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ORIGINAL ARTICLE

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P2-Receptor-mediated inhibition of noradrenaline release in the rat hippocampus

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Abstract Experiments on hippocampal slices were carried out in order to find out whether the release of noradrenaline in the hippocampus can be modulated through P2-receptors. The slices were preincubated with [^{3}H]-noradrenaline, superfused with medium containing desipramine (1 μM), and stimulated electrically, in most experiments by 4 pulses/100 Hz.

The adenosine A_1-receptor agonist N^6-cyclopentyladenosine (CPA) and the nucleotides ATP, adenosine-5'-O-(3-thiotriphosphate) (ATPγS) and adenosine-5'-O-(2-thiodiphosphate) (ADPβS) decreased the evoked overflow of tritium by up to 55 %. The adenosine A_{2a}-agonist 2-*p*-(2-carboxyethyl)-phenethylamino-5'-N-ethylcarboxamido-adenosine (CGS 21680; 0.003-0.3 μM) caused no change. The concentration-response curve of CPA was shifted to the right by the A_1-antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 3 nM) but not by the P2-receptor antagonists cibacron blue 3GA (30 μM) and reactive blue 2 (30 μM); the apparent pK_B value of DPCPX against CPA was 9.0. In contrast, the concentration-response curve of ATP was shifted to the right by DPCPX (3 nM), apparent pK_B 8.7, as well as by cibacron blue 3GA (30 μM), apparent pK_B 5.2, and reactive blue 2 (30 μM), apparent pK_B 5.6; the antagonist effects of DPCPX and cibacron blue 3GA were additive in a manner compatible with the blockade of two separate receptors for ATP. The same pattern was obtained with ATPγS: its concentration-response curve was shifted to the right by DPCPX as well as by cibacron blue 3GA and reactive blue 2. Suramin (300 μM) antagonized neither the effect of ATP nor that of ATPγS. The 5'-nucleotidase inhibitor α,β-methylene-ADP (100 μM) did not change the effect of ATP. Only cibacron blue 3GA (30 μM) but not reactive blue 2 (30 μM), given alone, consistently caused a small increase of the evoked overflow of tritium. Hippocampal slices degraded exogenous ATP, and this degradation was reduced by cibacron blue 3GA (30 μM), reactive blue 2 (30 μM) and suramin (300 μM).

The results indicate that the noradrenergic terminal axons of the rat hippocampus possess P2-receptors in addition to the known A_1-adenosine receptors. The presynaptic P2-receptors mediate an inhibition of noradrenaline release, are activated by nucleotides but not nucleosides, and are blocked by cibacron blue 3GA and reactive blue 2. ATP and ATPγS act at both the A_1- and the P2-receptors. An autoreceptor function of cerebral presynaptic P2-receptors remains doubtful.

Key words Rat hippocampus · Noradrenaline release · Presynaptic P1-receptor · Presynaptic P2-receptor · ATP breakdown · Cibacron blue 3GA · Reactive blue 2 · Suramin

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Introduction

Within their mosaic of release-modulating receptors (see Langer 1980; Starke 1981; Fuder and Muscholl 1995), axon terminals possess receptors for nucleosides and nucleotides. Presynaptic P1-(adenosine)-receptors have been known for some time (Hedqvist and Fredholm 1976; Vizi and Knoll 1976). Presynaptic P2-receptors were identified more recently. In peripheral sympathetically innervated tissues, activation of P2-receptors either decreases (e.g. mouse vas deferens: von Kügelgen et al. 1989) or, more rarely, increases transmitter release (e.g. guinea-pig ileum: Sperlágh and Vizi 1991; for review see Fuder and Muscholl 1995; von Kügelgen 1996). Some studies have suggested that presynaptic P2-receptors also occur in the CNS: noradrenergic and serotonergic axon terminals in the rat occipito-parietal cortex seem to possess release-inhibiting P2-receptors in addition to adenosine receptors (von Kügelgen et al. 1994b; Koch et al. 1995). Moreover there is evidence for release-enhancing P2-receptors at glutamatergic axons in the rat hippocampus (Motin and Bennett 1995). Presynaptic P2-receptors

were not detected at the noradrenergic axons of the rabbit occipito-parietal cortex (von Kügelgen et al. 1992).

In the hippocampus of rats, activation of A_1-adenosine receptors reduces the release of noradrenaline (Jonzon and Fredholm 1984). We have now examined the possibility of an additional modulation through P2-receptors in this area. The subject seemed of interest since ATP, the major endogenous agonist at P2-receptors, is released in the hippocampus upon electrical stimulation (Wieraszko et al. 1989; Pedata et al. 1990). Some results have been published in abstract form (Koch et al. 1996).

Methods

Male Wistar rats weighing 250-300 g (Savo Kisslegg, Germany) were killed by cervical dislocation. The brain was quickly removed and chilled. Frontal sections, 350 µm thick, were cut from both hippocampi using a tissue chopper. Slices weighed about 2-3 mg and contained about 0.8 mg protein.

The incubation and superfusion medium contained (mM): NaCl 118, KCl 4.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, ascorbic acid 0.57, disodium EDTA 0.03. It was saturated with 5% CO_2 in O_2. The pH was adjusted to 7.4 with NaOH 1 M. The medium used for superfusion contained desipramine (1 µM) in order to block uptake$_1$.

Tritium overflow. About 25 slices were preincubated at 37 °C for 30 min in 4 ml medium with (-)-[^{3}H]-noradrenaline (0.1 µM). One slice was then transferred to each of twelve superfusion chambers where it was held by a polypropylene mesh between platinum plate electrodes 4 mm apart. The slices were superfused with [^{3}H]-noradrenaline-free medium for 162 min at 0.6 ml min^{-1} and 37 °C. A Stimulator I (Hugo Sachs Elektronik, March-Hugstetten, Germany) operating in the constant current mode was used for electrical field stimulation. Five periods of stimulation were applied (rectangular pulses of 0.5 ms width and 70 mA current strength). The first, after 30 min of superfusion and consisting of 18 pulses/1 Hz, was not used for determination of tritium overflow. The following stimulation periods (S_1 to S_4) were applied after 58, 86, 114 and 142 min of superfusion and consisted of 4 pulses applied at 100 Hz unless stated otherwise. The collection of successive 4-min superfusate samples began 8 min before S_1. Some drugs (or solvents) were present in the medium throughout superfusion. Other drugs (or solvents) were added either 6 min before S_2 for the remainder of the experiment or, at increasing concentrations, from 6 min before to 22 min after S_2, S_3 and S_4. After superfusion, slices were solubilized, and tritium was measured in superfusate samples and solubilized tissues by liquid scintillation counting; quenching due to coloured drugs was corrected for.

The outflow of tritium was expressed as fractional rate (min^{-1}) and the electrically evoked overflow, obtained by subtraction of the estimated basal efflux, as a percentage of the tritium content of the slice, in analogy to previous works (von Kügelgen et al. 1992). Effects of drugs that were added after S_1 were evaluated as ratios of the overflow elicited by S_2, S_3 and S_4 and the overflow elicited by S_1 (S_n/S_1). S_n/S_1 ratios from individual experiments in which a test compound A was added after S_1 were also calculated as a percentage of the respective mean ratio in the appropriate control group (solvent instead of A). When the interaction of A, added after S_1, and a drug B, added throughout superfusion, was studied, the 'appropriate control' was a group in which B alone was used. Effects of drugs that were added after S_1 on basal tritium efflux were evaluated as ratios of the fractional rate immediately before S_2, S_3 and S_4 and the fractional rate before S_1 (b_n/b_1).

For ATP and CPA, the concentration-inhibition curves of which levelled off at high concentrations, the sigmoid-shaped function no. 25 of Waud (1976) was fitted to averaged concentration-inhibition data. The function yielded the maximal inhibition and the EC_{50} (concentration that caused 50 % of the maximal inhibition). The function was also fitted to averaged ATP and CPA concentration-inhibition data from experiments carried out in the presence of antagonists (DPCPX, cibacron blue 3GA or reactive blue 2); when asymptotic maxima were not reached in the presence of antagonists (e.g. Fig. 4), the maximal inhibition was taken to be that obtained in the absence of antagonists (cf. Kurz et al. 1993). pK_B (-log K_B) values of DPCPX, cibacron blue 3GA and reactive blue 2 were calculated from the increase in EC_{50} values (equation no. 16 of Waud 1976). Since only one antagonist concentration was used and a competitive character of the antagonism not verified, the values are *apparent* pK_B values.

Removal of ATP from the medium. Slices were initially incubated at 37 °C for 20 min in 30 ml medium. Two slices were then transferred to each of six test tubes with 3 ml medium (37 °C) bubbled with 5 % CO_2 in O_2 and containing either solvent or P2-receptor antagonists. ATP was added 20 min later, final concentrations 1, 3, 10 or 30 µM. Aliquots of 50 µl were removed 30 s before addition and 2.5, 5, 10, 15, 20, 30 and 60 min after addition of ATP and stored on ice. ATP was assayed by means of the luciferase technique using the ATP bioluminescence FL-AAM assay kit (Sigma, Deisenhofen, Germany) and a LB 953 luminometer (Berthold, Wildbad, Germany). A blank value obtained with fresh medium was subtracted from each experimental value. Calibration curves for ATP 0.1 to 30 µM were obtained with ATP standard and medium as solvent. Since P2-receptor antagonists can interfere with the luciferase assay (for example von Kügelgen and Starke 1994), separate calibration curves were determined for medium containing solvent or each of the antagonists.

Materials and statistical evaluation. The following drugs were used: suramin hexasodium salt (Bayer, Wuppertal, Germany); (-)-[ring-2,5,6-^{3}H]-noradrenaline, specific activity 2.0 to 2.6 TBq mmol^{-1} (Dupont, Dreieich, Germany); 2-*p*-(2-carboxyethyl)-phenethylamino-5'-N-ethylcarboxamido-adenosine HCl (CGS 21680), N^6-cyclopentyl-adenosine (CPA), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 2-hydroxysaclofen, reactive blue 2 (Research Biochemicals, Biotrend, Köln, Germany); yohimbine HCl (Roth, Karlsruhe, Germany); adenosine-5'-O-(2-thiodiphosphate) lithium salt (ADPβS), adenosine-5'-O-(3-thiotriphosphate) lithium salt (ATPγS), ATP disodium salt, (-)-bicuculline methiodide, cibacron blue 3GA, desipramine HCl, α,β-methylene-adenosine-5'-diphosphate disodium salt (α,β-methylene-ADP), tetrodotoxin (Sigma). Cibacron blue 3GA and reactive blue 2 are isomers; the sulphonic acid residue at the terminal benzene ring in cibacron blue 3GA is in the *o*-position, whereas reactive blue 2 is a mixture with the sulphonic residue either in the *m*- or *p*-position. Solutions of drugs were prepared with either distilled water, or (2-hydroxysaclofen) the KH_2PO_4- and $NaHCO_3$- containing stock solution of the medium, or (DPCPX) dimethyl sulphoxide (final concentration 58 µM), or (CPA) ethanol (final concentration 3.4 mM), or (tetrodotoxin) sodium acetate buffer (0.1 M; pH 4.8).

Means ± SEM are given throughout. Differences between means were tested for significance by the Mann-Whitney test. $P < 0.05$ was taken as the criterion of statistical significance. For multiple comparisons, *P* levels were adjusted according to Bonferroni. *n* is the number of brain slices (except in Fig. 7).

Results

Tritium overflow: general

Electrical stimulation by 4 pulses/100 Hz greatly increased the outflow of tritium (Fig. 1). The overflow elicited by S_1 averaged 1.22 ± 0.02 % of the tritium content of the tissue (corresponding to 43.8 ± 1.3 Bq; Table 1) and the fractional rate of outflow immediately before S_1

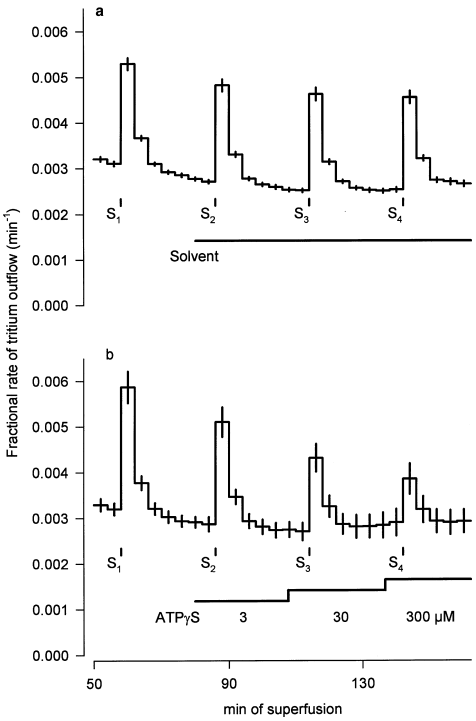

Fig. 1a, b Tritium outflow from slices of rat hippocampus and effect of ATPγS. After preincubation with [^{3}H]-noradrenaline, slices were superfused with medium containing desipramine (1 μM). They were stimulated four times by 4 pulses/100 Hz (S_1 to S_4). The superfusate was collected in 4-min samples. Solvent (**a**; n = 25) or ATPγS (**b**; n = 11) was added as indicated

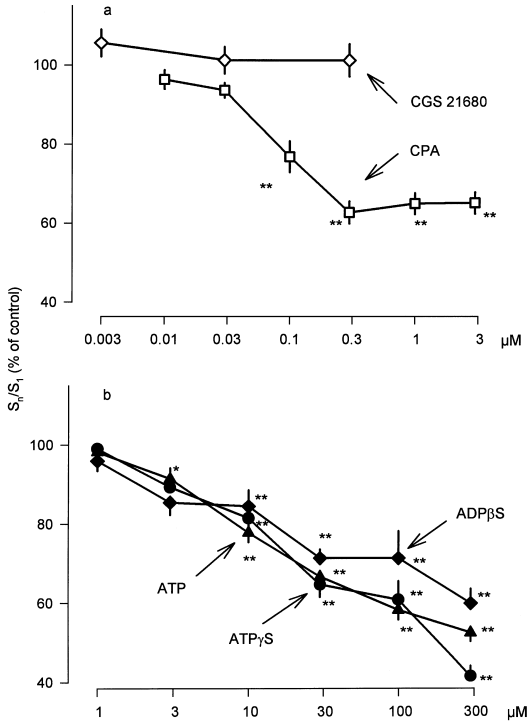

Fig. 2 Effects of adenine nucleosides **a** and nucleotides **b** on electrically evoked overflow of tritium. Slices were stimulated four times by 4 pulses/100 Hz (S_1 to S_4). CGS 21680, CPA, ATP, ATPγS or ADPβS was added at increasing concentrations from 6 min before to 22 min after S_2, S_3 and S_4. *Ordinates*, evoked tritium overflow: S_n/S_1 ratios obtained in individual tissue slices were calculated as a percentage of the corresponding average control S_n/S_1 ratio. Means ± SEM from 5-32 slices. Significant differences from control: $^*P < 0.05$ and $^{**}P < 0.01$

Table 1 Electrically evoked tritium overflow (S_1)

Drugs present throughout superfusion	S_1 (% of tissue tritium)	n
-	1.22 ± 0.02	139
DPCPX (3 nM)	1.27 ± 0.04	43
Cibacron blue 3GA (30 μM)	1.25 ± 0.05	61
DPCPX (3 nM) + cibacron blue 3GA (30 μM)	1.24 ± 0.05	32
Reactive blue 2 (30 μM)	1.51 ± 0.08**	35
Suramin (300 μM)	1.16 ± 0.03	36
α, β-Methylene-ADP (100 μM)	1.11 ± 0.08	12
2-Hydroxysaclofen (50 μM) + bicucullin (50 μM)	1.10 ± 0.05	25

After preincubation with [^{3}H]-noradrenaline, slices were superfused with medium containing the drugs indicated (in addition to desipramine 1 μM). S_1 was applied after 58 min of superfusion and consisted of 4 pulses/100 Hz. Means ± SEM from n slices. Significant differences from experiments, shown in first line, in which only desipramine was present: $^{**}P < 0.01$

(b_1) was 0.00319 ± 0.00003 min^{-1} (corresponding to 11.6 ± 0.4 Bq min^{-1}; n = 139).

When solvent was administered after S_1 (6 min before S_2), the S_2/S_1, S_3/S_1, and S_4/S_1 ratios were 0.96 ± 0.02, 0.95 ± 0.02 and 0.90 ± 0.02, and b_2/b_1, b_3/b_1, and b_4/b_1 ratios were 0.87 ± 0.01, 0.82 ± 0.01 and 0.82 ± 0.02, respectively (n = 25; Fig. 1a). Average S_n/S_1 ratios were also close to unity and b_n/b_1 ratios slightly below unity when the antagonists listed in Table 1 were present in the medium throughout superfusion and solvent was added after S_1.

Tetrodotoxin, when added after S_1 and then kept at a constant concentration (0.3 μM), abolished the evoked overflow of tritium (S_2 to S_4) whithout changing the basal outflow (n = 5).

Tritium overflow: effects of nucleosides and nucleotides

Adenine nucleosides and nucleotides were added after S_1 at increasing concentrations (e.g. Fig. 1b). They did not change the basal efflux of tritium (b_n/b_1) except for a slight (15 %) decrease by ATP (100 μM; not shown).

The selective adenosine A_1-receptor agonist CPA (Williams et al. 1986) reduced the evoked overflow of tritium, whereas the A_{2a}-receptor agonist CGS 21680 (Jarvis et al. 1989; 0.003 to 0.3 μM) caused no change (Fig. 2a). The EC_{50} value for CPA was 69 nM and the maximal inhibition 36 % (sigmoid curve fitting).

ATP decreased the evoked overflow of tritium with an EC_{50} value of 13 µM and a maximal inhibition by 48 % (Fig. 2b; sigmoid curve fitting). Two metabolically more stable nucleotides, ATPγS and ADPβS, also reduced the evoked overflow (Fig. 2b; ATPγS also in Fig. 1b). Their concentration-inhibition curves were similar to that of ATP but did not level off at high concentrations (Fig. 2b); it was not possible, therefore, to calculate EC_{50} and maximal inhibition values for these agonists by fitting sigmoid curves.

Tritium overflow: interactions

Drugs tested for their interaction with nucleosides and nucleotides were added throughout superfusion. Cibacron blue 3GA (30 µM) and reactive blue 2 (30 µM) increased the basal efflux of tritium by 45 and 20 %, respectively (b_1). Reactive blue 2 (30 µM) also increased the overflow evoked by S_1 (Table 1). The other drugs caused no change of b_1 (not shown) and S_1 (Table 1).

The adenosine A_1-receptor antagonist DPCPX (3 nM; Bruns et al. 1986; Lohse et al. 1987) shifted the concentration-inhibition curves of CPA, ATP and ATPγS by similar degrees to the right (Fig. 3). The shifts yielded apparent pK_B values of DPCPX against CPA and ATP of 9.0 and 8.7, respectively. (The shift of the ATPγS curve was not quantified because a sigmoid could not be fitted; see above.)

In contrast to DPCPX, the P2-antagonist cibacron blue 3GA (30 µM; see von Kügelgen et al. 1994a), which in some tissues preferentially blocks P2Y-receptors (Shirahase et al. 1991), shifted only the concentration-response curves of ATP and ATPγS to the right but not the curve of CPA (Fig. 4). The apparent pK_B value of cibacron blue 3GA against ATP was 5.2.

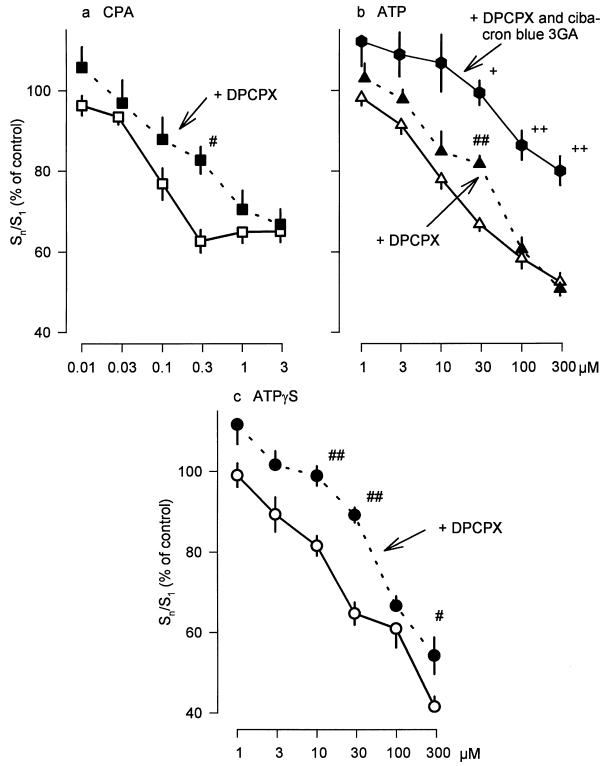

Fig. 3a-c Interaction of CPA, ATP and ATPγS with DPCPX or with cibacron blue 3GA + DPCPX. Slices were stimulated four times by 4 pulses/100 Hz (S_1 to S_4). CPA **a**, ATP **b** or ATPγS **c** was added at increasing concentrations from 6 min before to 22 min after S_2, S_3 and S_4. *Open symbols* represent experiments in which CPA, ATP or ATPγS was given alone (from Fig. 2). *Solid symbols* and *interrupted lines* represent experiments in which the medium contained DPCPX (3 nM) throughout superfusion. *Solid hexagons* and *uninterrupted lines* represent experiments in which the medium contained both cibacron blue 3GA (30 µM) and DPCPX (3 nM) throughout superfusion **b**. *Ordinates*, evoked tritium overflow: S_n/S_1 ratios obtained in individual tissue slices were calculated as a percentage of the corresponding average control S_n/S_1 ratio. Means ± SEM from 5-32 slices. Significant differences from experiments with CPA, ATP or ATPγS alone: $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$. Significant differences from experiments in which the medium contained only one antagonist: $^{+}P < 0.05$ and $^{++}P < 0.01$

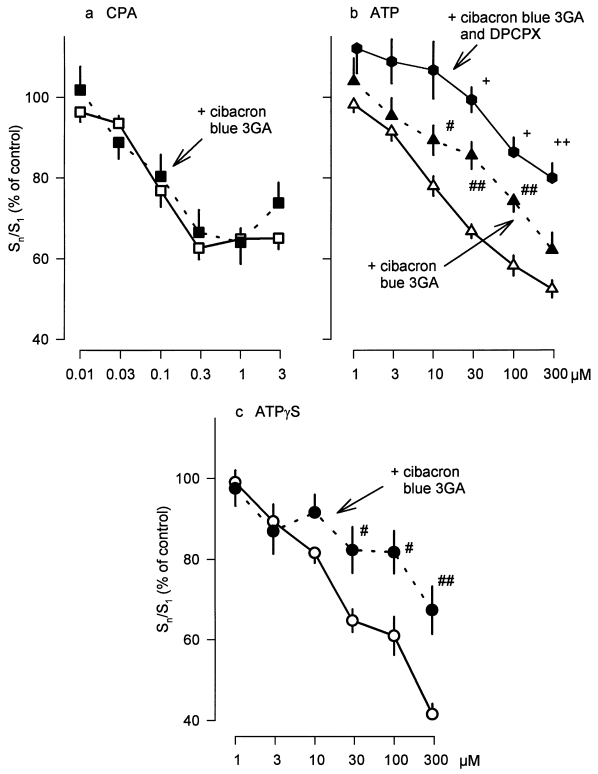

Fig. 4a-c Interaction of CPA, ATP and ATPγS with cibacron blue 3GA or with DPCPX + cibacron blue 3GA. *Open symbols* represent experiments in which CPA **a**, ATP **b** or ATPγS **c** was given alone (from Fig. 2). *Solid symbols* and *interrupted lines* represent experiments in which the medium contained cibacron blue 3GA (30 µM) throughout superfusion. *Solid hexagons* and *uninterrupted lines* represent experiments in which the medium contained both cibacron blue 3GA (30 µM) and DPCPX (3 nM) throughout superfusion (**b**; from Fig. 3). Means ± SEM from 4-32 slices. For other details see Fig. 3

Cibacron blue 3GA (30 µM) combined with DPCPX (3 nM) shifted the concentration-response curve of ATP beyond the antagonism caused by DPCPX alone (Fig. 3b) and cibacron blue 3GA alone (Fig. 4b). The shift beyond that produced by DPCPX alone (Fig. 3b) yielded an apparent pK_B of cibacron blue 3GA against ATP of 5.5, close to the 5.2 obtained in the absence of DPCPX.

Like cibacron blue 3GA, its isomer reactive blue 2 is P2Y-selective in some tissues (Burnstock and Warland 1987; Kennedy 1990). It behaved much like cibacron blue 3GA: at a concentration of 30 µM, it shifted the concentration-inhibition curves of ATP and ATPγS to the right but not the curve of CPA (Fig. 5). The apparent pK_B value of reactive blue 2 against ATP was 5.6.

In contrast to cibacron blue 3GA and reactive blue 2, suramin (300 µM), a non-subtype-selective P2-antagonist (Dunn and Blakeley 1988), failed to change the effects of ATP and ATPγS (Fig. 6a and b).

The blocker of 5'-nucleotidase α,β-methylene-ADP (Burger and Lowenstein 1970), administered at a concentration (100 µM) which inhibits the degradation of AMP to adenosine in rat hippocampal slices by about 90% (Cunha et al. 1992), had no effect on the concentration-inhibition curve of ATP (Fig. 6c).

In order to examine whether GABA was involved in the effect of ATP, some experiments were performed in the combined presence of the $GABA_A$ receptor antagonist bicuculline (50 µM) and the $GABA_B$ receptor antagonist 2-hydroxysaclofen (50 µM; Kerr et al. 1988) from the beginning of superfusion. The combination failed to attenuate the effect of ATP (1-300 µM; $n = 12$).

Tritium overflow: effects of P1- and P2-antagonists and yohimbine

DPCPX (3 nM), cibacron blue 3GA (30 µM) and suramin (300 µM) had not changed the evoked overflow of tritium (S_1) when added throughout superfusion, while reactive blue 2 (30 µM) had caused an increase (see above; Table 1). However, drug effects on the evoked overflow of tritium are better assessed when the drugs are given after S_1 so that S_1 is a reference value for each slice. In the following experiments the drugs to be tested were kept at a constant concentration from 6 min before S_2 onwards. Since their effect, if any, was similar at S_2, S_3 and S_4, responses to S_2 to S_4 were averaged (Table 2).

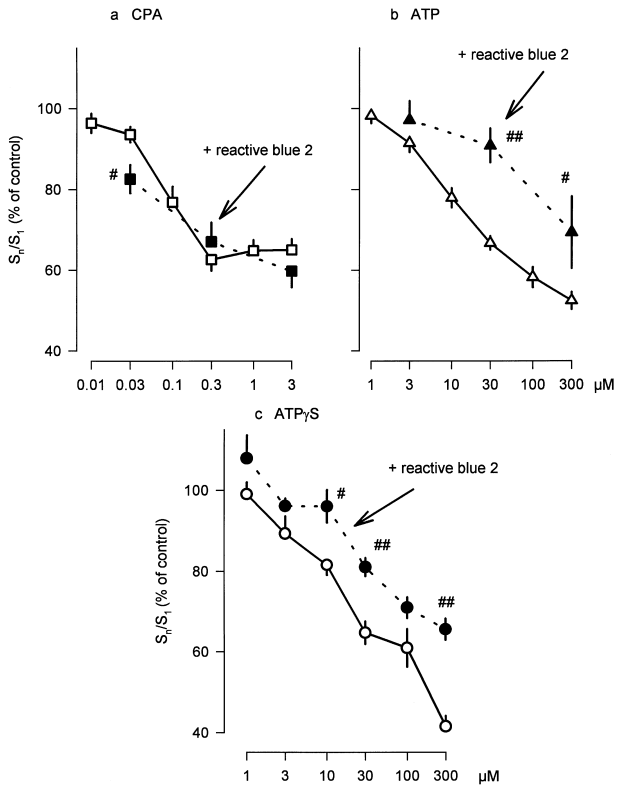

Fig. 5a-d Interaction of CPA, ATP and ATPγS with reactive blue 2. *Open symbols* represent experiments in which CPA **a**, ATP **b** or ATPγS **c** was given alone (from Fig. 2). *Solid symbols* represent experiments in which the medium contained reactive blue 2 (30 µM) throughout superfusion. Means ± SEM from 4-32 slices. For other details see Fig. 3

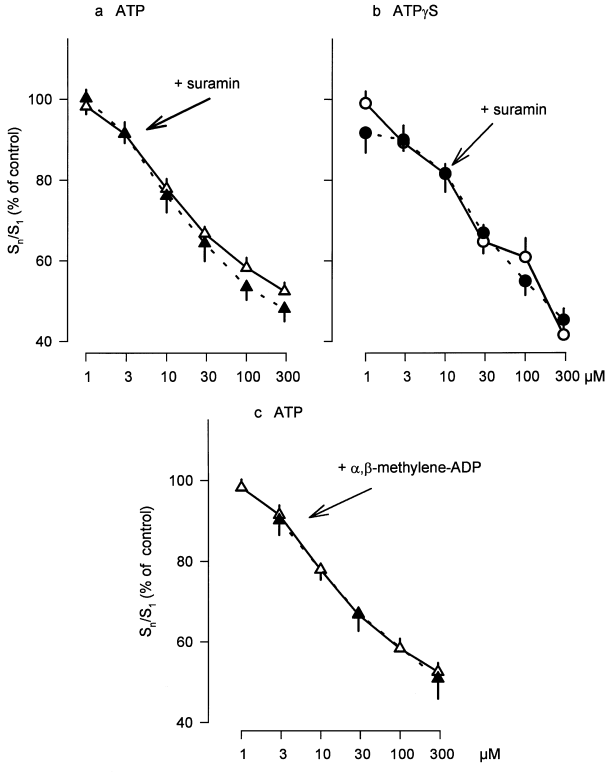

Fig. 6a, b Interaction of ATP and ATPγS with suramin, and interaction of ATP with α,β-methylene-ADP. *Open symbols* represent experiments in which ATP **a**, **c** or ATPγS **b** was given alone (from Fig. 2). *Solid symbols* represent experiments in which the medium contained either suramin (300 µM; **a**, **b**) or α,β-methylene-ADP (100 µM; **c**) throughout superfusion. Means ± SEM from 5-32 slices. For other details see Fig. 3

Table 2 Effects of P1- and P2-antagonists and yohimbine on electrically evoked tritium overflow

Drugs added 6 min before S_2	Evoked tritium overflow (average overflow at S_2 to S_4/ overflow at S_1; % of control)		
	4 pulses/100 Hz	30 pulses/1 Hz	30 pulses/1 Hz; yohimbine (1 μM) throughout superfusion
	100.0 ± 2.7 (10)	100.0 ± 2.2 (11)	100.0 ± 2.0 (8)
DPCPX (3 nM)	104.6 ± 3.3 (6)	99.1 ± 4.5 (4)	-
Reactive blue 2 (30 μM)	106.4 ± 7.0 (5)	105.7 ± 3.3 (12)	121.4 ± 3.7 (7)**
Cibacron blue 3GA (30 μM)	124.3 ± 4.0 (8)**	131.7 ± 4.0 (10)**	117.6 ± 5.1 (6)*
Yohimbine (1 μM)	108.4 ± 2.2 (4)	249.6 ± 4.0 (6)**	-

After preincubation with [^{3}H]-noradrenaline, slices were superfused with medium containing desipramine (1 μM) and, where indicated in heading, yohimbine (1 μM). They were stimulated four times (S_1 to S_4) at pulse numbers and frequencies indicated. DPCPX, reactive blue 2, cibacron blue 3GA or yohimbine was added 6 min before S_2 for the remainder of the experiment. Ratios "average overflow at S_2 to S_4/overflow at S_1" obtained in individual slices were calculated as a percentage of the corresponding average control ratio. Means ± SEM from (*n*) slices. Significant differences from corresponding control (first line): *$P < 0.05$ and **$P < 0.01$

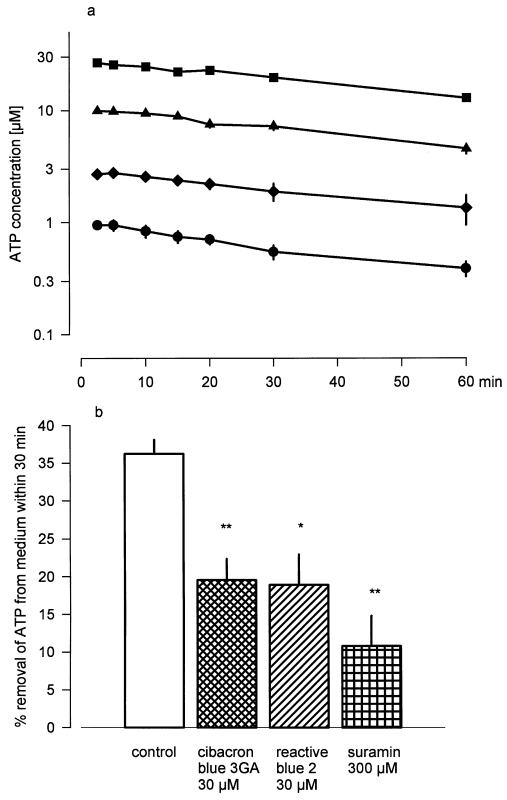

Fig. 7a, b Removal of ATP from the medium by hippocampal slices and effects of cibacron blue 3GA (30 μM), reactive blue 2 (30 μM) and suramin (300 μM). **a** shows the time course of the decline in ATP. ATP was added at an initial concentration of 1 (●), 3 (◆), 10 (▲) or 30 (■) μM and incubated with the slices for 60 min. **b** shows effects of P2-antagonists on the removal of ATP, initial concentration 30 μM, within 30 min. Means ± SEM from 9-13 observations. Significant differences from control: *$P < 0.05$ and **$P < 0.01$

DPCPX (3 nM), when administered in this manner, did not change the evoked overflow of tritium, irrespective of whether slices were stimulated by the usual 4 pulses/100 Hz trains or by 30 pulses/1 Hz. Reactive blue 2 (30 μM) also produced no change (yohimbine absent). Cibacron blue 3GA (30 μM) caused slight and similar increases at 4 pulses/100 Hz and 30 pulses/1 Hz (yohimbine absent; Table 2).

Yohimbine (1 μM) failed to increase the evoked overflow of tritium when slices were stimulated with 4 pulses/100 Hz, but markedly (by 150 %) increased it when the slices were stimulated with 30 pulses/1 Hz. When the medium contained yohimbine (1 μM) throughout superfusion, both reactive blue 2 (30 μM) and cibacron blue 3GA (30 μM) slightly increased the evoked overflow (30 pulses/1 Hz; Table 2).

Removal of ATP from the medium

When ATP (1, 3, 10 or 30 μM) was added to medium without tissue, its concentration remained constant for 60 min. In medium containing slices without addition of exogenous ATP, the concentration of ATP never exceeded 10 nM. Hippocampal slices removed added ATP from the medium, presumably by enzymatic degradation (Fig. 7a). At an initial ATP concentration of 30 μM, 36 % were degraded within the first 30 min (Fig. 7). Cibacron blue 3GA (30 μM), reactive blue 2 (30 μM), and to a non-significantly greater extent suramin (300 μM), inhibited the removal of ATP (Fig. 7b).

Discussion

As shown by its sensitivity to tetrodotoxin, the electrically evoked overflow of tritium reflected an action potential-induced, neuronal release of [^{3}H]-noradrenaline. Yohimbine increased the overflow elicited by 30 pulses/

1 Hz but not by 4 pulses/100 Hz indicating that α_2-autoinhibition operated during the long but not the short pulse train, as expected (Starke 1987; Singer 1988; Starke et al. 1989). The medium contained desipramine throughout superfusion, hence it is unlikely that any drug effect on the evoked overflow of tritium was due to interference with uptake$_1$.

Presynaptic adenosine receptors

Our experiments confirm the occurrence of release-inhibiting A_1-adenosine receptors at the noradrenergic axon terminals of the hippocampus (Jonzon and Fredholm 1984): the A_1-adenosine receptor agonist CPA decreased the evoked overflow of tritium, and DPCPX shifted the concentration-response curve of CPA to the right with an apparent pK_B value (9.0) similar to values found in other rat tissues (e.g. 9.3; Sebastiao et al. 1990).

The A_1-receptor mediated also part of the effects of ATP and ATPγS: their concentration-response curves were shifted to the right by DPCPX to an extent similar to the shift of the CPA curve (apparent pK_B value of DPCPX against ATP: 8.7). Blockade of 5'-nucleotidase by α,β-methylene-ADP did not change the effect of ATP indicating that ATP acted as such and not after conversion to adenosine. Evidence for direct activation of presynaptic A_1-receptors by ATP has also been obtained in other brain regions and in peripheral tissues (von Kügelgen et al. 1992, 1994a, 1994b; Fuder and Muth 1993; Kurz et al. 1993).

The A_{2a}-agonist CGS 21680, tested at concentrations which enhance noradrenaline release in other tissues (e.g. rat vas deferens: Gonçalves and Queiroz 1993; rat tail artery: Gonçalves and Queiroz 1996) and also the release of acetylcholine in the rat hippocampus (Cunha et al. 1994a), was without any effect in the present experiments. The apparent absence of A_{2a}-receptors agrees with observations on noradrenaline release in the rat occipito-parietal cortex (von Kügelgen et al. 1994b).

Presynaptic P2-receptors

In addition to activating the A_1-adenosine receptor, the nucleotides ATP and ATPγS, but not the nucleoside CPA, acted via a second receptor – a P2-receptor. In support of this view, the P2-antagonists cibacron blue 3GA and reactive blue 2 shifted the concentration-response curves of ATP and ATPγS to the right but not the curve of CPA. The apparent pK_B value of cibacron blue 3GA against ATP, 5.2, was close to values found at the presynaptic P2-receptors in rat atria (5.0; von Kügelgen et al. 1995), rat iris (4.7; Fuder and Muth 1993) and rat occipito-parietal cortex (5.0; von Kügelgen et al. 1994b); the apparent pK_B value of reactive blue 2 against ATP, 5.6, was close to the pK_B at the presynaptic P2-receptors in mouse vas deferens (5.3; von Kügelgen et al. 1994a). Cibacron blue 3GA produced a similar shift when tested against ATP in combination with DPCPX (beyond the shift caused by DPCPX alone; apparent pK_B 5.5), as predicted from theory for an agonist acting via two receptors.

In apparent contrast to a presynaptic P2 effect, the P2-antagonist suramin failed to attenuate the effects of ATP and ATPγS. However, no or very little antagonism of suramin against ATP and ATP analogues has also been found at the noradrenergic axons of the rat occipito-parietal cortex (von Kügelgen et al. 1994b), the rat vas deferens (Kurz et al. 1993) and the rat iris (Fuder and Muth 1993). Two explanations seem possible. First, suramin may possess low affinity for presynaptic P2-receptors in the rat. The second explanation is based on the degradation of some nucleotides such as ATP by ecto-nucleotidases. If a P2-antagonist slows down this degradation (Smolen and Weissmann 1978; Hourani and Chown 1989), the ensuing increase in nucleotide concentration will counteract the P2-receptor blockade. Depending on the antagonist's relative ecto-nucleotidase-inhibiting and P2 blocking potencies, the nucleotide effect may then (1) still be antagonized, or (2) remain unchanged, or (3) be even enhanced (see Crack et al. 1994; Bültmann et al. 1995). Possibility (1) seems to hold true for cibacron blue 3GA and reactive blue 2 which, although inhibiting the degradation of ATP during incubation with hippocampal slices (Fig. 7b), attenuated the presynaptic inhibitory effect of ATP (Fig. 4b and 5b). Possibility (2) may hold true for suramin, which inhibited the degradation of ATP to a slightly greater extent than cibacron blue 3GA and reactive blue 2 (Fig. 7b). Whether ATPγS is also broken down by rat hippocampal ecto-nucleotidases and the alternative explanation can be extended to the lack of effect of suramin against ATPγS remains open.

The evidence for presynaptic P2-receptors in the brain now extends to the noradrenergic (von Kügelgen et al. 1994b) and serotonergic (Koch et al. 1995) axons of the rat occipito-parietal cortex and the glutamatergic (Motin and Bennett 1995) and noradrenergic (present study) axons of the hippocampus. Given the P2Y selectivity of reactive blue 2 and cibacron blue 3GA in some tissues (Burnstock and Warland 1987; Kennedy 1990; Shirahase et al. 1991), the presynaptic P2-receptors in the rat hippocampus seem to belong to the P2Y group, like the receptors at other noradrenergic axons (von Kügelgen et al. 1989, 1994a, 1994b, 1995; Fuder and Muth 1993; Gonçalves and Queiroz 1996). No further classification is possible at this time.

It should be noted that we call the P2-receptors "presynaptic" in accord with the original functional definition of the term: modulating transmitter release (Starke and Langer 1979). That the receptors are anatomically presynaptic is not definitely proven. One indirect mode of presynaptic inhibition, by way of GABA, is excluded by the lack of an antagonist effect of bicuculline and 2-hydroxysaclofen against ATP. In the rat occipito-parietal cortex prostaglandins and nitric oxide also were excluded as mediators of the inhibitory effect of nucleotides (von Kügelgen et al. 1994b). In contrast to the ef-

fect of ATP itself on the release of noradrenaline (present study), an inhibition by ATP of the release of acetylcholine in rat hippocampal synaptosomes was shown to be mediated exclusively by breakdown to adenosine and subsequent activation of presynaptic A_1-adenosine receptors (Cunha et al. 1994b).

Endogenous input?

In peripheral sympathetically innervated tissues, P2-receptor antagonists, given alone, increase the release of noradrenaline (by up to about 100 %), indicating that the presynaptic P2-receptors are normally activated by an endogenous ligand, presumably ATP (e.g. rat iris: Fuder and Muth 1993; mouse and rat vas deferens: von Kügelgen et al. 1993, 1994a). In contrast, no increase by antagonists was obtained in the rat occipito-parietal cortex (von Kügelgen et al. 1994b). In the present study, cibacron blue 3GA consistently enhanced the release of noradrenaline (Table 2). However, the increase was small and contrasted with the lack of effect of reactive blue 2 (except in the presence of yohimbine; Table 2). Also, the effect of cibacron blue 3GA was quantitatively similar at 4 pulses/100 Hz and 30 pulses/1 Hz, in marked contrast to the effect of yohimbine which obeyed the well-established rules of presynaptic α_2-autoinhibition, as mentioned above. An autoreceptor function of the presynaptic P2-receptors at the noradrenergic axons of the rat hippocampus, hence, remains doubtful. The lack of any increase in noradrenaline release by DPCPX also excludes activation of the presynaptic A_1-adenosine receptors by an endogenous ligand under the conditions of these experiments.

Conclusion

The noradrenergic terminal axons of the rat hippocampus possess A_1-adenosine receptors as well as P2-receptors, both of which, when activated, decrease the release of noradrenaline. The presynaptic A_1-adenosine receptors are activated by nucleosides and some nucleotides such as ATP and are blocked by DPCPX. The presynaptic P2-receptors are activated by nucleotides but not by nucleosides and are blocked by cibacron blue 3GA and reactive blue 2. ATP is degraded in the rat hippocampus and this degradation is attenuated by cibacron blue 3GA, reactive blue 2 and suramin. In contrast to peripheral tissues where ATP, coreleased with noradrenaline, inhibits further release through activation of presynaptic P2-autoreceptors, an autoreceptor function of presynaptic P2-receptors in the brain remains doubtful.

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