (Krebs solution replaced at 15 min intervals), after which a preliminary contraction was induced with 60 mM KCl. The preparations were then contracted with either noradrenaline or KCl, and, when the contraction was stable, a cumulative concentration-response (relaxation) curve was obtained to one of the relaxant drugs. In most experiments the preparations were subsequently contracted with the other contractile agent (KCl or noradrenaline) and a second concentration-response curve to the same relaxant drug was obtained. Different relaxant drugs were examined on different preparations from any one rat. Isoprenaline was examined only on KCl-contracted preparations, because an α-adrenoceptor antagonist is required for valid concentration-response curves to this β-adrenoceptor agonist, and this precluded its examination on noradrenaline-contracted preparations.

The concentrations of noradrenaline and KCl were 0·01 μM and 15 mM, respectively, for the first series of experiments and 3 μM and 80 mM, respectively, for the second series. These concentrations were shown, in preliminary experiments, to give contractions which were submaximal and maximal, respectively, for each particular contractile agent (mean increases in tension: noradrenaline (n = 6) 0·01 μM 3·5 ± 0·52 mN, 3 μM 4·3 ± 0·58 mN; KCl (n = 9) 15 mM 3·1 ± 0·53 mN, 80 mM, 3·5 ± 0·45 mN). The KCl concentrations were achieved by adding the appropriate volume of 3 M KCl to 10 mL of Krebs solution in the tissue bath (Jones et al 1984).

Relaxant responses were expressed as % reversal of the induced contraction. The maximal relaxation was sometimes greater than 100%, indicating that some of the preparations had some inherent tone. From each curve the concentration producing a 50% relaxant response (IC50) was interpolated, and potency was

expressed as the mean negative log IC50. For each relaxant drug, any potency difference between noradrenaline-contracted and KCl-contracted preparations, respectively, was calculated as the ratio of IC50 values on KCl-contracted and NA-contracted preparations.

Drugs and solutions. The drugs used were: diltiazem (gift from Laboratoires d'Etudes et de Recherches Synthelabo), forskolin (Calbiochem-Behring), 3-isobutyl-1-methylxanthine (IBMX, Sigma), (−)-isoprenaline acid tartrate (Sigma), (−)-noradrenaline acid tartrate (Sigma), phenoxybenzamine hydrochloride (Smith, Kline & French), procaterol (Warner-Lambert), sodium nitrite (B.D.H. Chemicals Australia).

Stock solutions of isoprenaline or noradrenaline (100 mM) were prepared in 10 mM HCl, of diltiazem (10 mM), procaterol (10 mM) or sodium nitrite (1 M) in deionized water, of IBMX (10 mM) in 10 mM NaOH and of phenoxybenzamine (100 mM) in absolute ethanol containing 10 mM HCl. Forskolin was dissolved and diluted in absolute ethanol. Dilutions of the other drugs were made in Krebs solution. All dilutions were kept on ice during the course of the experiments.

The composition of the Krebs solution was (mM): NaCl 118; KCl 4·7; $CaCl_2$ 2·5; KH_2PO_4 1·2; $MgSO_4$ 1·2; $NaHCO_3$ 25; glucose 11·7; ascorbic acid 1·1.

Statistical analyses. Mean values of negative log IC50 are quoted together with their standard errors (s.e.), and differences between mean values have been assessed by Student's *t*-test. On the mean concentration-response curves, the s.e. of the mean responses are shown, to indicate the scatter of the data.

Results and discussion

The potency values (mean negative log IC50) for the various vascular relaxant drugs on preparations of aorta contracted both submaximally and maximally with either noradrenaline or KCl are shown in Table 1, together with values for the potency difference between noradrenaline-contracted and KCl-contracted preparations. Mean concentration-response curves corresponding to these data are illustrated in Fig. 1 to show the maximum relaxant responses achieved.

Diltiazem, a calcium entry blocking drug, was more potent on KCl-contracted preparations than on noradrenaline-contracted preparations. This difference in potency, which is a characteristic property of calcium entry blocking drugs on vascular smooth muscle (Bou et al 1983), occurred whether the preparations were submaximally or maximally contracted (Table 1, Fig. 1).

For the vasodilator drugs acting via cAMP or cGMP (forskolin, IBMX, β-adrenoceptor agonists and sodium nitrite) the results were different on submaximally and maximally contracted preparations. On submaximally contracted preparations the potency of the drugs was the same whether noradrenaline or KCl was used as the contractile agent (Table 1). On maximally contracted preparations, forskolin, IBMX and sodium nitrite were at least 20-fold less potent against KCl than against noradrenaline; procaterol relaxed noradrenaline-contracted but not KCl-contracted preparations (Table 1).

Table 1. Potency (mean negative log IC50 values) of forskolin, IBMX, procaterol and sodium nitrite on preparations of rat aorta contracted with noradrenaline (NA) or potassium chloride (KCl).

	Mean negative log IC50 ± s.e.		Potency diff. between NA- and KCl-contracted[a]
	NA-contracted	KCl-contracted	
A. Submaximally contracted preparations[a]			
Diltiazem	4·86 ± 0·27*** (5)[d]	6·75 ± 0·12 (5)	0·013
Forskolin	7·42 ± 0·11 (7)	7·35 ± 0·16 (6)	1·2
IBMX	5·85 ± 0·30 (6)	5·74 ± 0·09 (9)	1·3
Procaterol	7·13 ± 0·27 (3)	7·02 ± 0·07 (3)	1·3
Isoprenaline	—	7·40 ± 0·12 (4)	—
Sodium nitrite	4·37 ± 0·20 (6)	4·20 ± 0·09 (8)	1·5
B. Maximally contracted preparations[b]			
Diltiazem	4·05 ± 0·30** (4)	6·65 ± 0·18 (4)	0·003
Forskolin	7·17 ± 0·16** (4)	5·87 ± 0·22 (4)	20·0
IBMX	5·48 ± 0·17*** (4)	4·16 ± 0·11 (4)	20·9
Procaterol	Maximum relaxation < 50%	No relaxation	—
Isoprenaline	—	No relaxation	—
Sodium nitrite	3·65 ± 0·11** (6)	2·29 ± 0·31 (4)	22·9

[a] Preparations contracted with 0·01 μM NA or 15 mM KCl.
[b] Preparations contracted with 3 μM NA or 80 mM KCl.
[c] IC50 (KCl-contracted preparations)/IC50 (NA-contracted preparations).
[d] Numbers of observations.
Asterisks—value on NA-contracted preparations significantly different from that on KCl-contracted preparations ** $0·01 > P > 0·001$; *** $P < 0·001$.

The above results on maximally contracted preparations are in agreement with reports in the literature for drugs acting via cAMP or cGMP. For example, isoprenaline, sodium nitrite (Furchgott & Bhadrakom 1953), nitroprusside (Lincoln & Fisher-Simpson 1983), forskolin (Lincoln & Fisher-Simpson 1983; Jones et al 1984) and acetylcholine (Furchgott 1983) have each been described as being more potent on vascular preparations contracted with agonists, such as noradrenaline, than on those contracted with KCl. The results for submaximally contracted preparations (i.e. no potency difference) were not expected from the literature, and

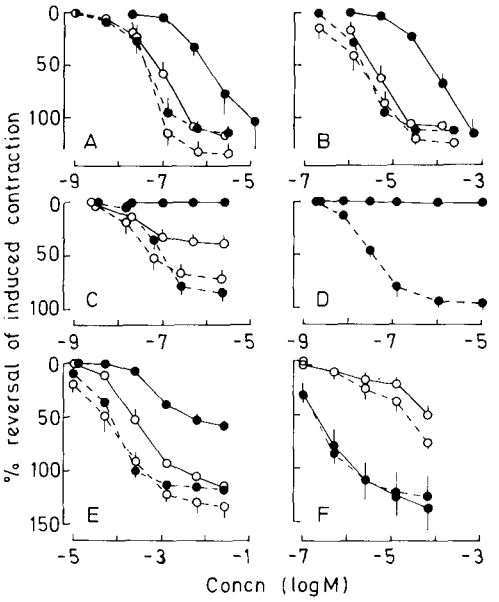

FIG. 1. Mean concentration-response (relaxation) curves for forskolin (A), IBMX (B), procaterol (C), isoprenaline (D), sodium nitrite (E) and diltiazem (F) on preparations of rat aorta contracted with KCl, 15 mM (●– –●) or 80 mM (●——●), or noradrenaline 0·01 μM (○– –○) or 3 μM (○——○). Relaxant responses are expressed as % reversal of the induced contractions. Standard errors of mean responses are shown by the vertical lines except when smaller than the symbols. Numbers of observations are as indicated in Table 1. The experiments with isoprenaline were carried out on preparations which had been treated with phenoxybenzamine (50 μM for 30 min followed by wash out) to block α-adrenoceptors.

indicate that not only the choice of contractile agent, but also its concentration, may affect responses to vascular relaxant drugs. This needs to be considered if a potency difference between preparations contracted with e.g. an α-adrenoceptor agonist and those contracted with KCl is to be used as an index for classifying vasodilator drugs, as proposed by Kent et al (1982).

The difference between the results on maximally and submaximally contracted preparations occurred because the location of the concentration-response curves changed markedly with different concentrations of KCl, but little, or not at all, with different concentrations of noradrenaline (Fig. 1). Interestingly, the effect on the vasodilator concentration-response curve of increasing the KCl concentration from 15 to 80 mM appeared to depend on the mechanism of action of the vasodilator drug. For the β-adrenoceptor agonists (which increase cAMP following β-adrenoceptor activation, Namm 1982) the response was abolished. For forskolin and IBMX (which increase cAMP by a mechanism distal to receptors) the potency, but not the maximum relaxation, was reduced. For sodium nitrite (which increases cGMP) both the potency and the maximum relaxation were reduced. A difference in the effects of high concentrations of KCl on relaxant responses to drugs which increase cAMP and those which increase cGMP has also been reported on guinea-pig trachea (Ito et al 1985), and this difference remains to be explained. It is possible that, for drugs that act via cGMP, high KCl concentrations inhibit the production of cGMP as well as inhibiting the relaxation that it induces (Rapaport et al 1985), whereas, for drugs that act via cAMP, high KCl concentrations may only inhibit the relaxation induced by cAMP with no effect on the production of this nucleotide (Lincoln & Fisher-Simpson 1983).

In conclusion, this study has shown that, on rat aorta, both the choice of contractile agent (noradrenaline or KCl) and also the concentration of contractile agent (especially of KCl) influence the potency of forskolin, IBMX, sodium nitrite and β-adrenoceptor agonists. In contrast, the potency of diltiazem was influenced only by the choice of contractile agent, and not by its concentration.

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