Diadenosine Polyphosphates Cause Contraction and Relaxation in Isolated Rat Resistance Arteries

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ABSTRACT

The effects of diadenosine polyphosphates (APnA; n = 3–6) and adenine nucleotides on contractile reactivity of isolated rat mesenteric resistance arteries (MrA) and superior epigastric arteries (SEA), which display a dense and sparse autonomic innervation, respectively, were evaluated. All agonists examined, except adenosine and AMP, induced contractions. The rank order of potency was similar in both arteries: α,β-methylene ATP (α,β-meATP) > AP5A > AP6A > AP4A > ATP > ADP > AP3A. Contractions were stable during several minutes in SEA but highly transient in MrA. They were reduced after exposure to 10 μM α,β-meATP and by 10 μM of the P2X antagonist pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid. During phenylephrine (10 μM)-induced contractions, the agonists induced a further contraction in SEA. In MrA, however, further contraction was followed by marked relaxation. The rank order of relaxing potency was comparable to that of the contractile potency of agonists. Also, the relaxing effects of APnA were blunted by 10 μM pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid and after exposure to α,β-meATP. In vitro and in vivo sympathectomy with 6-hydroxydopamine and removal of the endothelium did not modify the effects of APnA in MrA. Thus, the contractile effects of APnA in resistance arteries 1) are due to a P2X purinoceptor-mediated stimulation of the smooth muscle; 2) depend on the length of the phosphate chain; and 3) are followed by endothelium-independent relaxing effects in MrA but not SEA, which may involve receptors that are similar to those mediating contraction. The regional heterogeneity of APnA effects cannot be attributed to a direct neurogenic influence.

Diadenosine polyphosphates (APnA), which consist of two adenosine molecules linked together by a variable number of phosphate groups, are found in human and animal tissues. They can be released from brain synaptosomes (Pintor et al., 1993; Klishin et al., 1994) and are coreleased with catecholamines and ATP from the bovine adrenal medulla (Siller et al., 1994). This suggests that they may participate in sympathetic neurotransmission (Castillo et al., 1992). In binding assays APnA display affinity to P2X purinoceptors, a family of nonspecific cationic channels (Bo et al., 1994; Neely et al., 1996; Schafer and Reiser, 1997).

APnA have biphasic effects on blood pressure (Khattab et al., 1998) and diverse influences on the reactivity of isolated blood vessels and vascular beds (Pohl et al., 1991; Tepel et al., 1997; Westfall et al., 1997). Their vascular effect appears to vary with 1) the number of phosphate groups (Ralevic et al., 1995), 2) the presence and absence of endothelium (Busse et al., 1988), and 3) the type of vessel and vascular bed. With respect to the regulation of vascular resistance and blood flow, effects on small muscular arteries (diameter < 500 μm) and arterioles are most relevant (Bohlen, 1986; Mulvany and Aalkjaer, 1990). As is the case for various vasoactive compounds, the origin of the regional heterogeneity of the vascular actions of APnA is unknown. In view of the possible role of APnA in neurotransmission, it may be hypothesized that regional differences in the distribution of autonomic nerves contribute to the heterogeneity. In general, nerves influence the local supply and degradation of neurotransmitters and the presence of appropriate postjunctional receptors (Stassen et al., 1998).

In this study the effects of APnA (n = 3–6) were examined in densely innervated rat mesenteric resistance arteries (MrA) and sparsely innervated superior epigastric arteries (SEA) (Stassen et al., 1997a). Effects were evaluated on resting tension and during α1-adrenergic contraction. The effects

ABBREVIATIONS: APnA, diadenosine polyphosphates; MrA, mesenteric resistance artery; SEA, superior epigastric artery; 6-OHDA, 6-hydroxydopamine; α,β-me ATP, α,β-methylene ATP; PPADS, pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid; KRB, Krebs-Ringer-bicarbonate solution.
of APnA were compared with those of the candidate metabolites ATP, ADP, AMP, and adenosine (Lüthje and Ogilvie, 1988) and to those of the degradation-resistant agonist \(\alpha,\beta\)-methylene ATP (\(\alpha,\beta\)-meATP) (Burnstock and Kennedy, 1985; Delbro et al., 1985). To evaluate the contribution of P2X purinoceptors, the effects of the antagonist pyridoxal-phosphate-6-azophenyl-2', 4'-disulfonic acid (PPADS) (Lambrecht et al., 1992) and of pretreatment with a high concentration of \(\alpha,\beta\)-meATP (Burnstock and Kennedy, 1985; O’Connor et al., 1990) were determined. In addition, by comparing responses in densely and sparsely innervated arteries, the effects of acute and chronic sympathectomy with 6-hydroxydopamine (6-OHDA) (Aprigliano and Hermsmeyer, 1977) and of mechanical endothelium removal were investigated.

**Materials and Methods**

**Animals.** Male 16-week-old Wistar-Kyoto rats were obtained from a local inbred strain (Central Animal Facilities, Universiteit Maastricht, Maastricht, The Netherlands). They had free access to standard rat chow (Hope Farms, Woerden, The Netherlands) and tap water. The experimental studies were performed according to institutional guidelines and approved by the local Ethics Committee for the Use of Experimental Animals. Six of the 36 rats were chemically sympathomimetized with 6-OHDA (Aprigliano and Hermsmeyer, 1977). 6-OHDA (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% NaCl (50 mg/kg) brought to pH 4.7 with gluthathione (Merck, Darmstadt, Germany). 6-OHDA was injected twice i.p. with a 2-h interval, which was repeated after 3, 7, and 10 days to ensure sympathetic denervation. Effectiveness of this treatment was monitored by determination of tissue catecholamine content.

**Vessel Isolation.** Rats were sacrificed by cervical dislocation and exsanguination. The abdominal skin and muscles as well as the mesentery were removed by blunt dissection. The abdominal wall was placed inside up in a Petri dish coated with sylgard (Dow Corning, Seneffe, Belgium) and filled with Krebs-Ringer-bicarbonate solution (KRB). Skeletal muscle overlaying the left SEA was carefully removed under a dissecting microscope and 2-mm-long segments of this vessel were isolated just below the diaphragm. From the mesentery, third order side branches of the superior mesenteric artery (SMA) and inferior mesenteric artery (IMA) were isolated. The luminal diameters of SEA and MrA were of the same order of magnitude (diameter 200–300 \(\mu\)m), and both vessels are arterial anastomoses. The former interconnects the internal iliac artery to the inferior epigastric artery (side branches of the subclavian and common iliac artery, respectively) and gives rise to side branches perfusing the abdominal muscles. The latter interconnects second order mesenteric artery side branches of the superior mesenteric artery and gives rise to side branches that penetrate into the ileum. In some of the isolated vessels sympathetic nerves were acutely destroyed by incubation for 10 min in bicarbonate-free Krebs-Ringer containing 300 \(\mu\)g/ml 6-OHDA (Aprigliano and Hermsmeyer, 1977). In an additional series of isolated vessels the endothelium was mechanically removed by passing a human hair through the lumen (Osol et al., 1989). The success of this procedure was evaluated by recording the responses to 0.01 to 10 \(\mu\)M acetylcholine in arteries constricted with 10 \(\mu\)M phenylephrine. Absence of such responses proved effectiveness of the removal of the endothelium.

**Tension Measurements.** Arteries were mounted on two stainless steel wires (diameter 40 \(\mu\)m) as ring segments in an isometric myograph (model 410A; J.P. Trading, Aarhus, Denmark) between a force transducer (Kistler Morse DSC6, Seattle, WA) and a displacement device for recording of isometric force development (Mulvany and Aalkjaer, 1990). Arteries were stretched to their optimal luminal diameter with an active length tension protocol with 125 mM K+ as activating stimulus (De Mey and Brutsaert, 1984). During experimentation the vessels were kept in KRB that was maintained at 37°C and aerated with 95% \(O_2\) and 5% \(CO_2\).

**Experimental Protocols.** In initial experiments effects of 10 \(\mu\)M of the various agonists were evaluated at basal vessel tone. Next, in view of the transient nature of the contractile effect of the substances, a “single dose” concentration-response approach was used to determine agonist potency. Concentrations were separated by 45 to 60 min in drug-free solution. Only one type of agonist was tested in each arterial preparation. The single concentrations were chosen randomly. After EC50 concentrations had been determined, these were applied at intervals ranging from 10 to 90 min to determine the kinetics of homologous and heterologous “desensitization.”

In separate experiments, agonist effects were evaluated during contraction induced by 10 \(\mu\)M phenylephrine or 125 mM K+. Also in this case a single dose approach was used. Agonists were applied for at most 5 min during preconstriction and vessels were allowed to recover for 30 to 60 min. Some of the experiments were performed in vessels that had been exposed to 10 \(\mu\)M \(\alpha,\beta\)-meATP, which has been shown to irreversibly desensitize P2X purinoceptors (O’Connor et al., 1990); 2) in the presence of 10 \(\mu\)M PPADS, a putative P2X purinoceptor antagonist (Lambrecht et al., 1992); and 3) after removal of sympathetic nerves or endothelium.

**Catecholamine Content.** Tissue noradrenaline content was measured as an indicator of the density of adrenergic nerves. Arterial segments were placed in 1 ml of 0.1 N HCl containing 3 g/l gluthathione for 1 week, and the catecholamine content of the extract was determined by HPLC and fluorescent detection (van der Hoorn et al., 1989). Unlike for noradrenaline, the arterial contents of adrenaline and dopamine were below detection limits. After extraction of the catecholamines the preparations were solubilized in 1 ml of 1 N NaOH to determine their DNA content (Labarca and Paigen, 1980). Tissue noradrenaline content agreed with earlier histochemical observations in rat MrA and SEA (Stassen et al., 1997b).

**Compounds and Solutions.** The composition of KRB was as follows: 118.5 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l MgSO4, 1.2 mmol/l KH2PO4, 25.0 mmol/l NaHCO3, 2.5 mmol/l CaCl2, and 5.5 mmol/l glucose. In high K+ solution (125 mM K+) all NaCl was replaced by an equimolar concentration of KCl. All agonists and pharmacological tools were obtained from Sigma Chemical Co. except for PPADS, which was obtained from Research Biochemicals International (Natick, MA). Stock solutions were prepared on the day of use in double distilled water.

**Data Analysis.** Contractile reactivity was measured as active wall tension (active force divided by twice the vessel segment length) and expressed as a percentage of the tissue response to 125 mM K+ at the beginning of the experimental protocols. Concentration-response curves were analyzed in terms of sensitivity (pD2 = \(-\log EC_{50}\) determined by least-squares sigmoidal curve fitting of individual curves (GraphPad Prism 1.00; GraphPad, San Diego, CA). Differences between agonists and between types of vessels were evaluated by ANOVA followed by t test according to Bonferroni with \(P < .05\) denoting statistical significance. Data are shown as mean ± S.E.

**Results**

**General Tissue Characteristics.** Optimal luminal diameter and maximal contractile responses to high K+ and to phenylephrine were of the same order of magnitude in isolated third order side branches of the MrA and in SEA of the rat, but noradrenaline content was 100 times higher in MrA than in SEA (Table 1). After chemical sympathectomy with 6-OHDA in vivo, optimal luminal diameter and maximal response to phenylephrine were not modified in MrA, whereas the tissue noradrenaline content was markedly reduced (Table 1). MrA and SEA of the rat are thus of comparable size but differ markedly in their density of sympathetic nerves.
Effects of APnA on Basal Tension. AP3A, AP4A, AP5A, or AP6A caused contraction in both types of vessels. In SEA these responses were stable during a 5-min period, whereas they were highly transient in MrA (Fig. 1). The vessels were therefore exposed at 60-min intervals to single doses of APnA to determine the contractile potency of the compounds. As judged from pD2 values, the order of potency was AP6A > AP5A > AP4A > AP3A in SEA and AP5A > AP6A > AP4A > AP3A in MrA with the potencies of AP6A, AP4A, and AP3A being comparable in SEA and MrA (Table 2; Fig. 2). In SEA the maximal contractile responses to agonists were comparable, whereas in MrA responses to AP3A were larger than those to the other APnA.

Although up to 1 mM AMP or adenosine did not modify wall tension, α,β-meATP, ATP, and ADP caused contractions (Fig. 3). Comparable with the vascular responses to the APnA also these three agonists caused stable vasoconstrictions in SEA but highly transient contractile responses in MrA. Again a single dose approach was used to determine contractile potency. In both types of vessel α,β-meATP was more potent than AP6A or AP5A. ATP and ADP were equipotent with AP4A and AP3A (Table 2; Fig. 3).

Shortly after resistance arteries had been exposed to APnA or adenine nucleotides contractile responses to a second addition of the same agonist were temporarily but drastically reduced (Fig. 1). Table 3 summarizes the time required for recovery of contractile responsiveness of MrA to EC50 concentrations of APnA and adenine nucleotides. Determined by this approach, the desensitization that was induced by AP6A and AP5A lasted significantly longer than that induced by AP4A, AP3A, ATP, ADP, and α,β-meATP. Similar results were obtained in SEA (data not shown). It is noteworthy that after exposure, for instance, to AP5A not only responses to AP5A but also those to AP6A, AP4A, AP3A, ATP, ADP, and α,β-meATP were markedly suppressed. In MrA, not only contractile responses to APnA but also contractile responses to 10 μM phenylephrine were temporarily suppressed after exposure to AP5A. Potassium-induced contractions (125 mM) were, however, not influenced by prior administration of AP5A.

Collectively, these observations at basal tone indicate that resistance arteries can initially respond to APnA and subsequently become refractory to the agonists. Although the order of potency was comparable, the time course of the contractile effects differed markedly between the sparsely

![Fig. 1. Original records of myograph experiments with SEA (top) and MrA (bottom) at basal tone showing force development by application of high K+ solution (K+ 125 mM; 100%), phenylephrine (Phe; 10 μM), and AP5A (10 μM). AP5A causes stable constrictions in SEA and highly transient constrictions in MrA. Repeated applications of AP5A lead to desensitization of the arteries that ceased after approximately 40 min.](image1)

![Fig. 2. Concentration-response curves for the contractile effects of AP3A, AP4A, AP5A, and AP6A in SEA (top) and MrA (bottom). Effects are presented as a percentage of the response to 125 mM K+ and are shown as mean ± S.E. (n = 7).](image2)

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>SEA (EC50 ± log M)</th>
<th>MrA (EC50 ± log M)</th>
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<tbody>
<tr>
<td>α,β-meATP</td>
<td>6.75 ± 0.06</td>
<td>6.54 ± 0.05</td>
</tr>
<tr>
<td>AP6A</td>
<td>5.10 ± 0.11</td>
<td>5.06 ± 0.04</td>
</tr>
<tr>
<td>AP5A</td>
<td>5.01 ± 0.21</td>
<td>5.73 ± 0.15</td>
</tr>
<tr>
<td>AP4A</td>
<td>4.23 ± 0.12</td>
<td>4.33 ± 0.15</td>
</tr>
<tr>
<td>ATP</td>
<td>3.78 ± 0.17</td>
<td>4.20 ± 0.04</td>
</tr>
<tr>
<td>ADP</td>
<td>3.78 ± 0.01</td>
<td>3.71 ± 0.08</td>
</tr>
<tr>
<td>AP3A</td>
<td>3.32 ± 0.15</td>
<td>3.43 ± 0.10</td>
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innervated SEA and the densely innervated MrA. To evaluate whether the temporary blunting of APnA-induced contraction was due to a long-lasting relaxing effect, rather than to receptor desensitization, we studied preconstricted tissues.

**Responses to APnA in Preconstricted Arteries.** Contractile responses to 10 μM phenylephrine were stable for several minutes in both MrA and SEA (Fig. 4). Administration of APnA during these α₁-adrenergic contractions had different effects in the two types of resistance arteries.

In preconstricted SEA APnA induced a rapid and transient relaxation of variable amplitude followed by a further increase in tone that faded during the 5-min exposure period (Fig. 4, top). Qualitatively similar findings were obtained with α,β-meATP, ATP, and ADP and the order of potency was comparable to that noted for the contractile effects in resting SEA (Fig. 3).

In phenylephrine-contracted MrA, the responses to APnA were again triphasic (Fig. 4, bottom). An initial brisk and transient relaxation was followed by a secondary increase in tone to levels that exceeded that of the α₁-adrenergic contraction and finally by a marked reduction of the phenylephrine-induced contraction. Although up to 0.3 mM adenosine and AMP did not modify the contractile responses to phenylephrine, effects of α,β-meATP, ATP, and ADP were qualitatively similar to those of the APnA. pD₂ values for the final relaxing effects were determined from single dose concentration-response curves (Fig. 5; Table 4). They indicate that the rank order of relaxing potency is α,β-meATP > AP5A > AP6A > AP4A > ATP > AP3A > ADP. The rank order of the relaxing potency was comparable to that of the contractile potency of the compounds. In general, the pD₂ values for the relaxing effects were, however, slightly higher than those for the contractile effects (Tables 2 and 4). Obviously, in MrA the temporary blunting of APnA-induced contraction was due to a long-lasting relaxing effect, rather than only to receptor desensitization.

**Role of the Endothelium and of Sympathetic Nerves.** The triphasic effects of APnA on phenylephrine-induced contractions in MrA were not significantly altered by the presence of the cyclooxygenase inhibitor indomethacin (3 μM) or of the nitric oxide synthase inhibitor N(G)-nitro-L-arginine (100 μM; data not shown). Furthermore, mechanical removal of the endothelium did not significantly modify contractile effects of APnA and natural adenine nucleotides in MrA at basal tension (data not shown) or the relaxing effect of these compounds during phenylephrine-induced contraction in MrA (Fig. 6). Removal of the endothelium only increased the maximum relaxation to α,β-meATP.

Acute exposure of MrA to 6-OHDA in vitro did not modify the contractile and relaxing effects of AP5A (data not shown). When MrA were obtained from rats that had been chronically innervated SEA and the densely innervated MrA. To evaluate whether the temporary blunting of APnA-induced contraction was due to a long-lasting relaxing effect, rather than to receptor desensitization, we studied preconstricted tissues.

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Acute exposure of MrA to 6-OHDA in vitro did not modify the contractile and relaxing effects of AP5A (data not shown). When MrA were obtained from rats that had been chronically

**TABLE 3**

<table>
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<tr>
<th>Nucleotide</th>
<th>Maximum Time (min)</th>
<th>Average Time (min ± S.E.)</th>
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<tr>
<td>AP6A</td>
<td>50</td>
<td>43 ± 6</td>
</tr>
<tr>
<td>AP5A</td>
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<td>40 ± 6</td>
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<tr>
<td>ADP</td>
<td>30</td>
<td>23 ± 6</td>
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<td>AP4A</td>
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<tr>
<td>ATP</td>
<td>20</td>
<td>20 ± 0</td>
</tr>
<tr>
<td>AP3A</td>
<td>20</td>
<td>17 ± 6</td>
</tr>
<tr>
<td>α,β-meATP</td>
<td>20</td>
<td>17 ± 6</td>
</tr>
</tbody>
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sympathectomized with 6-OHDA in vivo, the relaxing effects of APnA or of ATP were comparable to those in vessels from intact rats (data not shown).

Effects of PPADS and α,β-meATP. In intact MrA, the presence of the candidate P2X receptor antagonist PPADS (10 µM) diminished the contractile effect of 10 µM AP5A on basal tension and the contractile and relaxing effect of 10 µM AP5A during phenylephrine-induced contraction (Fig. 7). Furthermore, after exposure of MrA during 10 min to 10 µM α,β-meATP contractile responses to phenylephrine recovered within 60 min but contractile responses to 10 µM AP5A could not be obtained again during at least the next 300 min.

**Discussion**

Exogenous APnA induced contraction in isolated resistance arteries. In MrA, but not in SEA, APnA-induced contraction was rapidly followed by a marked relaxation. Contractile and relaxing effects of APnA seem to be mediated by similar P2X purinoceptors on the arterial smooth muscle cells. The regional heterogeneity of the arterial actions of APnA cannot be attributed to degradation of the agonists, endothelial effects, or to acute and chronic influences of perivascular sympathetic nerves.

In line with findings in various isolated vascular beds and arteries of several species (Schlüter et al., 1994; Davies et al., 1995; Tepel et al., 1997; Westfall et al., 1997; Khattab et al., 1998) exogenous administration of APnA and adenine nucleotides induced contraction in rat SEA and MrA. The rank order of potency was the same for both vessels and comparable to those reported for the perfused rat kidney (van der Giet et al., 1997) and the perfused rat mesenteric arterial bed (Ralevic et al., 1995). High concentrations of AP3A induced stronger maximal concentrations than the more potent substances AP5A and AP6A. This observation may be due to rapid desensitizing effects coming into being before the potential maximum of the purinergic contraction was achieved because desensitizing effects of AP5A and AP6A are considerably stronger than those of AP3A. In line with the higher affinity of especially AP5A and AP6A for P2X receptors in ligand-binding studies (Bo et al., 1994), the larger APnA were more potent contractile agonists than ATP. P2X purinoceptors were previously demonstrated by functional analysis (Lagaud et al., 1996) and autoradiography (Bo and Burnstock, 1993) in rat MrA. Mimicry of the effects by α,β-meATP and blockade of APnA-induced contractions by PPADS and by prior exposure to a high concentration of α,β-meATP strengthen the suggestion that P2X purinoceptors mediate the contractile responses induced by APnA, these P2X purinoceptors seem to be located on the resistance artery smooth muscle cells.

Contractile effects of APnA and adenine nucleotides were comparable in the two types of vessel except for their duration of action. Responses were maintained for several minutes in the epigastric vessels and highly transient in the MrA. This does not seem to be due to regional differences in the degradation of the agonists or to endothelium-dependent relaxation. Nerve-related degradation, which may be the case for candidate neurotransmitters, can be ruled out because sympatheticotomy did not modify the time course of the responses. Furthermore, differences in time course were not only observed with APnA but also with the degradation-resistant α,β-meATP. In view of our findings we suggest that a regionally selective secondary relaxing effect of APnA is responsible for the transient nature of the contractile responses in the MrA.

<table>
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<tr>
<th>Nucleotide</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (log M)</th>
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<td>α,β-meATP</td>
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<td>AP6A</td>
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<td>ATP</td>
<td>4.93 ± 0.17</td>
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<tr>
<td>AP4A</td>
<td>3.81 ± 0.12</td>
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**Fig. 6.** Ultimate relaxing responses in MrA with (closed symbols) and without (open symbols) endothelium during contraction induced by 10 µM phenylephrine. Mean ± S.E. (n = 5).

**Fig. 7.** Contractile effect of 10 µM AP5A (left) and relaxing effect of 10 µM AP5A during contraction induced by 10 µM phenylephrine (right) in mesenteric resistance arteries in the absence (□) and presence (■) of 10 µM PPADS. Mean ± S.E. (n = 5).
APnA and adenosine nucleotides have previously been observed to induce arterial relaxation in several vascular beds (Sumiyoshi et al., 1997; van der Giet et al., 1997). Ralevic et al. (1995) reported relaxing effects of APnA in the agonist-constricted rat mesenteric arterial bed and noted that the order of potency differed from that of the contractile effect of the compounds under basal conditions. In this study of isolated MrA preconstricted with phenylephrine we observed 1) dynamic responses consisting of contraction and relaxation at identical agonist concentrations and 2) comparable agonist potency orders for the contractile and the relaxing effects. This discrepancy between both studies may find its origin in the use of bolus injections in the first and stable agonist concentrations maintained during several minutes in this study. It is unlikely that the relaxing responses would be mediated by metabolites generated by asymmetric cleavage of the compounds (Lüthje and Ogilvie, 1987).

The possible metabolites of APnA (ATP, ADP, AMP, and adenosine) were considerably less potent or ineffective relaxing agents than AP5A and AP6A and not only APnA but also the degradation-resistant P2X purinoceptor agonist α,β-meATP elicited mesenteric arterial relaxation after an initial further increase in tone.

In MrA, APnA-induced contraction and APnA-induced relaxation displayed notable similarities. The agonist potency orders were comparable and both effects 1) could be reproduced by a low concentration of α,β-meATP, 2) were blocked in the presence of PPADS, 3) were persistently blunted after pretreatment with a high concentration of α,β-meATP, and 4) were not modified by sympathectomy or endothelium removal. This suggests that both effects are mediated by the same sarcolemmal P2X purinoceptors or by closely related receptors. Molecular analyses revealed the existence of several P2X purinoceptor subtypes (Humphrey et al., 1998). To firmly establish the role of one of these subtypes in either or both the contractile and relaxing effects may require either more specific purinoceptor agonists and antagonists or, e.g., antisense gene transfer techniques.

APnA-induced arterial contraction most likely involves Ca2+ influx through receptor-operated channels (Lagaud et al., 1996; Tepel et al., 1996), but the smooth muscle mechanism that leads to relaxation remains to be established. It is noteworthy that in MrAs contractions induced by depolarizing high K+ solution could not be attenuated by APnA. This is compatible with a role for sarcolemmal Ca2+-activated K+ channels as was reported for the direct relaxing effect of APnA in porcine coronary artery (Sumiyoshi et al., 1997). The observed dual effects of APnA in rat MrA are in line with findings that these compounds stimulate Ca2+ influx and blunt effects of angiotensin II on intracellular Ca2+ concentration in isolated arterial smooth muscle cells (Tepel et al., 1996). More importantly, we note in vitro observations are also in line with the blood pressure response to APnA in anesthetized rats, which consists of an initial transient pressor response followed by a long-lasting hypotension (Khattab et al., 1998). The direct effects of APnA on the arterial smooth muscle in vascular beds that govern systemic vascular resistance seem to suffice to explain the complex blood pressure response. This could even be demonstrated in humans by Kikuta et al. (1999) who reported about the use of AP4A in anesthetized humans to reduce elevated blood pressure, again, indicating the prevalence of the vasorelaxing effects of APnA.

In contrast to the dual responses in MrA, SEA contracted but failed to relax in response to APnA. The order of contractile potency of APnA and adenosine nucleotides and the inhibitory effects of PPADS and α,β-meATP were comparable in both arteries, suggesting that similar P2X purinoceptors are involved. The time course of the contractile effects differed, however, markedly between both vessels despite similar kinetics of recovery of desensitization. This is most likely due to underlying relaxing responses in the mesenteric resistance but not in the epi gastric arteries. The SEA were studied because they lack sensory motor and sympathetic nerves (Stassen et al., 1997a). These structures are candidate sources and sites of action of endogenous adenosine nucleotides and APnA and can influence the presence of postjunctional receptors for neurotransmitters (Stassen et al., 1998). However, neither acute nor chronic sympathectomy influenced the regional diversity of the arterial responses to APnA.

In summary, these observations suggest that APnA directly constricts rat resistance arterial smooth muscle through P2X purinoceptors and subsequently trigger vascular relaxation in some vascular beds through similar receptors or as a direct consequence of the initial contractile mechanism. These effects and their regional heterogeneity do not involve the endothelium or an acute or chronic sympathetic nervous influence. The identification of the subtypes of purinoceptors involved in these contractile and relaxing effects of APnA in the two resistance arteries is addressed more specifically in Steinmetz et al. (2000).

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References


