J. Pharm. Pharmacol. 1999, 51: 1183–1190
Received April 19, 1999
Accepted April 29, 1999

Differences between the Vasorelaxant Activity of Adenosine-receptor Agonists on Guinea-pig Isolated Aorta Precontracted with Noradrenaline or Phenylephrine

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Abstract

The relaxant effect of adenosine and 5′-(*N*-ethylcarboxamido)adenosine (NECA) against α-adrenoceptor-mediated contractile tone in guinea-pig isolated aortic rings has been examined to determine if this A_{2B}-receptor-mediated relaxation was dependent upon the contracting agent, and whether the contractions were dependent upon intracellular or extracellular calcium.

Relaxation responses were consistently greater for aortic rings pre-contracted with phenylephrine (3×10^{-6} M) than for rings pre-contracted with noradrenaline (3×10^{-6} M). Maximum inhibition by NECA was significantly greater for phenylephrine-contracted aortae than for noradrenaline-contracted (81·9 ± 2·8% compared with 25·0 ± 1·5%). These differences persisted in the presence of β- and α_2-adrenoceptor blockade and could not, therefore, be attributed to stimulation of these receptors by noradrenaline. The ratio of the contractions obtained before and in the presence of adenosine or NECA was compared with the control ratio obtained before and after vehicle. Experiments were performed both in the presence of normal calcium levels and under calcium-free conditions. In normal-calcium medium, NECA inhibited phenylephrine-induced contractions (test ratio, 76·7 ± 3·9%; control ratio, 133·1 ± 9·8%) to a greater extent than noradrenaline-induced contractions (108·4 ± 4·1 and 123·4 ± 4·9%); adenosine similarly inhibited phenylephrine-induced contractions more than those induced by noradrenaline. Under calcium-free conditions, adenosine (36·7 ± 11·9 and 110·7 ± 26·6%) and NECA (55·2 ± 9·1 and 87·1 ± 14·9%) were only effective against phenylephrine-induced contractions. This suggests that activation of the A_{2B}-receptor by these agonists inhibited intracellular mobilization of calcium for phenylephrine-induced contractions only. The effects on extracellular calcium influx were examined for phenylephrine- and noradrenaline-induced contractions in normal-calcium medium but in the presence of ryanodine to prevent intracellular calcium mobilization. NECA inhibited phenylephrine-induced contractions (77·3 ± 12·4 and 111·4 ± 9·3%), presumably by interfering with influx of calcium through receptor-operated calcium channels. In contrast, NECA failed to reduce noradrenaline-induced contractions (121·5 ± 10·7 and 122·4 ± 11·6%), suggesting that the effect on noradrenaline is predominantly via interaction with intracellular calcium. Adenosine was consistently a more effective relaxant than NECA, possibly because of an additional intracellular component of the response.

We conclude that adenosine receptor agonists inhibit phenylephrine-induced contractions of guinea-pig aorta more selectively than noradrenaline-induced contractions. A_{2B}-receptor stimulation might reveal a fundamental difference between the modes of contraction elicited by these two α-adrenoceptor agonists.

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Adenosine elicits relaxation in all vascular beds studied except kidney and placenta (Olsson & Pearson 1990). This vasorelaxation is mediated through interaction with extracellular A_2-adenosine receptors (Collis & Brown 1983; Headrick & Berne 1990) which in guinea-pig aorta have been identified as the A_{2B}-subtype (Martin 1992; Alexander et al 1994). An intracellular P-site adenosine receptor might also be involved in the vasodilation by adenosine in guinea-pig aorta (Collis & Brown 1983) and rat mesenteric artery (Prentice et al 1997). In the current study, extracellular A_{2B}-adenosine receptor stimulation of the guinea-pig aorta was studied by use of both adenosine and 5′-(*N*-ethylcarboxamido)adenosine (NECA), which is not a substrate for the purine transport system (Collis & Brown 1983) and does not, therefore, exert an intracellular action. Stimulation of A_2-adenosine receptors is thought to evoke vasorelaxation by reducing the amount of calcium available for contraction (Young & Merrill 1983; Ramagopal & Mustafa 1988; Zawadzki & Weiss 1990), possibly by inhibition of membrane receptor-operated calcium channels (Collis & Brown 1983; Ramagopal & Mustafa 1988; Urquhart & Broadley 1991), resulting in reduced entry of extracellular calcium ions. Previous studies have shown that adenosine inhibits both intra- and extracellular Ca^{2+} mobilization in the relaxation of coronary arteries precontracted with $PGF_{2\alpha}$, acetylcholine or noradrenaline (Ramagopal et al 1989; Ramagopal & Mustafa 1990). We have shown that NECA inhibits the contraction of guinea-pig aorta induced by noradrenaline by interfering with influx of extracellular Ca^{2+} only (Ford & Broadley 1996) whereas the phenylephrine-induced contractions were inhibited by interfering with both intra- and extracellular Ca^{2+} (Ford & Broadley 1999).

In the current study the effects of NECA and adenosine on phenylephrine- or noradrenaline-induced contraction of guinea-pig aorta have been examined. Adenosine or NECA were added to the tissues either after the contractions had reached a plateau or before the induction of the contraction. The contractions of aortic smooth muscle induced by α-adrenoceptor agonists consists of an initial fast phase, as a result of intracellular Ca^{2+} release, followed by a slower-developing contraction, which is dependent upon calcium influx through receptor-operated channels (Rinaldi et al 1991). To determine whether NECA and adenosine cause vasorelaxation by interacting with intracellular or extracellular calcium mobilization, the aortae were set up in normal-calcium and Ca^{2+}-free media. In addition, the effects of NECA and adenosine against contractions dependent upon extracellular Ca^{2+} only were examined in the presence of ryanodine to block the release of intracellular Ca^{2+} (Sorrentino & Volpe 1993).

Materials and Methods

Drugs and solutions

Adenosine (free base), 5′-(*N*-ethylcarboxamido) adenosine, (−)-noradrenaline bitartrate salt, (−)-phenylephrine hydrochloride, and idazoxan hydrochloride were from Sigma (Poole, UK), ryanodine was from Biomolecular Research Laboratories (Cambridge, UK) and (−)-propranolol hydrochloride was from Zeneca (Macclesfield, Cheshire, UK).

Tissue preparation

Male Dunkin–Hartley guinea-pigs, 250–500 g, were killed by a blow to the back of the head then exsanguination under running water. The rib cage was removed and the thoracic aorta exposed. The aorta was cleared of connective tissue in-situ and then excised. The endothelial lining was mechanically removed by gently rubbing the luminal surface with a softwood stick. Rings (5 mm) were then cut and mounted under 1-g tension in 50-mL tissue baths containing Krebs-bicarbonate solution, in double distilled water, of composition (mM): NaCl 118, KCl 4.7, $CaCl_2.2H_2O$ 2·5, $MgSO_4{\cdot}7H_2O$ 1·2, $KH_2PO_4.2H_2O$) 1·2, $NaHCO_3$ 25, glucose 11·7 (''normal'' Krebs). The Krebs solution was maintained at 37°C and oxygenated continuously with 95% O_2–5% CO_2. Tension was measured by use of Dynamometer UF1 isometric force transducers (sensitivity 2 oz) and displayed on a Devices M19 chart recorder. Tissues were left to equilibrate for 60 min before drug addition.

Study of cumulative concentration–response curves

Cumulative concentration–response curves were constructed for noradrenaline or phenylephrine to determine a submaximum concentration suitable for use for preconstriction. Noradrenaline (3×10^{-6} M) or phenylephrine (3×10^{-6} M) was used to preconstrict the aortic rings and when the tension had stabilized to a plateau cumulative concentration–response curves were constructed for the relaxation elicited by NECA or adenosine. The concentrations of NECA and adenosine resulting in submaximum inhibition of tension were determined for use in subsequent experiments.

Adrenoceptor antagonists or ryanodine, where used, were added 20 min before addition of agonist.

Study of contractions in Ca^{2+}-free and normal-Ca^{2+} medium

The effects of the test drugs were examined against contractions obtained in normal Krebs solution and in Ca^{2+}-free Krebs solution (contractions dependent upon intracellular Ca^{2+}). Four aortic rings were taken from the same guinea-pig and mounted in separate tissue baths. Tissue viability was established by initiating a contraction to either noradrenaline (3×10^{-6} M) or phenylephrine (3×10^{-6} M). After the contraction had reached a plateau the tissues were washed with normal Krebs solution and incubated until tension had returned to baseline levels. The steps subsequent to this priming stage are shown in Figure 1. Two of the four preparations were washed in modified Krebs solution from which the calcium had been omitted and 4 mM EDTA (disodium salt) added (calcium-free). The other two preparations were washed in normal Krebs solution. The tissues were incubated for 10 min and then submaximum contractions to noradrenaline or phenylephrine were induced in all four preparations (C1). After the contractions had peaked, all preparations were washed three times in normal Krebs-bicarbonate solution and then incubated for 10 min. After this time the normal Krebs in the calcium-free preparations was replaced with calcium-free solution and the normal Krebs in the normal preparations was replaced with normal Krebs. Adenosine or NECA were immediately added to one of each of the two types of preparation (test) and vehicle was added to the others (control). After 10 min incubation, a second contraction to noradrenaline or phenylephrine was obtained (C2). A representative trace is shown in Figure 1.

The use of EDTA to achieve Ca^{2+}-free conditions did not seem to have any undue deleterious effect upon the tissues. For example, in control experiments the second contraction (C2) to phenylephrine in Ca^{2+}-free medium was usually of the same magnitude as the first contraction (C1) (e.g. Table 2).

Study of contractions dependent upon extracellular calcium

To study the effects of NECA and adenosine on contractions dependent upon extracellular calcium, experiments were performed in the presence of ryanodine (10^{-5} M) added 20 min before C1 in normal calcium medium only.

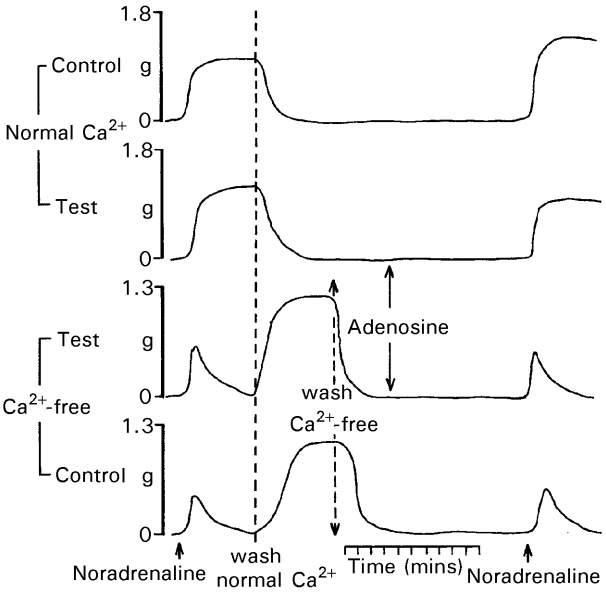

Figure 1. Example traces from an experiment designed to examine the effect of adenosine (10^{-3} M) against contractions induced by noradrenaline (3×10^{-6} M) in guinea-pig aortic rings, in normal and calcium-free Krebs solution.

Data and analysis

EC50 values (molar concentration inducing 50% of the maximum inhibition of tension) were calculated from individual concentration–response curves for noradrenaline, phenylephrine, NECA and adenosine, and the geometric mean calculated, with its 95% confidence limits. In experiments on contractions in response to single doses of noradrenaline or phenylephrine the second contraction was expressed as a percentage of the first contraction ((C2/C1) × 100) in each experiment and the mean ± s.e.m. quoted. Statistical differences between test and control experiments were estimated by use of Student's paired *t*-tests in which the variation of the two samples was assumed to be equal. Values of $P < 0.05$ were taken as indicative of significant differences. Although four preparations from the same animal were used to examine Ca^{2+}-free and normal-Ca^{2+} media, statistical comparisons were made between the test (NECA or adenosine) and its paired control only. Thus, failure of a tissue to respond in either the normal-Ca^{2+} or Ca^{2+}-free group resulted in rejection of the paired tissue only, not the entire group.

Results

Cumulative concentration–response curves

Noradrenaline and phenylephrine caused concentration-related increases in guinea-pig aortic tension (Figure 2); EC50 values and maximum

increases in tension are shown in Table 1. From these concentration–response curves, the concentration of noradrenaline and phenylephrine selected for precontracting the aortic rings was 3×10^{-6} M. There was no significant difference between the absolute size of contractions induced by single additions of this concentration of noradrenaline ($0{\cdot}96 \pm 0{\cdot}22$ g, n = 4) or phenylephrine ($1{\cdot}02 \pm 0{\cdot}2$ g, n = 4).

NECA relaxed both phenylephrine- and noradrenaline-preconstricted guinea-pig aortic rings in a concentration-dependent manner (Figure 3). The maxima of the NECA concentration–response curves for noradrenaline-precontracted aortic rings were significantly less than for phenylephrine-precontracted rings (Table 1). The EC50 values, however, did not differ significantly (Table 1).

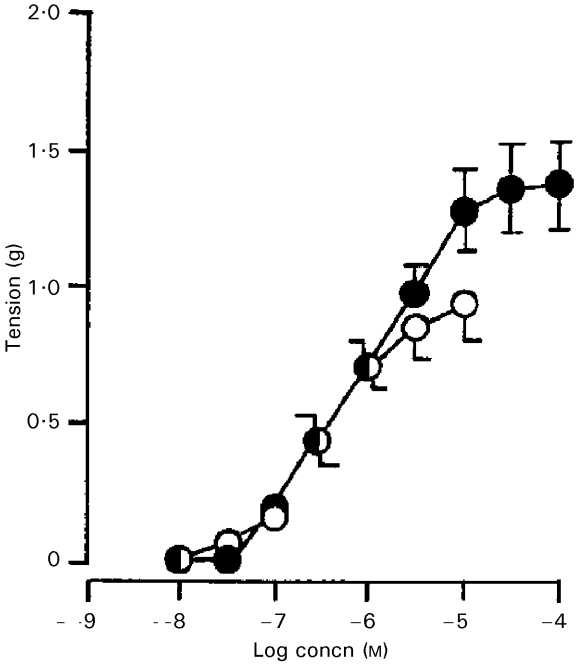

Figure 2. Mean cumulative concentration–response curves for noradrenaline (●, n = 4) and phenylephrine (○, n = 7) in guinea-pig aortic rings. Results are expressed as absolute values with the vertical bars representing s.e.m.

The presence of 10^{-6} M propranolol had no significant effect on either the maximum response or the EC50 value for the effect of NECA on noradrenaline-precontracted guinea-pig aortic rings. Similarly the α_2-adrenoceptor antagonist idazoxan (10^{-6} M) had no significant effect on the response to NECA of aortic rings precontracted with 10^{-5} M noradrenaline (Table 1).

Adenosine also caused concentration-dependent relaxation of noradrenaline- and phenylephrine-induced guinea-pig aortic tone (Figure 3B). There was no significant difference between the maximum response to adenosine of noradrenaline- and phenylephrine-precontracted guinea-pig aortic rings, although EC50 values for phenylephrine-precontracted tissues were significantly lower than for noradrenaline-preconstriction (Table 1).

NECA in calcium-free and normal media

Figure 1 shows an example trace of contractions induced by noradrenaline in normal and calcium-free Krebs solution. Control contractions obtained with noradrenaline (3×10^{-6} M) in normal-calcium medium were larger ($0{\cdot}98 \pm 0{\cdot}18$ g) than under calcium-free conditions ($0{\cdot}36 \pm 0{\cdot}06$ g). The control contractions to noradrenaline in calcium-free Krebs were phasic whereas the contractions in normal Krebs reached a steady plateau.

In normal-calcium medium, NECA (10^{-5}M) significantly reduced noradrenaline-induced contraction (ratio $108{\cdot}4 \pm 4{\cdot}1\%$, n = 5) compared with

Table 1. EC50 values and maximum responses for the contractile responses to phenylephrine and noradrenaline and relaxation responses to the adenosine receptor agonists 5′-(*N*-ethylcarboxamido)adenosine and adenosine in guinea-pig aortic rings.

Agonist	Preconstriction agent and antagonist[a]	n	EC50[b] (M)	Maximum response[c]
Phenylephrine		7	$3{\cdot}0\ (2{\cdot}3–4{\cdot}0) \times 10^{-7}$	$0{\cdot}9 \pm 0{\cdot}1$ g
Noradrenaline		4	$7{\cdot}8\ (3{\cdot}7–16{\cdot}8) \times 10^{-7}$	$1{\cdot}4 \pm 0{\cdot}4$ g
5′-(*N*-Ethylcarboxamido)adenosine	Phenylephrine	4	$8{\cdot}2\ (5{\cdot}3–12{\cdot}6) \times 10^{-7}$	$81{\cdot}9 \pm 2{\cdot}8\%$
	Noradrenaline	4	$1{\cdot}4\ (0{\cdot}5–3{\cdot}9) \times 10^{-6}$	$25{\cdot}0 \pm 1{\cdot}5\%$
	+ propranolol	4	$7{\cdot}8\ (5{\cdot}9–10{\cdot}2) \times 10^{-7}$	$18{\cdot}3 \pm 4{\cdot}4\%$
	+ idazoxan	4	$8{\cdot}0\ (4{\cdot}0–16{\cdot}1) \times 10^{-7}$	$12{\cdot}9 \pm 7{\cdot}1\%$
Adenosine				
	Phenylephrine	4	$5{\cdot}5\ (4{\cdot}8–6{\cdot}3) \times 10^{-5}$	$95{\cdot}1 \pm 4{\cdot}1\%$
	Noradrenaline	4	$9{\cdot}4\ (4{\cdot}1–21{\cdot}4) \times 10^{-4}$	$92{\cdot}0 \pm 16{\cdot}2\%$

[a]The concentrations of noradrenaline and phenylephrine were 3×10^{-6} M except for noradrenaline in the presence of idazoxan (10^{-5} M). The concentrations of propranolol and idazoxan were 10^{-6} M. [b]Molar EC50 values (concentrations producing 50% of the maximum inhibition of tension) with 95% confidence limits in parentheses. [c]Maximum responses for relaxing agents are expressed as % of the phenylephrine- or noradrenaline-induced contraction.

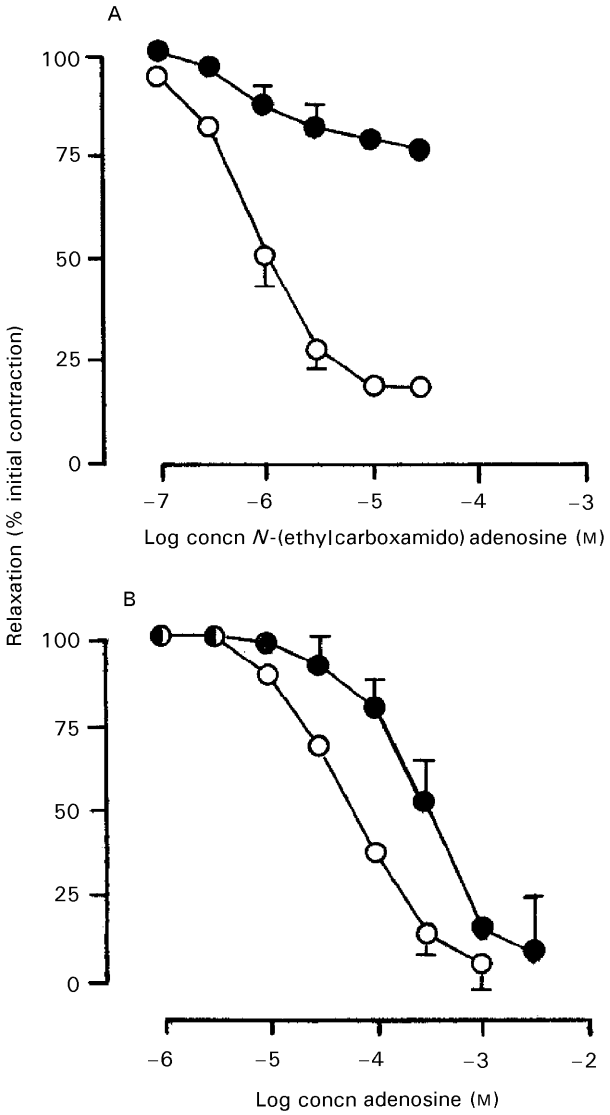

Figure 3. Mean concentration–response curves for A. 5′-(*N*-ethylcarboxamido) adenosine and B. adenosine in guinea-pig aortic rings precontracted with 3×10^{-6} M noradrenaline (●, n = 4) and 3×10^{-6} M phenylephrine (○, n = 4). Results are expressed as percentages of the initial contraction, with the vertical bars representing s.e.m.

the control (ratio 123·4 ± 4·9%, n = 5) (Table 2). In calcium-free Krebs, however, NECA had no effect on the contraction to noradrenaline, the test (73·8 ± 8·7%, n = 5) and control ratios (68·7 ± 6·3%, n = 5) not differing significantly (Table 2).

When phenylephrine (3×10^{-6} M) was used to contract the tissue, the first contractions (C1) in control experiments were again larger in normal Krebs solution (0·89 ± 0·12 g, n = 5) than in calcium-free Krebs (0·28 ± 0·06 g, n = 5). NECA (10^{-5} M) significantly reduced the test ratio in both normal (76·7 ± 3·9%, n = 9) and calcium-free Krebs solution (55·2 ± 9·1%, n = 9) compared with their respective controls (normal 133·1 ± 9·8%, n = 9; calcium-free 87·1 ± 14·9%, n = 9) (Table 2).

Table 2. The effects of 5′-(*N*-ethylcarboxamido)adenosine (NECA) and adenosine on the contractions of guinea-pig isolated aortic rings to noradrenaline and phenylephrine.

Relaxant	Constrictor	n	Test ratio (%)	Control ratio (%)
Normal calcium				
NECA	Noradrenaline	5	108·4 ± 4·1*	123·4 ± 4·9
	Phenylephrine	9	76·7 ± 3·9*	133·1 ± 9·8
Adenosine	Noradrenaline	6	57·1 ± 12·9*	129·3 ± 9·1
	Phenylephrine	4	29·5 ± 5·4*	166·9 ± 20·0
Calcium-free				
NECA	Noradrenaline	5	73·8 ± 8·7	68·7 ± 6·3
	Phenylephrine	9	55·2 ± 9·1*	87·1 ± 14·9
Adenosine	Noradrenaline	6	64·2 ± 10·2	77·1 ± 8·6
	Phenylephrine	8	36·7 ± 11·9*	110·7 ± 26·6

Test values are the ratios of the second contraction (in the presence of NECA or adenosine to noradrenaline) to the first contraction (in their absence). Control values are the same ratios using vehicle alone. *$P < 0.05$ compared with control ratios.

Adenosine in calcium-free and normal media

In the presence of adenosine (10^{-3} M) the contractions induced by noradrenaline (3×10^{-6} M) in normal medium were significantly reduced (test ratio 57·1 ± 12·9%, n = 6) compared with the control (129·3 ± 9·1%, n = 6) (Table 2). In calcium-free medium adenosine did not significantly reduce the test ratio (64·2 ± 10·2%, n = 6, compared with 77·1 ± 8·6%, n = 6, for the control ratio).

In the presence of adenosine (10^{-3} M), the contractions induced by phenylephrine (3×10^{-6} M) were significantly reduced in both normal (29·5 ± 5·4%, n = 4) and calcium-free (36·7 ± 11·9%, n = 8) media compared with their respective controls (normal 166·9 ± 20·0%, n = 4; calcium-free 110·7 ± 26·6%, n = 8; Table 2).

NECA in the presence of ryanodine

In the presence of ryanodine (10^{-5} M), NECA (10^{-5} M) had no significant effect on contractions induced by noradrenaline (121·5 ± 10·7%, n = 16, compared with 122·4 ± 11·6%, n = 16, for control). However, pretreatment with NECA significantly reduced contractions induced by phenyl-ephrine in the presence of ryanodine (77·3 ± 12·4%, n = 5, compared with 111·4 ± 9·3%, n = 5, for the control).

Discussion

Cumulative concentration–response curves

The increases in aortic tone induced by both noradrenaline and phenylephrine are mediated via activation of α-adrenoceptors. This causes an

increase in the intracellular calcium concentration via activation of the inositol phosphate cascade, releasing Ca^{2+} from intracellular stores and stimulating extracellular calcium entry through receptor-operated calcium channels (ROCs) (Rasmussen et al 1987; Minneman 1988). NECA reduced the tone induced by both noradrenaline and phenylephrine in a concentration-dependent manner. Although NECA is not selective for adenosine receptor subtypes (Bruns et al 1986), it has been shown that it is the A_2-receptor subtype which mediates vasorelaxation and in guinea-pig aorta the receptor is of the A_{2B}-subtype (Martin 1992; Alexander et al 1994). The EC50 values for the relaxant effect of NECA in noradrenaline- and phenylephrine-precontracted preparations were not significantly different, although maximum inhibition of phenylephrine-induced tone was substantially and significantly greater than for noradrenaline-induced tone. Such a difference might arise if the sizes of the contractions induced by noradrenaline and phenylephrine were substantially different; this was not so in this work. It is also possible that interaction between α_1-adrenoceptor and A_{2B}-receptor agonists is one of functional antagonism. Consequently, the relaxation response of rabbit aorta to NECA has been shown to be dependent on the concentration of phenyl-ephrine; as the concentration was increased, the NECA response was reduced (Wiener et al 1993). The relaxation was inversely proportional to the fractional occupancy of the α_1-adrenoceptor. Therefore, if noradrenaline and phenylephrine produce equal responses with different fractional occupancies, this could explain the different effects of NECA. However, we have shown that the use of different concentrations of noradrenaline (3×10^{-6} M and 10^{-5} M) to preconstrict the aorta (which would provide different occupancy values) did not result in maximum relaxation responses of different magnitude ($10{\cdot}9 \pm 3{\cdot}8$ and $8{\cdot}7 \pm 4{\cdot}1\%$, respectively) (unpublished observation). Adenosine also reduced both noradrenaline- and phenylephrine-induced tone in a concentration-dependent manner. The mechanism of vasorelaxation by adenosine has been attributed to both extracellular and intracellular sites (Collis & Brown 1983; Young & Merrill 1983; Long & Stone 1987; Zawadzki & Weiss 1990), the extracellular site being the A_{2B}-receptor (Martin 1992). Adenosine was consistently a more effective relaxant than NECA, suggesting that adenosine has an additional action, presumably at the intracellular site. The EC50 values for adenosine-mediated vasorelaxation of noradrenaline- and phenylephrine-induced tone in guinea-pig aorta differed significantly whereas the maximum inhibition achieved did not. Thus, both adenosine and NECA produced greater relaxation responses against phenylephrine-induced contractions than against those induced by noradrenaline. This phenomenon is indicative of a fundamental difference between the modes of contraction elicited by these two α-adrenoceptor agonists which NECA and adenosine are capable of distinguishing. The possibility exists that NECA and adenosine exert their effect by interfering with only one of several mechanisms of contraction, and that this component is more pronounced in the phenylephrine- induced contraction.

One possibility is that noradrenaline, but not phenylephrine, was simultaneously stimulating β-adrenoceptors that exert an underlying relaxant effect (Broadley 1996). Because β-adrenoceptor- and A_2-receptor-mediated vasodilation both depend on activation of adenylyl cyclase and accumulation of cAMP (Lefkowitz et al 1983; Olsson & Pearson 1990), it is possible that if noradrenaline has already activated this system, further effects of NECA might be reduced. This possibility, however, was discounted because blockade of β-adrenoceptors with propranolol did not affect the relaxation of the noradrenaline-contracted tissue by NECA and did not unmask a greater relaxation.

Another possibility is that noradrenaline also activates α_2-adrenoceptors. Although the guinea-pig aorta does not seem to be a preparation in which stimulation of α_2-adrenoceptors causes a contraction (McGrath et al 1989), it is possible that a small population of α_2-adrenoceptors exists, as they do in the rat vasculature (Ruffolo et al 1991). α_2-Adrenoceptors are negatively coupled to adenylyl cyclase via a G_i regulatory protein, their stimulation inducing a fall in cyclic AMP levels (Bylund 1988). NECA, through the extracellular A_{2B}-purinoceptor, is thought to exert its vasorelaxant effects by elevating cAMP levels as a result of stimulation of adenylyl cyclase (Bruns et al 1986). Thus, in the presence of noradrenaline NECA would have to overcome any inhibitory effects on adenylyl cyclase mediated via α_2-adrenoceptors, before relaxation could be evoked. The response would consequently be less than in phenylephrine-precontracted tissues. Idazoxan, to inhibit α_2-adrenoceptors (Doxey et al 1983) did not, however, modify the response to NECA of noradrenaline precontracted tissues. Stimulation of α_2-adrenoceptors by noradrenaline does not, therefore, explain the different actions on noradrenaline- and phenylephrine-contracted aortae.

Effects of NECA on contractions dependent upon intracellular calcium

Phenylephrine- and noradrenaline-induced contractions in Ca^{2+}-free Krebs solution are dependent

upon intracellular calcium (Karaki & Weiss 1988; Koch et al 1990; Rinaldi et al 1991) rather than on heterogeneous sources of intra- and extracellular Ca^{2+}, as for normal Ca^{2+} medium (Young & Merrill 1983). Previous studies by others have shown that adenosine and NECA inhibit vascular tone already induced by noradrenaline (Ramagopal & Mustafa 1990) or $PGF_{2\alpha}$ (Ramagopal et al 1989) under Ca^{2+}-free conditions. However, the contractions induced by noradrenaline and phenylephrine in Ca^{2+}-free medium in the current study were not sustained (see Figure 1) and it was not possible to determine effects of adenosine and NECA after induction of a contraction. Pretreatment with NECA or adenosine resulted in a significant reduction in the test ratio for phenylephrine-induced contractions compared with the control ratio, under both normal and calcium-free conditions. This implies that stimulation of extracellular A_{2B}-receptors is capable of inhibiting contractions dependent upon intracellular calcium. When noradrenaline was used as the contracting agent, pretreatment with NECA or adenosine under normal conditions also caused a reduction in the test ratio compared with the control value, but under calcium-free conditions there was no reduction by NECA or adenosine; this agrees with our previous observations (Ford & Broadley 1996).

Effects of NECA on contractions dependent upon extracellular calcium

Ryanodine was used to prevent intracellular calcium release (Sorrentino & Volpe 1993) in contractions induced by noradrenaline and phenylephrine. We have previously shown that at the concentration used, ryanodine substantially inhibits contractions induced by phenylephrine under calcium-free conditions, the effect being smaller under normal-calcium conditions (Ford & Broadley 1999). Contractions initiated by the two noradrenergic agonists in the presence of ryanodine were therefore dependent upon extracellular calcium influx. NECA caused a significant reduction of the phenylephrine-induced contractions in the presence of ryanodine. Previous studies have shown that NECA has little effect on contractions dependent upon calcium influx through voltage-operated calcium channels, for example by increasing K^+ (Young & Merrill 1983; Urquhart & Broadley 1991). Furthermore, K^+-induced influx of $^{45}Ca^{2+}$ in bovine coronary artery rings (Ramagopal & Mustafa 1988) and rabbit aorta (Zawadzki & Weiss 1990) was relatively resistant to inhibition by adenosine and NECA. NECA seems, therefore, to inhibit phenylephrine-induced contractions by preventing influx of extracellular Ca^{2+} through the so-called receptor-operated calcium channels. In contrast, NECA had no effect on the noradrenaline-induced contractions under identical conditions, suggesting that Ca^{2+} influx induced by noradrenaline was not inhibited by NECA. Earlier observations with normal Krebs had, however, indicated inhibition of the noradrenaline contraction; this must have been because of an effect on intracellular Ca^{2+} release, because there was no action in Ca^{2+}-free medium. The reason for this discrepancy is unclear. NECA seems to inhibit Ca^{2+} influx, as evidenced by the result in the presence of ryanodine. Perhaps different Ca^{2+} channels are opened by noradrenaline and phenylephrine.

The greater effects of NECA and adenosine on phenylephrine-induced contractions than on noradrenaline-induced contraction might arise because NECA discriminates between α_1-adrenoceptor subtypes stimulated by noradrenaline and phenylephrine. Two subtypes of α_1-adrenoceptor have been identified which utilise different mechanisms for increasing intracellular Ca^{2+}. The α_{1A}-adrenoceptor causes extracellular calcium influx via opening of a G protein-linked, dihydropyridine-sensitive calcium channel. The α_{1B}-adrenoceptor is linked to the release of Ca^{2+} from intracellular storage sites initiated by the increased turnover of phosphatidylinositol and IP_3 accumulation (Han et al 1987; Ruffolo et al 1991). Noradrenaline and phenylephrine might activate these pathways differently and this might explain the greater activity of NECA against phenylephrine. The effectiveness of NECA against phenylephrine in calcium-free medium suggests an interaction primarily with α_{1B}-adrenoceptor-mediated calcium mobilization pathways. These speculations, however, require further study using contractions selectively mediated via α_{1A}- or α_{1B}-adrenoceptor subtypes. Furthermore, this generalization might not apply strictly to vascular α_1-adrenoceptor-mediated responses; in particular, the aorta might contain an additional subtype of α_1-adrenoceptor with characteristics distinct from either the α_{1A} or α_{1B} subtypes (Oriowo & Ruffolo 1992; Bylund et al 1994).

Acknowledgements

This work was supported by a grant from SmithKline Beecham Ltd.

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