ATP Modulates Noradrenaline Release by Activation of Inhibitory P2Y Receptors and Facilitatory P2X Receptors in the Rat Vas Deferens

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ABSTRACT
The role of ATP on the modulation of noradrenaline release elicited by electrical stimulation (100 pulses/8 Hz) was studied in the prostatic portion of rat vas deferens preincubated with [3H]noradrenaline. In the presence of P1 antagonists, the nucleotides 2-methylthioadenosine-5′-triphosphate (2-MeSATP), 2-methylthioadenosine 5′-diphosphate (2-MeSADP), ADP, and ATP decreased electrically evoked tritium overflow up to 44%, with the following order of potency: 2-MeSATP > 2-MeSADP > ADP. The P2Y antagonists reactive blue 2 (RB2) and 2-methylthioadenosine 5′-monophosphate (2-MeSAMP) increased, whereas the P2X antagonist pyridoxal-5′-phosphate-6-[(2′-naphthylazo-6′-nitro-4′,8′-disulfonate) (PPNDS) decreased evoked tritium overflow. The inhibitory effect of 2-MeSATP was antagonized by RB2 (10 μM) and by 2-MeSAMP (10 μM) but not by the selective P2Y1 receptor antagonist 2′-deoxy-N6-methyladenosine 3′,5′-bisphosphate (MRS 2179; 10 μM). When, besides P1 receptors, inhibitory P2Y receptors were blocked with RB2, α,β-methyleneadenosine 5′-triphosphate (α,β-meATP), β,γ-imidoadenosine 5′-triphosphate (β,γ-imidoATP), β,γ-methyleneadenosine 5′-triphosphate (β,γ-meATP), 2-MeSATP, and ATP enhanced tritium overflow up to 140%, with the following order of potency: α,β-meATP > 2-MeSATP = ATP = β,γ-meATP > β,γ-imidoATP. The facilitatory effects of α,β-meATP and β,γ-imidoATP were prevented by PPNDS. Under the same conditions, apyrase attenuated, whereas the ectonucleotidase inhibitor 6-N,N-diethyl-α,β-γ-dibromomethylene 5′-triphosphate enhanced tritium overflow, an effect that was prevented by PPNDS. In the prostatic portion of the rat vas deferens, endogenous ATP exerts a dual and opposite modulation of noradrenaline release: an inhibition through activation of P2Y receptors with a pharmacological profile similar to that of the P2Y12 and P2Y13 receptors and a facilitation through activation of P2X receptors with a pharmacological profile similar to that of P2X1 and P2X3, or P2X2/P2X3 receptors.

In the sympathetic nervous system, ATP is stored and released with noradrenaline from postganglionic nerve terminals and acts not only as a transmitter (Burnstock, 1990; Westfall et al., 2002) but also as a presynaptic modulator (von Kugelgen et al., 1999). The released ATP exerts effects on nerve terminals and on postsynaptic cells, by activation of (von Kugelgen et al., 1999). The released ATP exerts effects on nerve terminals and on postsynaptic cells, by activation of (von Kugelgen et al., 1999). The released ATP exerts effects on nerve terminals and on postsynaptic cells, by activation of (von Kugelgen et al., 1999). The released ATP exerts effects on nerve terminals and on postsynaptic cells, by activation of (von Kugelgen et al., 1999).

ABBREVIATIONS: 2-MeSATP, 2-methylthioadenosine-5′-triphosphate; 2-MeSADP, 2-methylthioadenosine-5′-diphosphate; RB2, reactive blue 2; 2-MeSAMP, 2-methylthioadenosine 5′-monophosphate; MRS 2179, 2′-deoxy-N6-methyladenosine 3′,5′-bisphosphate; α,β-meATP, α,β-methyleneadenosine 5′-triphosphate; β,γ-imidoATP, β,γ-imidoadenosine 5′-triphosphate; β,γ-meATP, β,γ-methyleneadenosine 5′-triphosphate; PPNDS, pyridoxal-5′-phosphate-6-[(2′-naphthylazo-6′-nitro-4′,8′-disulfonate); DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; ZM 241385, 4-(2-[7-amino-2-[2-furyl]1,2,4-triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)-phenol; NF 279, 8,8′-[carbonylbis(imino)-4,1-phenylecaryonolimino-4,1-phenylecaryonolimino]-bis-1,3,5-naphthalenetrifussonic acid; ARL 67156, 6-N,N-diethyl-α,β,γ-dibromomethylene 5′-triphosphate; PPADS, pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid.
activation of presynaptic inhibitory P2Y receptors (von Kugelgen et al., 1999). In addition to presynaptic inhibitory P2Y receptors, sympathetic nerve terminals may also be endowed with excitatory P2 receptors. Cultured rat sympathetic neurons (Boehm, 1999; Nörenberg et al., 1999) and postganglionic sympathetic nerves that innervate the heart (Sperlágh et al., 2000; Sesti et al., 2002, 2003) express P2X receptors that are present on axon terminals and mediate an enhancement of noradrenaline release.

In rat vas deferens, a sympathetic innervated tissue, a P2 receptor-mediated inhibition of noradrenaline release has been demonstrated previously (Kurz et al., 1993). However, the receptor subtype involved has not been identified. Furthermore, immunohistochemical studies have shown that, in this tissue, P2X receptors are also present on nerve terminals (Vulchanova et al., 1996; Lee at al. 2000), but their putative involvement on modulation of noradrenaline release was never investigated.

Therefore, the aims of the present study were 1) to study the role of ATP on modulation of noradrenaline release, 2) to characterize pharmacologically the P2 receptor subtypes that mediate the inhibitory effects of ATP on noradrenaline release, and 3) to investigate whether P2X receptors also participate in the modulation of noradrenaline release in the rat vas deferens. In this study, only the prostatic portion of rat vas deferens was used because in this preparation the importance of ATP as transmitter is particularly relevant (Sneddon and Machaly, 1992).

Materials and Methods

Experimental Protocol. Adult male Wistar rats (290–340 g; IBMC, Porto, Portugal) were used. Handling and care of animals were conducted according to the European Union guiding principles in animal research (86/609/EU). Animals were killed by cervical dislocation and exsanguination. Prostatic halves of vas deferens were dissected out, cleaned of connective tissue, and divided longitudinally into preparations. Eight tissue preparations were then incubated in 2 ml of medium containing 0.1 µM [3H]noradrenaline, for 40 min at 37°C. Individual preparations were placed in superfusion chambers between platinum electrodes and superfused with [3H]noradrenaline-free medium at a rate of 1 ml min⁻¹. Successive 5-min samples of the superfusate were collected from t = 55 min onwards (t = 0 min being the start of superfusion). At the end of the experiments, tritium was determined in superfusate samples and in tissues by scintillation spectrometry (LS 6500; Beckman Coulter, Fullerton, CA). The medium contained 118.6 mM NaCl, 4.70 mM KCl, 2.52 mM CaCl₂, 1.23 mM MgSO₄, 25.0 mM NaHCO₃, 10.0 mM glucose, 0.3% ascorbic acid, 0.031 mM disodium EDTA and was saturated with 95% O₂, 5% CO₂ and kept at 37°C. The superfusion medium also contained desipramine (400 nM; to inhibit neuronal uptake of noradrenaline) and yohimbine (1 µM; to block α₂-autoreceptors). Up to five identical periods of electrical stimulation were applied (Stimulator II; Hugo Sachs Elektronik, March-Hugstetten, Germany; constant current mode, rectangular pulses, 1-ms width, 50-mA current strength, and 18 V cm⁻¹ voltage drop between electrodes). The first, starting at t = 30 min (S₀) was not used for determination of tritium overflow. The subsequent periods (S₁ to S₄), also consisting of 100 pulses at 8 Hz, started at t = 60 min with 30-min intervals. Concentration-response curves for P2 receptor agonists were obtained by adding the agonists at increasing concentration 8 min before S₂, S₃, and S₄ up to the end of each stimulation period. Concentration-response curves for P2 receptor antagonists were obtained by adding antagonists 20 min before S₂, S₃, and S₄ at increasing concentrations. When indicated, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (100 nM; to block adenosine A₁ receptors), 4-(2-[7-aminomethyl-2-(1,2,4-triazolo[2,3-e][1,3,5]triazin-5-ylamino)ethyl]phenol (ZM 241385) (100 nM; to block adenosine A₂A receptors), and RB2 (10 µM; to block P2Y receptors) were added throughout superfusion.

Data Evaluation. The outflow of tritium was expressed as fraction of the tissue tritium content at the onset of the respective collection period (fractional rate of outflow, minute⁻¹). Effects of drugs on basal tritium outflow were estimated by the b₂/b₁ and b₄/b₃ ratios, and were expressed as percentage of the mean ratio obtained in the appropriate control; b₂ was the fractional rate of outflow in the 5-min period before S₂, S₃, and S₄ and b₄ (b₂, b₃, and b₄, respectively), and b₁ was the fractional rate of outflow in the 5-min period before S₁. The overall duration of tritium evoked by electrical stimulation was calculated as the difference between “total tritium outflow during the 10-min period after start of stimulation” and the estimated “basal outflow,” being expressed as percentage of tritium content of the tissue at the onset of stimulation. Effects of drugs added after S₁ on tritium overflow were evaluated as ratios of the overflow elicited by S₂, S₃, and S₄ (S₃) and the overflow elicited by S₁ (S₃/S₁). S₃/S₁ values obtained in individual experiments in which a test compound A was added after S₁ were calculated as percentage of the respective mean ratio in the appropriate control group (solvent instead of A). When interaction of drug A, added after S₁, and a drug B added either after S₃ or at the beginning of superfusion, was studied, the “appropriate control” was a group in which B alone was used.

Drugs. Levo-Ç-ring-2,5,6-3[H]noradrenaline, specific activity 46.8 Ci mm⁻¹ was from DuPont-NEN (Gural, Lisboa, Portugal); ADP, ATP, apyrase grade VI (EC 3.6.1.5), DPCPX, desipramine hydrochloride, 2MeSADP, 2MeSAMP, 2MeSATP, β,γ-methylATP, γ-methylATP, suramin hexasodium, RB2 (basilen blue E-3G), and yohimbine hydrochloride were from Sigma (Sintra, Portugal); ZM 241385, 8,8′-[carboxylbis(mino-4,1-phenylecarbonylimino)-4,1-phenylecarbonylimino]bis-1,3,5-naphtalene-trisulfonic acid hexasodium (NF 279), 2′-deoxy-N6-methyladenosine 3′,5′-bisphosphate tetrammonium (MRS 2179), 6-N,N-diethyl-d-b-dibromomethylene 5′-triphosphate trimmonium (ARL 67156), pyridoxalphosphate-6-azophenyl-2′-disulfonic acid tetrasodium (PPADS), and PPNDs were from Tocris (Bristol, UK). Stock solutions up to 10 mM were made in dimethyl sulfoxide or water and kept at −20°C. Solutions of drugs were prepared immediately before use and solvent was added to the superfusion medium in parallel control experiments.

Statistical Analysis. Results are presented as means ± S.E.M.; n is the number of tissue preparations. Effect of drugs on basal tritium outflow and evoked tritium overflow was tested for significance by an analysis of variance followed by the Dunnett's multiple comparison test. P values lower than 0.05 were taken to indicate significant differences.

Results

General Observations. The fractional rate of basal tritium outflow and electrically evoked tritium overflow from prostatic portions of rat vas deferens in different experimental conditions are shown in Table 1. Blockade of adenosine receptors with the adenosine A₁ receptor antagonist DPCPX (Lohse et al., 1987) and with the adenosine A₂A receptor antagonist ZM 241385 (Poucher et al., 1995) did not change either basal tritium outflow or evoked tritium overflow. When, in addition to P1 antagonists, the P2Y antagonist RB2 (10 µM) was added throughout superfusion, basal tritium outflow and evoked tritium overflow were increased (Table 1). Basal tritium outflow and evoked tritium overflow remained constant throughout the experiment, regardless of the drugs added throughout superfusion, with b₂/b₁ and S₃/S₁ values close to unity (not shown). Basal tritium outflow
was not changed by drugs added after S1, except by the P2 antagonists PPADS, PPNDS, and RB2 (see below).

**Influence of P2 Receptor Agonists on Tritium Overflow.** Electrical stimulation of prostatic portion of rat vas deferens by 100 pulses/8 Hz caused an overflow of tritium that was modified by several purine nucleotides. The nucleotides were tested in the presence of the P1 antagonists DPCPX (100 nM; to block adenosine A1 receptors) and ZM 241385 (100 nM; to block adenosine A2 receptors), to avoid contribution of these receptors to the effects of nucleotides. Under these conditions, α,β-meATP and β,γ-methioATP slightly increased, β,γ-imidooATP did not change, whereas 2-MeSATP, 2-MeSADP, ADP, and ATP decreased evoked tritium overflow in a concentration-dependent manner (Fig. 1). Adenosine (1 mM) only caused a small decrease of evoked tritium overflow (S2/S1 = 87 ± 3%; n = 4; P < 0.05). The apparent order of potency of nucleotides that decreased evoked tritium overflow was: 2-MeSATP > 2-MeSADP > ADP > ATP. As shown in Fig. 2, the effect of 2-MeSATP was prevented by the nonselective P2Y antagonist RB2 (10 μM) and by the P2Y12 and P2Y13 antagonist 2-MeSAMP (10 μM; Hollopeter et al., 2001; Zhang et al., 2002) but not by the selective P2Y1 antagonist MRS 2179 (10 μM; Boyer et al., 1998).

**Influence of P2 Receptor Agonists on Tritium Overflow, in the Presence of RB2.** The results obtained with nucleotides suggest that, in addition to inhibitory P2Y receptors, facilitatory P2 receptors may also be involved in the modulation of tritium overflow. Because there are no selective agonists for each of the P2 receptors, the effects of nucleotides were reevaluated in the presence of 10 μM RB2 (added at beginning of superfusion and kept throughout) to block inhibitory P2Y receptors.

When inhibitory P2Y receptors were blocked, α,β-meATP, β,γ-methioATP, 2-MeSATP, β,γ-imidoATP, and ATP, but not 2-MeSADP, caused a concentration-dependent increase on evoked tritium overflow (Fig. 3). The facilitatory effects of α,β-methioATP and β,γ-imidoATP were prevented by the P2X antagonist PPADS (3 μM; Lambrecht, 2000; Fig. 4), suggesting an involvement of facilitatory P2X receptors on the modulation of evoked tritium overflow.

**Influence of P2 Receptor Antagonists on Tritium Overflow.** The effect of several P2 antagonists on evoked tritium overflow was also tested to investigate whether the ATP released was tonically activating the P2 receptors under the stimulation conditions used.
PPNDS and PPADS (Lambrecht 2000) was due to blockade of a facilitation of noradrenaline release mediated by endogenous ATP, the effect of apyrase (an enzyme that metabolizes ATP; Zimmermann, 1999) and of the ectonucleotidase inhibitor ARL 67156 (Crack et al., 1995) was investigated. Apyrase and ARL 67156 were tested in the presence of DPCPX (100 nM) and ZM 241385 (100 nM), and RB2 (10 μM; the concentration that prevented the inhibitory effect of 2-MeSATP and caused a concentration-dependent increase on tritium overflow; Figs. 2 and 5), to remove the influence of P1 receptors and inhibitory P2Y receptors, respectively. Apyrase (5 U/ml) decreased, whereas ARL 67156 (50 μM) increased evoked tritium overflow (Fig. 6). The facilitatory effect of ARL 67156 was prevented by PPNDS (3 μM; Fig. 6), supporting the observation that endogenous ATP may increase evoked tritium overflow by activation of facilitatory P2X receptors.

**Discussion**

The electrically evoked tritium overflow from tissue preparations of rat vas deferens preincubated with [3H]noradrenaline was assumed to reflect action potential-evoked neuronal release of noradrenaline. In accordance with previous findings (Kurz et al., 1993), noradrenaline release was inhibited by ATP and by other nucleotides such as 2-MeSATP and enhanced by the P2 antagonists suramin and RB2. It is unlikely that effects of ATP and other nucleotides tested on noradrenaline release are due to adenosine because they were still observed in the presence of the P1 antagonists DPCPX and ZM 241385 (Fig. 1). Therefore, changes in tritium overflow caused by nucleotides and P2 antagonists are, most likely, mediated by prejunctional P2 receptors, with the inhibitory effects being mediated by prejunctional P2Y receptors.

**Inhibitory P2Y Receptors.** From the P2Y receptors already cloned, only the P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, and P2Y14 subtypes have been shown to occur in mammalian tissues (Ralevic and Burnstock, 1998; Abbracchio et al., 2003). Involvement of P2Y2, P2Y4, P2Y6, and P2Y11 subtypes on the inhibition of noradrenaline release may be excluded by the following reasons: 1) P2Y2 is only weakly activated by 2-MeSATP, 2-MeSADP, and ADP (Nicholas et al., 1996), nucleotides that caused a marked inhibition of noradrenaline release; 2) P2Y4 is insensitive to suramin (Charlton et al., 1996), an antagonist that caused a facilitation of noradrenaline release in the present study, possibly by preventing a tonic activation of inhibitory P2Y receptors; 3) P2Y6 is selective for uridine nucleotides, is very weakly activated by 2-MeSATP, and is not activated by ATP and ADP (Nicholas et al., 1996), whereas, in the present study, 2-MeSATP was the most potent compound at the inhibitory P2Y receptors; and 4) the P2Y11 is sensitive to suramin (Communi et al., 1997, 1999) that, in the present study, caused a concentration-dependent inhibition of noradrenaline release.

The P2Y1, P2Y12, and P2Y13 subtypes are receptors with a very similar agonist profile. The order of potency of nucleotides that activate these receptors is 2-MeSADP > ADP > ATP (Boyer et al., 1996; Hollopeter et al., 2001; Zhang et al., 2002). This agonist profile is very similar to that observed for inhibitory P2Y2 receptors in the present study: 2-MeSATP > ATP > ADP > ADP (Communi et al., 1997, 1999).
of noradrenaline for 40 min and electrically stimulated with five trains of 100 pulses/8 Hz (S₀-S₄). The P₁ antagonists DPCPX (100 nM) and ZM 241385 (100 nM) and the P₂Y antagonist RB2 (10 μM) were added at the beginning of superfusion and kept throughout. PPADS (3 μM) was added 20 min before S₂ and kept throughout. For evaluation of effects of drugs, Sₙ/S₁ ratios obtained in the presence of antagonists were expressed as percentage of the average of the corresponding Sₙ/S₁ control value (see Materials and Methods). Values are means ± S.E.M. from four to six tissue preparations. Significant differences from respective control (solvent): *, P < 0.05 and ***, P < 0.01.

TABLE 2
Effects of P₂ antagonists on electrically evoked tritium overflow from prostatic portion of rat vas deferens

<table>
<thead>
<tr>
<th>Drug Added after S₁</th>
<th>μM</th>
<th>Sₙ/S₁ % control</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Added after S₁</td>
<td>10</td>
<td>109 ± 6</td>
<td>3</td>
</tr>
<tr>
<td>Suramin</td>
<td>30</td>
<td>120 ± 6***</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>131 ± 9***</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>140 ± 5***</td>
<td>6</td>
</tr>
<tr>
<td>RB2</td>
<td>3</td>
<td>122 ± 5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>139 ± 6**</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>126 ± 6*</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>86 ± 7</td>
<td>6</td>
</tr>
<tr>
<td>PPADS</td>
<td>3</td>
<td>37 ± 4**</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>37 ± 4**</td>
<td>6</td>
</tr>
<tr>
<td>PPADS</td>
<td>10</td>
<td>65 ± 2**</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>29 ± 3**</td>
<td>9</td>
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<tr>
<td></td>
<td>1</td>
<td>95 ± 2</td>
<td>6</td>
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<td></td>
<td>3</td>
<td>78 ± 3**</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>39 ± 2**</td>
<td>6</td>
</tr>
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</table>

Significant differences from respective control: *P < 0.05 and ***, P < 0.01.

2-MeSADP > ADP ≥ ATP. Therefore, P₂Y₁, P₂Y₁₂, and P₂Y₁₃ subtypes are the most serious candidates to mediate that inhibition of noradrenaline release.

P₂Y₁ receptors are blocked by MRS 2179, which is selective for this receptor subtype (Boyer et al., 1998) and also by RB2, suramin, and PPADS, with similar potencies (Boyer et al., 1994). Furthermore, it has been shown that P₂Y₁ recep-
tors can close N-type Ca\(^{2+}\) channels in neurons (Filippov et al., 2000), a mechanism that may explain the inhibitory effect of nucleotides on transmitter release. The lack of antagonism by MRS 2179 of inhibitory effects caused by 2-MeSATP, combined with the observation that, per se, MRS 2179 did not change noradrenaline release, whereas PPADS decreased noradrenaline release, exclude the involvement of P2Y1 receptors on inhibition of noradrenaline release in the prostatic portion of the rat vas deferens. Furthermore, the observation that nucleotides, such as 2-MeSATP and 2-MeSADP, decreased noradrenaline release even in the presence of the adenosine A\(_1\) receptor antagonist DPCPX (at a concentration that is about 200 times higher than its affinity constant at the adenosine A\(_1\) receptor; Lohse et al., 1987) excludes the involvement of the recently described heteromers of P2Y1 and adenosine A\(_1\) receptors (Yoshioka et al., 2001) or the P3 receptor (Forsyth et al., 1991) on the inhibition of noradrenaline release caused by these nucleotides.

P2Y12 and P2Y13 subtypes are both blocked by 2-MeSAMP, with similar potency (Hollopet at 2001; Zhang et al., 2002). Because 2-MeSAMP prevented the inhibitory effects of 2-MeSATP, the PY12 and/or the P2Y13 receptor subtypes are the strongest candidates to mediate an inhibition of noradrenaline release in the prostatic portion of rat vas deferens. Contribution of P2Y12 receptors can be questioned because ATP is an antagonist at P2Y12 receptors (Hollopet et al., 2001). However, the involvement of the P2Y12 receptors cannot be completely excluded because there is the possibility that ATP may be partly metabolized to ADP that is an agonist at these receptors.

Participation of P2Y13 receptors, and eventually P2Y12 receptors, on modulation of noradrenaline release is compatible with their known distribution and coupling system. P2Y13 receptors are widely expressed (Zhang et al., 2002) and like P2Y12 receptors, are negatively coupled to adenylate cyclase through activation of a G\(_{i/o}\) protein (Hollopet et al., 2001; Kublick and von Kügelgen, 2002; Zhang et al., 2002). Recently, several studies have shown that P2Y12- and P2Y13-like receptors may mediate an inhibition of neuronal Ca\(^{2+}\) channels (Powell et al., 2000; Kulick and von Kügelgen, 2002; Unterberger et al., 2002; Kubista et al., 2003). This signaling pathway is also activated by other presynaptic receptors that inhibit transmitter release (Boehm and Kubista, 2002).

**Facilitatory P2X Receptors.** Our results also show that some purine nucleotides including ATP may also enhance noradrenaline release in the prostatic portion of rat vas deferens. This conclusion is supported by the observation that nucleotides, such as α,β-meATP, increased noradrenaline release, even when inhibitory P2Y receptors were blocked, and by the observation that the P2X antagonists PPADS, PPNDs, and NF 279 decreased noradrenaline release, an effect that is most likely due to an attenuation of a release-enhancing effect of endogenous ATP. Evidence that endogenous ATP was activating facilitatory P2 receptors was obtained by changing the levels of endogenous ATP under conditions of low influence of inhibitory P2Y receptors. The ATP-metabolizing enzyme apyrase decreased noradrenaline release, most likely by preventing the release enhancing effects of endogenous ATP, whereas the ecto-ATPase inhibitor ARL 67156, which prevents ATP degradation, increased noradrenaline release, most likely by favoring the release-enhancing effects of endogenous ATP.

P2Y11 receptors can couple to intracellular pathways that may lead to a facilitation of transmitter release (Communi et al., 1997) and can be activated by α,β-meATP (van der Weyden et al., 2000), but their involvement on the facilitation of noradrenaline release is unlikely because they are blocked by RB2 but not by PPADS (Communi et al., 1999; van der Weyden et al., 2000). Therefore, the P2 receptors that mediate facilitation of noradrenaline release seem to belong to the P2X receptor subtype, as observed in other sympathetic sympathetic innervated tissues (Sperlàgh et al., 2000; Sesti et al., 2003, 2003) and in cultured sympathetic neurons (Boehm, 1999).

From all functional P2X receptors known, homomeric P2X1 or P2X3 receptors and heteromeric receptors composed of P2X2/P2X3, P2X1/P2X5, and P2X4/P2X6 subunits are activated by α,β-meATP (Nörenberg and Illes, 2000). Immuno-histochemical studies have shown that only the P2X1, P2X2, and P2X3 subunits are expressed in rat vas deferens (Lee et al., 2000) and that some of them, namely, the P2X2 and P2X3 subunits, are found on nerves fibers and nerve terminals (Vulchanova et al., 1996; Lee et al., 2000).

The antagonist profile of facilitatory P2 receptors that modulate noradrenaline release is similar to that described for homomeric P2X1 or P2X3 receptors and for heteromeric P2X2/P2X3 receptors (Lambrecht, 2000). The antagonist profile observed, α,β-meATP > 2-MeSATP = ATP > β,γ-meATP = β,γ-imidoATP, is slightly different from that described for homomeric P2X1 or P2X3 receptors and heteromeric P2X2/P2X3 receptors (2-MeSATP ≥ ATP > α,β-meATP; Nörenberg and Illes, 2000). This discrepancy may not indicate the presence of a different P2X receptor subtype but is just a consequence of the coexistence of P2Y and P2X receptors that are activated by the same nucleotides; 2-MeSATP may also activate inhibitory receptors disturbing a clear definition of the order of potency of agonists at facilitatory P2X receptors.

In conclusion, endogenous ATP exerts a dual and opposite modulation of noradrenaline release in the prostatic portion of the rat vas deferens: an inhibition through activation of P2Y receptors with a pharmacological profile similar to that of the recently cloned P2Y12 and/or P2Y13 subtypes and a facilitation through activation of P2X receptors that have a pharmacological profile similar to that of homomeric P2X1 and P2X3 or heteromeric P2X2/P2X3 receptors.

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**References**


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