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Pharmacological characterization of P_2 purinoceptor types in rat locus coeruleus neurons

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Abstract

The frequency of spontaneous action potentials of locus coeruleus neurons was recorded extracellularly in pontine slices of the rat brain. The adenosine 5′-triphosphate (ATP) analogues α,β-methylene ATP (α,β-meATP) and 2-methylthio ATP increased the firing rate with a similar potency, while uridine 5′-triphosphate (UTP) was inactive. Diadenosine 5′-pentaphosphate (Ap_5A), diadenosine 5′-tetraphosphate (Ap_4A) and diadenosine 5′-triphosphate (Ap_3A) all facilitated the firing. When equimolar concentrations were compared, Ap_5A had the largest effect followed by Ap_4A and Ap_3A. Suramin markedly inhibited responses to α,β-meATP and 2-methylthio ATP; the effect of Ap_4A was only slightly depressed by suramin. Pyridoxalphosphate-6-azophenyl-2,4-disulfonic acid (PPADS) strongly antagonized α,β-meATP, but failed to alter the effects of 2-methylthio ATP and Ap_4A. Reactive blue 2 weakly antagonized α,β-meATP and did not interfere with 2-methylthio ATP and Ap_4A. Moreover, suramin depressed responses to (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartic acid (NMDA), but not to substance P. PPADS failed to affect the AMPA- and NMDA-induced increases in firing. Hence, locus coeruleus neurons may possess receptors for adenosine nucleotides (P_{2X} and P_{2Y} purinoceptors) and dinucleotides (P_{2D} purinoceptors); receptors for uridine nucleotides (P_{2U} purinoceptors or pyrimidinoceptors) are probably absent.

Keywords: Purinoceptor; Pyrimidinoceptor; Excitatory amino acid receptor; Locus coeruleus; Extracellular recording

1. Introduction

Adenosine 5′-triphosphate (ATP) has been shown to activate P_2 purinoceptors which can be differentiated from adenosine-sensitive P_1 purinoceptors by means of specific antagonists (Burnstock and Buckley, 1985). The potency orders of structural analogues of ATP (α,β-methylene ATP [α,β-meATP], 2-methylthio ATP) have been used to subdivide the P_2 purinoceptors of multicellular preparations into the P_{2X} (α,β-meATP > 2-methylthio ATP) and P_{2Y} types (2-methylthio ATP > α,β-meATP). However, it is difficult to determine a reliable rank order of agonist potencies in such preparations, since ATP and the prototypic agonist 2-methylthio ATP are rapidly degraded by ectonucleotidases, while α,β-meATP is rather resistant to enzymatic breakdown (Kennedy and Leff, 1995). The antagonists suramin and reactive blue 2 act at both P_2 purinoceptor types with comparable affinities (Kennedy, 1990; Kennedy and Leff, 1995; Humphrey et al., 1995). By contrast, pyridoxalphosphate-6-azophenyl-2,4-disulfonic acid (PPADS) is selective for P_{2X} over P_{2Y} purinoceptors in a certain range of concentrations (Ziganshin et al., 1994; Humphrey et al., 1995). Finally, it has been shown that the P_{2X} and P_{2Y} purinoceptors represent different structural and functional entities, the former being a ligand-activated cationic channel, the latter a G protein-coupled receptor (Abbracchio and Burnstock, 1994; Kennedy and Leff, 1995).

Additional G protein-coupled P_2 purinoceptors belong to the P_{2U} (sensitive to both ATP and uridine 5′triphosphate [UTP], and widely distributed), P_{2T} (sensitive to ADP and localized on platelets) and P_{2D} classes (sensitive to diadenosine polyphosphates, and localized on chromaffin cells and brain synaptosomes) (Dalziel and Westfall, 1994; Fredholm et al., 1994). Finally, ATP^{4-} activates P_{2Z} purinoceptors of mast cells and macrophages which open

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membrane pores of up to 900 Da (Dalziel and Westfall, 1994; Fredholm et al., 1994). It is noteworthy that a separate receptor recognizing pyrimidine nucleotides, for example UTP (pyrimidinoceptor), has been described in some tissues (Seifert and Schulz, 1989; Connolly, 1994; Abbracchio and Burnstock, 1994).

P_2 purinoceptors and pyrimidinoceptors appear to be widely distributed in the peripheral nervous system (Illes and Nörenberg, 1993; Illes et al., 1995). Much less is known about the effects of ATP and other nucleotides in the brain and spinal cord. Central noradrenergic neurons of the locus coeruleus represent one of the few well investigated areas. Locus coeruleus neurons were suggested to possess two types of P_2 purinoceptor, one opening non-selective cationic channels (P_{2X}) and the other closing K^+ channels probably via G protein activation (P_{2Y}) (Harms et al., 1992; Shen and North, 1993; Illes et al., 1995). The aim of the present study was threefold. Firstly, we confirmed the excitatory effects of the P_2 purinoceptor agonists α,β-meATP and 2-methylthio ATP on the spontaneous firing rate of rat locus coeruleus neurons (Tschöpl et al., 1992). Secondly, we searched for diadenosine polyphosphate- and UTP-sensitive receptors. Thirdly, we investigated the interaction of the P_2 purinoceptor antagonists suramin, PPADS and reactive blue 2 with α,β-meATP, 2-methylthio ATP and diadenosine tetraphosphate (Ap_4A).

2. Materials and methods

2.1. Brain slice preparation

The preparation and maintenance of midpontine slices of the rat brain were previously described (Tschöpl et al., 1992). In brief, male Wistar rats (150–220 g) were anaesthetized with ether and decapitated. Slices of 300–400 μm thickness, containing the caudal part of the locus coeruleus were prepared in oxygenated medium at 1–4°C with a Lancer vibratome. A single slice was placed in a recording chamber and was superfused at a rate of 2 ml/min with medium saturated with 95% O_2 + 5% CO_2 and maintained at 35–36°C. The medium had the following composition (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 24 and glucose 11.

2.2. Identification of locus coeruleus neurons and extracellular recording

The locus coeruleus appeared under a binocular microscope as a translucent oval area on the ventrolateral border of the fourth ventricle. Locus coeruleus cells fire spontaneously with a constant rate of 0.2–5 Hz. They were easily identified by their electrophysiological and pharmacological characteristics (Tschöpl et al., 1992). Since α_2-adrenoceptor agonists depress the firing (Cedarbaum and Aghajanian, 1977), a single test concentration of noradrenaline (30 μM), which produced complete and reversible inhibi-

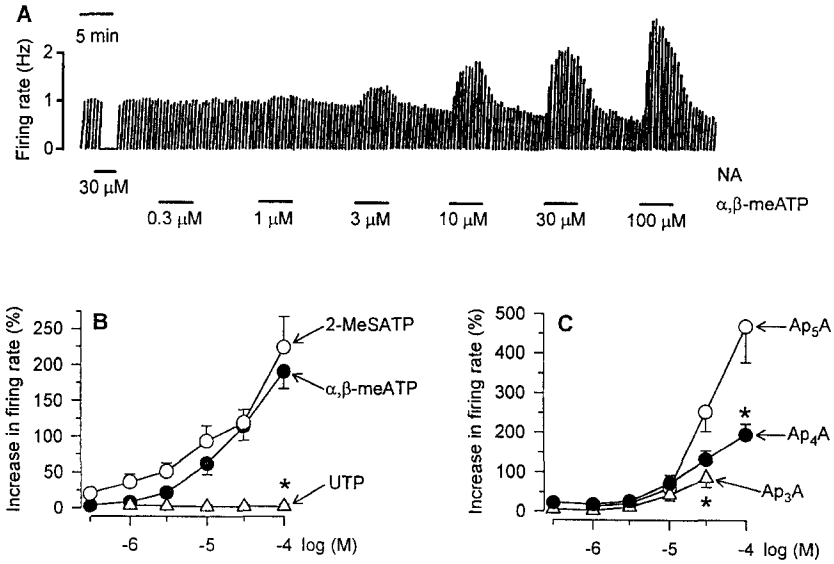

Fig. 1. Effects of ATP analogues, UTP and diadenosine polyphosphates on the firing rate of locus coeruleus neurons. Pontine slices of the rat locus coeruleus were prepared and the frequency of spontaneous action potentials was recorded extracellularly as consecutive 30-s samples. (A) Concentration-dependent effect of α,β-methylene ATP (α,β-meATP). Representative tracing out of 9 similar experiments. Noradrenaline (NA; 30 μM) was used to functionally identify locus coeruleus neurons; it abolished the firing rate reversibly. Noradrenaline and α,β-meATP were superfused as indicated by the horizontal bars. (B) Concentration-response curves of α,β-meATP (●; $n = 9$), 2-methylthio ATP (2-MeSATP) (○; $n = 5$) and UTP (△; $n = 6$). * $P < 0.05$; significant difference from the effect of 2-methylthio ATP at 100 μM. (C) Concentration-response curves of diadenosine pentaphosphate (Ap_5A) (○; $n = 9$) tetraphosphate (Ap_4A) (●; $n = 9$) and triphosphate (Ap_3A) (△; $n = 5$). * $P < 0.05$; significant differences from the effect of Ap_5A and Ap_4A at 100 and 30 μM, respectively. Means ± S.E.M. of n experiments both in B and C.

tion of the firing rate, was applied at the beginning of each experiment (Fig. 1A).

Glass microelectrodes filled with 4 M NaCl and having a tip resistance of 2–4 MΩ were used for recording the firing rate. The electrode signals were passed through a Grass P16 high-impedance amplifier, filtered and displayed on a Tektronix 5113 oscilloscope. The spikes were gated and counted by means of a WPI 121 window discriminator coupled to an electronic ratemeter and a Watanabe WTR 311 pen-recorder. Firing rate was recorded as consecutive 30-s samples.

2.3. Application of drugs and evaluation of data

Different drugs were applied by changing the superfusion medium by means of three-way taps. At the constant flow rate of 2 ml/min about 30 s were required until the drug reached the bath.

Concentration-response curves of all agonists were made by applying increasing concentrations of the agonists for 5 min. The intervals between subsequent applications were either 10 min (P_2 purinoceptor agonists) or 15 min (excitatory amino acid receptor agonists and substance P). Previous experiments demonstrated that repetitive application of α,β-meATP (Tschöpl et al., 1992), *N*-methyl-D-aspartic acid (NMDA), (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and substance P (Fröhlich et al., 1994) with the above protocol reproducibly increases the firing rate. Potentiation of the discharge of action potentials was measured at its maximum (average of two counting periods), irrespective of drug contact-time, and was expressed as percentage of the average spike discharge during the 2 min immediately before addition (average of four counting periods). Since a clear maximum of the concentration-response curves was not reached with any of the agonists, the concentration that produced 50% of the maximum effect (EC_{50}) could not be determined. Consequently, it was not possible to quantify antagonist effects as concentration ratios obtained from rightward shifts, but they are described by the inhibitory potencies of various antagonists on agonist effects.

Concentration-response curves of agonists were also determined in the presence of P_2 purinoceptor antagonists, which were applied 15 min (suramin, reactive blue 2) or 20 min (PPADS; Ziganshin et al., 1994) before the addition of the lowest agonist concentration and were then present for the entire duration of the experiment. None of the antagonists altered the firing rate on their own (for suramin see also Tschöpl et al., 1992). Only one concentration-response curve of one agonist was made on a single cell of a brain slice.

2.4. Drugs

The following drugs were used: substance P (Bachem, Bubendorf, Switzerland); suramin hexasodium salt (Bayer, Wuppertal, Germany); (−)noradrenaline hydrochloride (Hoechst, Frankfurt am Main, Germany); 2-methylthioadenosine 5′-triphosphate tetrasodium salt, reactive blue 2, pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid tetrasodium salt (RBI, Natick, MA, USA); α,β-methyleneadenosine 5′-triphosphate dilithium salt, P^1,P^3-di(adenosine-5′)triphosphate ammonium salt, P^1,P^4-di(adenosine-5′)tetraphosphate ammonium salt, P^1,P^5-di(adenosine-5′)pentaphosphate ammonium salt, uridine 5′-triphosphate tetrasodium salt, *N*-methyl-D-aspartic acid (Sigma, Deisenhofen, Germany); (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (Tocris Cookson, Bristol, UK).

Stock solutions (1–10 mM) of all drugs were prepared with distilled water except for AMPA which was dissolved in 0.5 M HCl. Further dilutions were made with medium. The highest concentration of suramin (100 μM) was directly dissolved in medium. Equivalent quantities of the solvent had no effect.

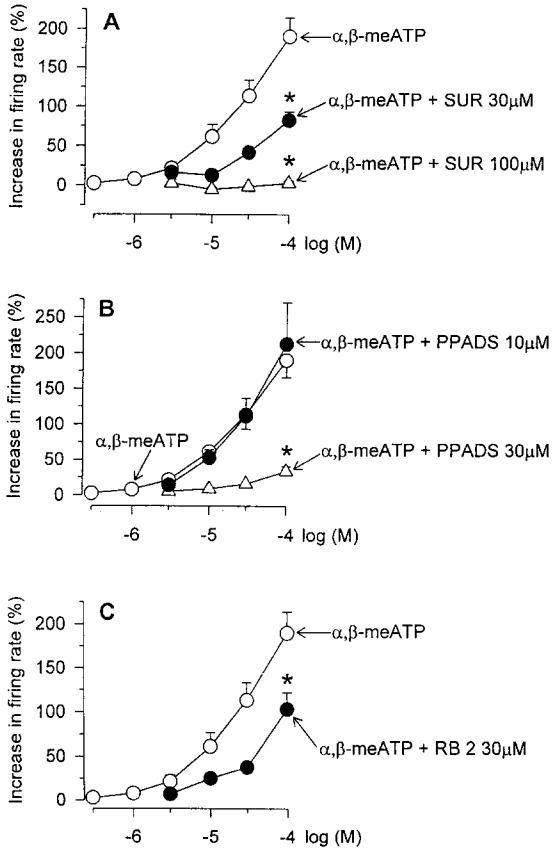

Fig. 2. Interaction of suramin, PPADS and reactive blue 2 with α,β-meATP on locus coeruleus neurons. (A) Concentration-response curves of α,β-meATP were determined in the absence (○; $n = 9$) or in the presence of suramin (SUR; 30 μM) (●; $n = 6$) or suramin (100 μM) (△ $n = 5$). (B) Concentration-response curves of α,β-meATP were determined in the absence (○; $n = 9$) or in the presence of PPADS (10 μM) (○; $n = 6$) or PPADS (30 μM) (△; $n = 5$). (C) Concentration-response curves of α,β-meATP were determined in the absence (○; $n = 9$) or in the presence of reactive blue 2 (RB 2; 30 μM) (●; $n = 8$). Means ± S.E.M. of n experiments in A, B and C. * $P < 0.05$; significant difference from the effect of α,β-meATP at 100 μM.

2.5. Statistics

Means ± S.E.M. are given throughout. The one way analysis of variance followed by the Bonferroni *t*-test method was used for comparison of the means (SigmaStat, Jandel, Erkrath, Germany). A probability level of 0.05 or less was considered statistically significant.

3. Results

All locus coeruleus neurons included in this study discharged spontaneous action potentials with an average rate of 1.1 ± 0.05 Hz ($n = 172$).

3.1. Effects of P_2 purinoceptor agonists

The prototypic P_2 purinoceptor agonists α,β-meATP (0.3–100 μM) and 2-methylthio ATP (0.3–100 μM) concentration dependently increased the firing rate of locus coeruleus neurons (Fig. 1A,B). The concentration-response curves of the two agonists were practically identical. By contrast, UTP was inactive over a wide range of concentrations (1–100 μM; Fig. 1B). Diadenosine 5′-triphosphate (Ap_3A; 0.3–100 μM), diadenosine 5′-tetraphosphate (Ap_4A; 0.3–100 μM) and diadenosine 5′-pentaphosphate (Ap_5A; 0.3–30 μM) all increased the firing (Fig. 1C). While the adenosine polyphosphates had similar activities at low concentrations, their effects markedly differed from each other above 10 μM (Fig. 1C). At 100 μM Ap_5A caused a larger response than Ap_4A, and at 30 μM Ap_5A was more active than Ap_3A.

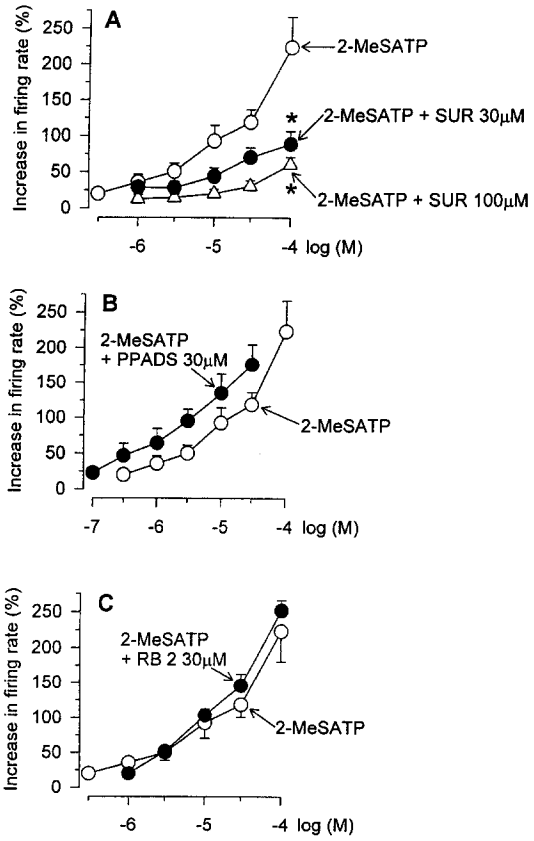

Fig. 3. Interaction of suramin, PPADS and reactive blue 2 with 2-methylthio ATP on locus coeruleus neurons. (A) Concentration-response curves of 2-methylthio ATP (2-MeSATP) were determined in the absence (○; $n = 5$) or in the presence of suramin (SUR; 30 μM) (●; $n = 4$) or suramin (100 μM) (△; $n = 4$). (B) Concentration-response curves of 2-methylthio ATP were determined in the absence (○; $n = 5$) or in the presence of PPADS (30 μM) (●; $n = 5$). (C) Concentration-response curves of 2-methylthio ATP were determined in the absence (○; $n = 5$) or in the presence of reactive blue 2 (RB 2; 30 μM) (●; $n = 5$). Means ± S.E.M. of n experiments in A, B and C. * $P < 0.05$; significant differences from the effect of 2-methylthio ATP at 100 μM.

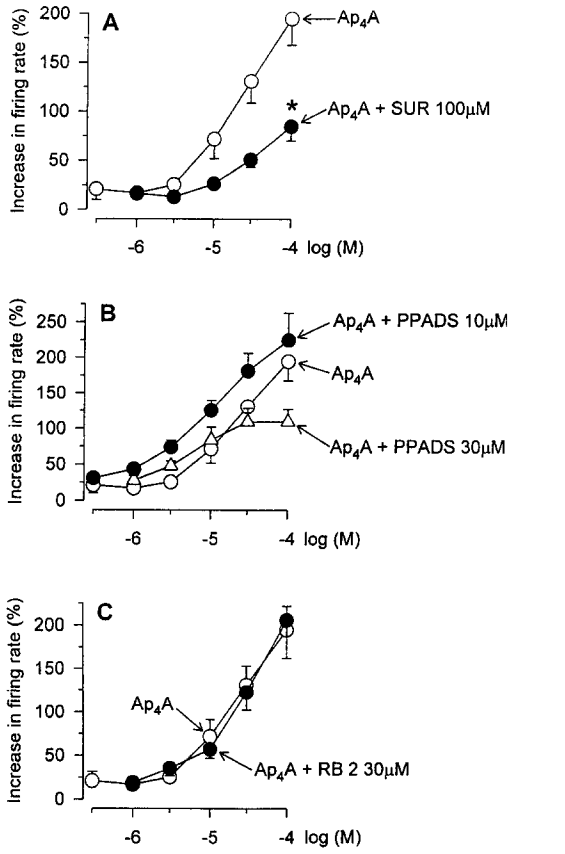

Fig. 4. Interaction of suramin, PPADS and reactive blue 2 with Ap_4A on locus coeruleus neurons. (A) Concentration-response curves of Ap_4A were determined in the absence (○; $n = 9$) or in the presence of suramin (SUR; 100 μM) (●; $n = 6$). (B) Concentration-response curves of Ap_4A were determined in the absence (○; $n = 9$) or in the presence of PPADS (10 μM) (●; $n = 5$) or PPADS (30 μM) (△; $n = 6$). (C) Concentration-response curves of Ap_4A were determined in the absence (○; $n = 9$) or in the presence of reactive blue 2 (RB 2; 30 μM) (●; $n = 7$). Means ± S.E.M. of n experiments in A, B and C. * $P < 0.05$; significant difference from the effect of Ap_4A at 100 μM.

3.2. Interaction of P_2 purinoceptor agonists and antagonists

Suramin depressed the α,β-meATP (3–100 μM)-induced increase in firing rate at 30 μM and virtually

abolished it at 100 μM (Fig. 2A). PPADS did not alter the effect of α,β-meATP (3–100 μM) at 10 μM, but markedly inhibited it at 30 μM (Fig. 2B). Finally, reactive blue 2 (30 μM) also antagonized the responses to α,β-meATP (3–100 μM) (Fig. 2C).

Suramin (30, 100 μM) concentration dependently inhibited the effect of 2-methylthio ATP (1–100 μM; Fig. 3A). By contrast, PPADS (30 μM) and reactive blue 2 (30 μM) did not interfere with 2-methylthio ATP (0.1–30 μM and 1–100 μM, respectively; Fig. 3B,C). A rather high concentration (100 μM) of suramin which markedly depressed the effects of α,β-meATP and 2-methylthio ATP (Fig. 2A and Fig. 3A) only moderately attenuated the effect of Ap_4A (1–100 μM; Fig. 4A). Both PPADS (10, 30 μM) and reactive blue 2 (30 μM) failed to antagonize the responses to Ap_4A (1–100 μM) (Fig. 4B,C).

The maximum agonist concentrations in the presence of suramin were not high enough to obtain full concentration-response curves for α,β-meATP (Fig. 2A), 2-methylthio ATP (Fig. 3A) and Ap_4A (Fig. 4A). Hence, it is unclear whether the shifts to the right were parallel or not.

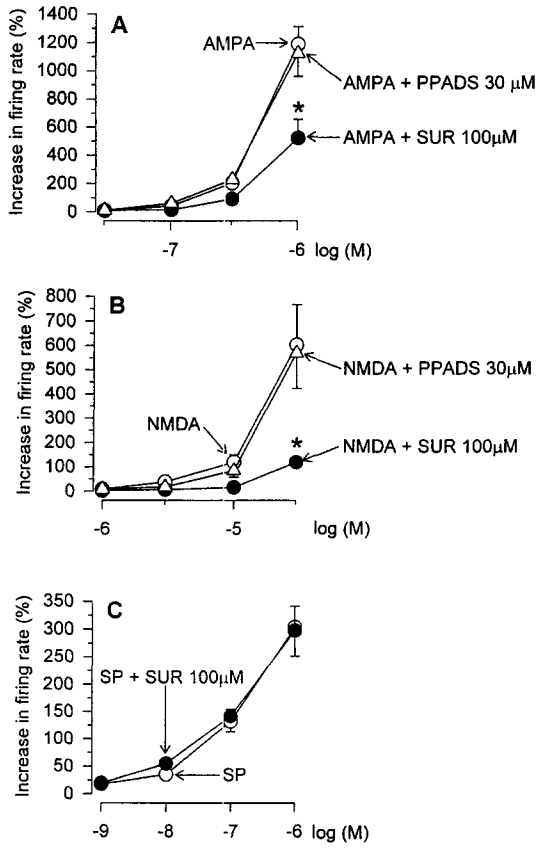

Fig. 5. Interaction of suramin and PPADS with AMPA, NMDA and substance P on locus coeruleus neurons. (A) Concentration-response curves of AMPA were determined in the absence (○; $n = 8$) or in the presence of suramin (SUR; 100 μM) (●; $n = 6$) or PPADS (30 μM) (△; $n = 7$). * $P < 0.05$; significant difference from the effect of AMPA at 1 μM. (B) Concentration-response curves of NMDA were determined in the absence (○; $n = 10$) or in the presence of suramin (100 μM) (●; $n = 7$) or PPADS (30 μM) (△; $n = 6$). * $P < 0.05$; significant difference from the effect of NMDA at 30 μM. (C) Concentration-response curves of substance P (SP) were determined in the absence (○; $n = 8$) or in the presence of suramin (100 μM) (●; $n = 5$). Means ± S.E.M. of n experiments in A, B and C.

3.3. Effects of NMDA, AMPA and substance P, and their interaction with P_2 purinoceptor antagonists

AMPA (0.03–1 μM), NMDA (1–30 μM), and substance P (0.001–1 μM) markedly facilitated the firing rate (Fig. 5). Suramin (100 μM) interfered with AMPA (0.03–1 μM) and NMDA (1–30 μM), but not with substance P (0.001–1 μM) (Fig. 5). PPADS (30 μM) did not alter the excitatory effects of AMPA (0.3–1 μM) and NMDA (1–30 μM) (Fig. 5A,B).

4. Discussion

The ATP analogues α,β-meATP, 2-methylthio ATP and the diadenosine polyphosphates Ap_5A, Ap_4A and Ap_3A all facilitated the discharge of spontaneous action potentials in rat locus coeruleus neurons. ATP itself was not tested, since it is known to increase the firing rate only when given in the presence of the P_1 purinoceptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; Tschöpl et al., 1992). Hence, a direct excitatory P_2 effect is counteracted by an indirect (mediated by the degradation product, adenosine) inhibitory P_1 effect (Illes and Nörenberg, 1993).

The purinoceptor-type preferential agonists (Burnstock and Kennedy, 1985) α,β-meATP (P_{2X}) and 2-methylthio ATP (P_{2Y}) were equipotent in locus coeruleus neurons. This finding may indicate the co-existence of P_{2X} and P_{2Y} purinoceptors. It was reported recently that in cell culture systems or after the blockade of ectonucleotidases 2-methylthio ATP has a higher potency than α,β-meATP both at P_{2X} and P_{2Y} purinoceptors (Kennedy and Leff, 1995). However, in the present study a multicellular preparation was used and the enzymatic degradation of 2-methylthio ATP was not inhibited. Moreover, intracellular measurements (Harms et al., 1992) and whole-cell patch-clamp recordings (Shen and North, 1993) suggested that locus coeruleus neurons are endowed with both P_{2X} and P_{2Y} purinoceptors, utilizing ligand-gated cationic channels and G proteins, respectively (Illes et al., 1995). Hence, the α,β-meATP- and 2-methylthio ATP-induced increases in firing rate may be due to the preferential activation of the two different purinoceptor types.

UTP failed to increase the firing rate of locus coeruleus cells. Central and peripheral noradrenergic neurons are different in this respect. Experiments with extracellular electrophysiological methods (Connolly and Harrison, 1994; Connolly, 1994, 1995) and with the measurement of [^{3}H]noradrenaline release (Boehm et al., 1995) both indicated the presence of separate receptors for ATP and UTP

in rat superior cervical ganglia. Of course, although unlikely, the possibility remains that receptors for UTP are present at locus coeruleus neurons, but UTP is rapidly degraded by ectonucleotidases.

Adrenal chromaffin cells, which derive from the same part of the neuronal crest as sympathetic neurons, resembled locus coeruleus cells in another respect. The diadenosine polyphosphates are co-stored with catecholamines in the secretory granules of chromaffin cells and inhibit the carbachol-induced catecholamine release via P_{2D} purinoceptors (Pintor and Miras-Portugal, 1993; Zimmermann, 1994). Moreover, binding sites for Ap_4A and Ap_5A were demonstrated both in bovine chromaffin cells (Pintor et al., 1991) and rat brain synaptosomes (Pintor et al., 1993). In the present experiments, various diadenosine polyphosphates increased the firing rate of locus coeruleus neurons; Ap_5A had the strongest activity, followed by Ap_4A and Ap_3A. Hence, P_{2X}, P_{2Y} and P_{2D} purinoceptors may coexist in the locus coeruleus.

Suramin and reactive blue 2 do not differentiate between P_2 purinoceptor types (Kennedy, 1990; Kennedy and Leff, 1995; Humphrey et al., 1995). Accordingly, concentrations of suramin which are known to block P_2 purinoceptor-mediated reactions (Blakeley et al., 1991; Bültmann and Starke, 1994), reduced the effects of both α,β-meATP and 2-methylthio ATP; responses to Ap_4A were also depressed by suramin although to a lower extent than those to α,β-meATP and 2-methylthio ATP. Even a rather high concentration of reactive blue 2 (Nakazawa et al., 1991; Boehm, 1994) did not interfere with 2-methylthio ATP and Ap_4A and was only a weak antagonist of α,β-meATP. In addition, both suramin and reactive blue 2 are known to inhibit kainate-, NMDA- and GABA-induced currents in rat hippocampal neurons (Nakazawa et al., 1995) and suramin reduces the dimethylphenypiperazinium-induced release of [^{3}H]noradrenaline from chick sympathetic neurons (Allgaier et al., 1995; Humphrey et al., 1995). In agreement with these findings responses to the ionotropic glutamate receptor agonists AMPA and NMDA were antagonized by suramin. It is noteworthy that the Ap_4A-induced facilitation of the firing rate was attenuated by suramin to a similar extent as the AMPA- and NMDA-induced facilitation. The effect of substance P was, however, not altered.

The maximum agonist concentrations in the presence of suramin were not high enough to obtain full concentration-response curves and to decide whether the prerequisites for a competitive antagonism (parallel shift, no change in maximum) are fulfilled. Our decision not to increase agonist concentrations above 100 μM is easily explained by economic reasons and by the endeavour to avoid possible non-selective effects. We assume that the shifts would be non-parallel and suggest that high concentrations of suramin block ATP effects not only selectively at the recognition sites of P_{2X} and P_{2Y} purinoceptors but also non-selectively at the cationic channels of P_{2X} purinoceptors, an effect also observed with other ligand-gated cationic channels (AMPA, NMDA). A further non-selective effect of suramin may lead to inhibition at the effector binding site of the α subunit of G proteins (Freissmuth et al., 1996) and thereby to interruption of the transduction mechanism of P_{2Y} purinoceptors. Eventually, suramin probably fails to interfere with the recognition sites of the Ap_4A-sensitive (P_{2D}) purinoceptor or of the NMDA and AMPA receptors. High concentrations of reactive blue 2 may also act at the cationic channels of P_{2X} purinoceptors, but not at the recognition sites of any purinoceptor (P_{2X}, P_{2Y}, P_{2D}) present in the locus coeruleus.

The existence of two different P_2 purinoceptor types in locus coeruleus neurons activated either by α,β-meATP (P_{2X}) or 2-methylthio ATP (P_{2Y}) is also supported by the fact that PPADS in concentrations selective to P_{2X} purinoceptors (Ziganshin et al., 1994; Humphrey et al., 1995) attenuated the effect of α,β-meATP only. Moreover, PPADS did not interfere with Ap_4A, AMPA or NMDA probably because in contrast to suramin and reactive blue 2 it does not alter the properties of cationic channels or G proteins.

In conclusion, on the basis of this extracellular study, it is tentatively proposed that rat locus coeruleus neurons like rat superior cervical ganglion neurons possess purinoceptors identical with the P_{2X2} receptor recently isolated by molecular biology methods from rat PC12 cells (Brake et al., 1994; Surprenant et al., 1995). P_{2X2} receptors were suggested to be present in various areas of the rat brain (nucleus tractus solitarii, Ueno et al., 1992; tuberomamillary nucleus, Furukawa et al., 1994; Kennedy and Leff, 1995). This receptor has a rather low sensitivity to ATP and especially to α,β-meATP, and exhibits little desensitization to P_2 purinoceptor agonists. In contrast to P_{2X2} receptors, P_{2X1} receptors isolated from the rat vas deferens show a higher sensitivity to α,β-meATP and strongly desensitize (Valera et al., 1994). P_{2X4} receptors isolated from the rat superior cervical ganglion are not blocked by suramin (Buell et al., 1996). Finally, locus coeruleus neurons may possess a G-protein-coupled P_{2Y} purinoceptor similar to the recently isolated P_{2Y1} receptor (Webb et al., 1993; Barnard et al., 1994).

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