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SPECIAL REPORT

UTP- and ATP-triggered transmitter release from rat sympathetic neurones via separate receptors

[1]Stefan Boehm, Sigismund Huck & *Peter Illes

Institute of Neuropharmacology, University of Vienna, Waehringerstrasse 13a, A-1090 Vienna, Austria and *Institute of Pharmacology and Toxicology, University of Leipzig, Haertelstrasse 16-18, D-04107 Leipzig, Germany

In rat cultured sympathetic neurones, UDP, UTP and ATP at micromolar concentrations triggered Ca^{2+}-dependent and tetrodotoxin-sensitive [^{3}H]-noradrenaline release. The overflow evoked by UTP or ATP was similar at 100 μmol l^{-1}, the concentration used in all subsequent experiments. Pre-exposure of the neurones to 100 μmol l^{-1} UTP significantly reduced ensuing secretory effects of UTP but not of ATP. Conversely, pre-exposure to ATP diminished the overflow due to ATP but not that due to UTP. In the presence of 10 μmol l^{-1} pyridoxal-5'-phosphate or 30 μmol l^{-1} suramin, the secretory response to ATP was reduced, but the effect of UTP was unaltered. Zn^{2+} (10 μmol l^{-1}) reduced the overflow triggered by UTP, but increased the overflow due to ATP. These results indicate the presence of separate receptors for pyrimidine nucleotides and for purine nucleotides which both trigger transmitter release.

Keywords: ATP; UTP; P_2 purinoceptor; pyrimidinoceptor; rat sympathetic neurone; noradrenaline release

Introduction One of the subtypes of P_2 purinoceptors is the P_{2u} receptor which is activated by extracellular ATP and UTP (Fredholm *et al.*, 1994). However, the existence of a receptor activated by UTP, but not by ATP has also been postulated (for review see, Seifert & Schultz, 1989). Evidence for such a pyrimidinoceptor has lately been obtained in a glioma cell line (Lazarowski & Harden, 1994), but still awaits experimental confirmation in cultured neurones. Recent electrophysiological data suggested the presence of distinct receptors for ATP and UTP in rat superior cervical ganglia (see e.g. Connolly, 1995).

In primary cultures of rat sympathetic neurones, ATP evokes noradrenaline release by activation of P_2 purinoceptors (Boehm, 1994). The present study investigates the effects of pyrimidine nucleotides in these cultures and presents evidence for separate receptors for ATP and UTP which both trigger transmitter release.

Methods Superior cervical ganglia were dissected from 2 to 6 day old Sprague Dawley rat pups, dissociated, and plated onto 5 mm polystyrol discs coated with collagen as described by Boehm (1994). After 7 days *in vitro*, culture discs were incubated in 0.03 μmol l^{-1} [^{3}H]-noradrenaline in culture medium with 1 mmol l^{-1} ascorbic acid for 60 min and subsequently superfused at a rate of about 1.0 ml min^{-1} with a buffer containing (mmol l^{-1}): NaCl 117, KCl 6.0, $CaCl_2$ 2.0, $MgCl_2$ 2.0, glucose 20, HEPES 10, fumaric acid 0.5, Na-pyruvate 5.0, ascorbic acid 0.57, adjusted to pH 7.4 with NaOH; the buffer was kept at 25°C. After 60 min of washout, collection of 4 min superfusate fractions was started. Cultures were stimulated by 60 s exposures to UTP after 72 min of superfusion (S_{UTP}) and to ATP after 92 min (S_{ATP}), at concentrations of 0.1 μmol l^{-1} to 1 mmol l^{-1}. In a separate series of experiments, neurones were first exposed to UDP (after 72 min) and then to UTP (after 92 min) at 10 μmol l^{-1} to 1 mmol l^{-1}. When desensitization was investigated, cultures were pre-exposed to either 100μmol l^{-1} UTP or ATP from min 72 to 80, and then overflow was triggered by 60 s exposure to 100μmol l^{-1} UTP or ATP after 92 min of superfusion. Suramin (30μmol l^{-1}), pyridoxal-5'-phosphate (10μmol l^{-1}), $ZnCl_2$ (10μmol l^{-1}), or tetrodotoxin (1μmol l^{-1}) were added to, or extracellular Ca^{2+} was removed from, the medium after 52 min of superfusion. Thereafter, these changes in the composition of the superfusion medium were maintained until the end of experiments. Finally, the radioactivity remaining in the cells was extracted by perchloric acid and sonication, and the radioactivity in extracts and collected fractions was determined by liquid scintillation counting.

The fractional rate of ^{3}H outflow was obtained by dividing the radioactivity of a 4 min sample by the total radioactivity of cultures at the beginning of the corresponding 4 min collection period. The rate of outflow per minute was obtained by a subsequent division by 4. Stimulation-evoked overflow was calculated as the difference between the total ^{3}H outflow in the fractions during and immediately after stimulation and the estimated basal outflow, which was assumed to decline linearly from the sample preceding stimulation to the sample 8-12 min after the beginning of the stimulus. This difference was expressed as a percentage of the total radioactivity in the cultures at the beginning of the stimulation (S_{UDP}%, S_{UTP}%, S_{ATP}%). All data represent arithmetic means ± s.e.mean. Significance of differences was evaluated by the Mann-Whitney-test.

(−)-[Ring-2,5,6-^{3}H)-noradrenaline (56.9 Ci $mmol^{-1}$) was obtained from NEN, Dreieich, Germany; ATP-Na_2, UDP-Na, UTP-Na_3, tetrodotoxin, pyridoxal-5'-phosphate were from Sigma, München, Germany; suramin hexasodium salt was a gift from Bayer, Austria.

Results Basal ^{3}H efflux measured after 68 min of superfusion amounted to 0.0024 ± 0.0001 min^{-1}, corresponding to 0.0486 ± 0.0021 nCi (n = 130). This rate of outflow was not altered in the presence of 30 μmol l^{-1} suramin, 10 μmol l^{-1} pyridoxal-5'-phosphate, 1 μmol l^{-1} tetrodotoxin or in the absence of extracellular Ca^{2+} (not shown), but slightly increased by 10 μmol l^{-1} Zn^{2+} (0.0028 ± 0.0002 min^{-1}, n = 9; P < 0.05). Exposure to UDP, UTP or ATP for 60 s caused concentration-dependent increases in ^{3}H outflow: UTP was about equipotent to UDP and apparently more potent than ATP (Figure 1a and b). UTP and ATP evoked similar overflow (S_{UTP}% 2.311 ± 0.132; S_{ATP}% 2.089 ± 0.124; n = 20; P > 0.05) at a concentration of 100 μmol l^{-1}, which was used in all subsequent experiments.

Pre-exposure to 100 μmol l^{-1} UTP for 8 min markedly reduced subsequent UTP-, but not ATP-evoked overflow. By contrast, 8 min pre-exposure to 100 μmol l^{-1} ATP reduced ATP- but not UTP-evoked overflow (Figure 1c). In the absence of extracellular Ca^{2+} (n = 6) and in the presence of 1 μmol l^{-1} tetrodotoxin (n = 6), either nucleotide failed to induce ^{3}H overflow. Suramin (30 μmol l^{-1}) diminished ATP-

[1] Author for correspondence.

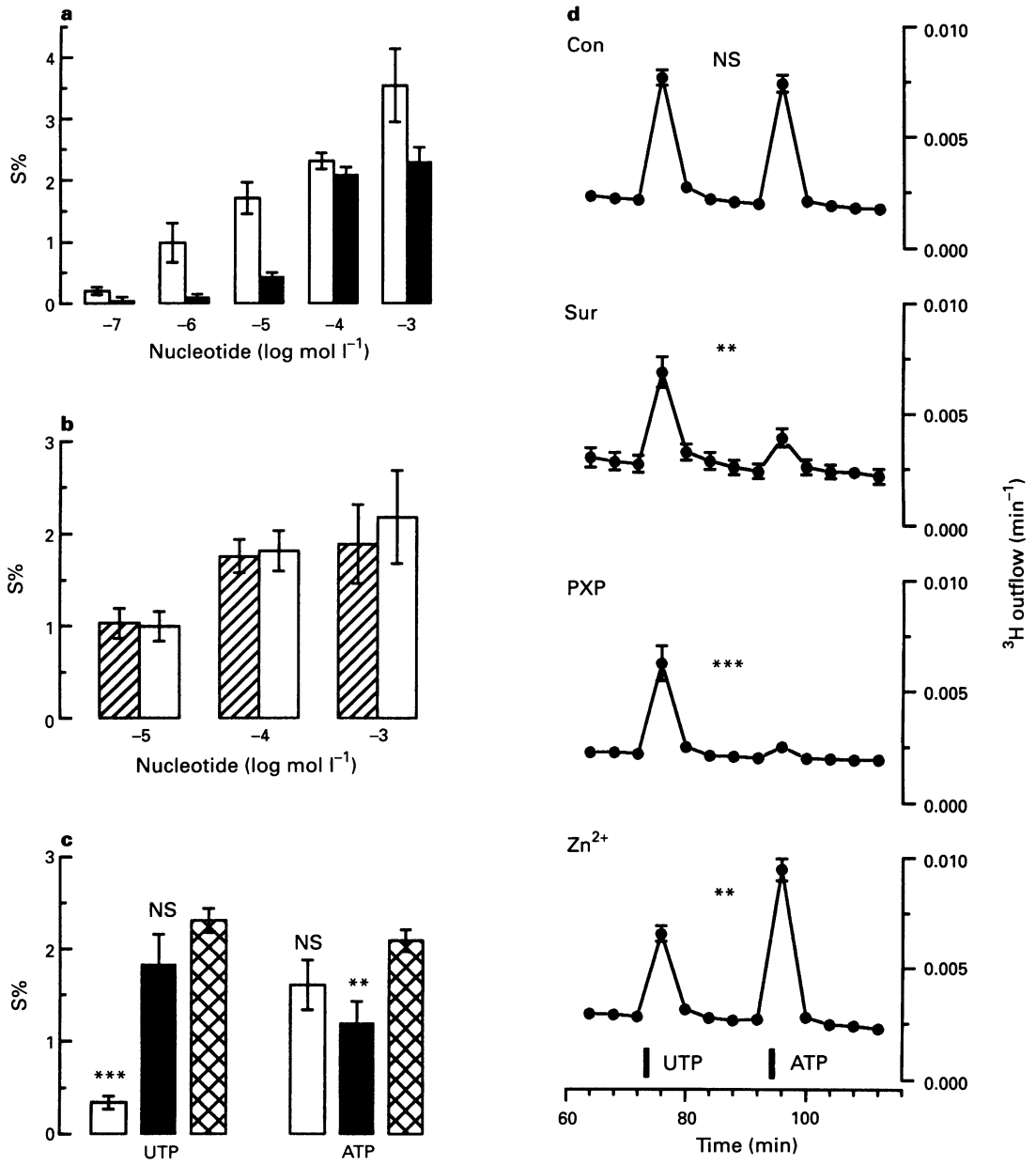

Figure 1 Tritium overflow evoked by UDP, UTP and ATP from rat sympathetic neurones labelled with [^{3}H]-noradrenaline. After labelling, cell cultures were superfused and 4 min fractions of superfusate were collected subsequent to a 60 min washout period. (a) UTP (open columns) and ATP (solid columns), at the concentrations indicated, were included in the medium for 60 s after 72 min (UTP) and 92 min (ATP) of superfusion, respectively. Overflow is presented as % of the total radioactivity in the cultures at the beginning of exposure to nucleotides (S%). $n=6$, with the exception of 100 μmol l^{-1} ($n=20$). (b) UDP (hatched columns) and UTP (open columns), at the concentrations indicated, were included in the medium for 60 s after 72 min (UDP) and 92 min (UTP) of superfusion, respectively. Results are presented as % of the total radioactivity (S%). $n=5-6$. (c) Overflow evoked by 100 μmol l^{-1} of UTP (left-hand 3 columns) or ATP (right-hand 3 columns) present for 60 s (after 92 min of superfusion) subsequent to pre-exposure to the same concentration of UTP (open columns) or ATP (solid columns) from min 72 to 80. Results are shown as % of the total radioactivity (S%), $n=8$ to 9. **$P<0.01$, ***$P<0.001$ vs. the corresponding control values (hatched columns; taken from a, $n=20$); NS no significant difference. (d) ^{3}H outflow and UTP- as well as ATP-evoked overflow under control conditions (Con) or in the presence of 30 μmol l^{-1} suramin (Sur), 10 μmol l^{-1} pyridoxal-5'-phosphate (PXP), or 10 μmol l^{-1} $ZnCl_2$ (Zn^{2+}), added to the medium after 52 min of superfusion. UTP and ATP (100 μmol l^{-1}) were applied for 60 s as indicated by the bars; $n=6$ to 9, and $n=20$ in the case of controls. Significant differences between the overflow evoked by UTP and ATP: **$P<0.01$, ***$P<0.001$, respectively; NS no significant difference.

evoked overflow by approximately 70% (S_{ATP}% 0.675 ± 0.080; $n=6$, $P<0.001$ vs control), but left the overflow due to UTP unchanged ($S_{UTP\%}$%; 1.921 ± 0.275; $n=6$; $P>0.05$). Pyridoxal-5'-phosphate (10 μmol l^{-1}) reduced ATP-induced overflow by about 90% (S_{ATP}% 0.207 ± 0.087; $n=8$; $P<0.001$ vs control) but failed to alter UTP-evoked overflow (S_{UTP}% 1.761 ± 0.350; $n=8$; $P>0.05$). Zn^{2+} (10 μmol l^{-1}) reduced UTP-evoked overflow (S_{UTP}% 1.627 ± 0.220; $n=9$; $P<0.05$ vs control), but increased ATP-induced overflow (S_{ATP}% 2.801 ± 0.247; $n=9$; $P<0.05$ vs control; Figure 1d).

Discussion Both UTP and ATP elicited [^{3}H]-noradrenaline release from rat sympathetic neurones in a Ca^{2+}-dependent and tetrodotoxin-sensitive manner. In numerous cells, UTP and ATP elicit various actions by activating the same receptor, the P_{2u} purinoceptor (Fredholm *et al.*, 1994). In PC12 cells, for example, UTP and ATP mobilise intracellular Ca^{2+} via a P_{2u} purinoceptor, although activation of this receptor fails to induce catecholamine secretion (Barry & Cheek, 1994). In the present study, UTP and ATP triggered transmitter release, but acted upon separate receptors, as shown by the following: (i)

The secretory response to either UTP or ATP showed homologous desensitization, but not cross-desensitization, i.e. overflow evoked by UTP was significantly reduced after pre-exposure to UTP, but not after pre-exposure to ATP, and *vice versa*; (ii) the P_2 purinoceptor antagonist, suramin (Dunn & Blakeley, 1988) and pyridoxal-5'-phosphate (Trezise *et al.*, 1994) reduced the overflow evoked by ATP, but not that evoked by UTP; (iii) Zn^{2+} (10 μmol l^{-1}), which enhances ATP-induced currents (Cloues *et al.*, 1993), increased transmitter release evoked by ATP, but reduced the release evoked by UTP.

ATP evokes noradrenaline release from rat cultured sympathetic neurones by eliciting inward currents through P_{2x} purinoceptors (Boehm, 1994). UTP, by contrast, does not evoke inward currents in these neurones (unpublished observation) and induces transmitter release via a distinct receptor. Since UDP, which activates pyrimidinoceptors but not P_{2u} purinoceptors (Von Kügelgen & Starke, 1990; Lazarowski & Harden 1994), was about as potent as UTP, rat sympathetic neurones obviously possess pyrimidinoceptors triggering noradrenaline release.

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References

BARRY, V.A. & CHEEK, T.R. (1994). Extracellular ATP triggers two functionally distinct calcium signalling pathways in PC12 cells. *J. Cell. Sci.*, **107**, 451–462.

BOEHM, S. (1994). Noradrenaline release from rat sympathetic neurones evoked by P_2-purinoceptor activation. *Naunyn-Schmied. Arch. Pharmacol.*, **350**, 454–458.

CLOUES, R., JONES, S. & BROWN, D.A. (1993). Zn^{2+} potentiates ATP-activated currents in rat sympathetic neurones. *Pflüger's Arch.*, **424**, 152–158.

CONNOLLY, G.P. (1995). Differentiation by pyridoxal-5-phosphate, PPADS and iso-PPADS between responses mediated by UTP and those evoked by α,β-methylene-ATP on rat sympathetic ganglia. *Br. J. Pharmacol.*, **114**, 727–731.

DUNN, P.M. & BLAKELEY, A.G.H. (1988). Suramin: a reversible P_2-purinoceptor antagonist in the mouse vas deferens. *Br. J. Pharmacol.*, **93**, 243–245.

FREDHOLM, B.B., ABBRACHIO, M.P., BURNSTOCK, G., DALY, J.W., HARDEN, T.K., JACOBSON, K.A., LEFF, P. & WILLIAMS, M. (1994). Nomenclature and classification of purinoceptors. *Pharmacol. Rev.*, **46**, 143–156.

LAZAROWSKI, E.R. & HARDEN, T.K. (1994). Identification of a uridine nucleotide-selective G-protein-linked receptor that activates phospholipase C. *J. Biol. Chem.*, **269**, 11830–11836.

SEIFERT, R. & SCHULTZ, G. (1989). Involvement of pyrimidinoceptors in the regulation of cell functions by uridine and by uracil nucleotides. *Trends Pharmacol. Sci.*, **10**, 365–369.

TREZISE, D.J., BELL, N.J., KHAKH, B.S., MICHEL, A.D. & HUMPHREY, P.P.A. (1994). P_2 purinoceptor antagonist properties of pyridoxal-5-phosphate. *Eur. J. Pharmacol.*, **259**, 295–300.

VON KÜGELGEN, I. & STARKE, K. (1990). Evidence for separate vasoconstriction-mediating nucleotide receptors, both distinct from the P_{2x}-receptor, in rabbit basilar artery: a receptor for pyrimidine nucleotides and a receptor for purine nucleotides. *Naunyn-Schmied. Arch. Pharmacol.*, **341**, 538–546.

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