British Journal of Pharmacology (1995) 116, 2764-2770

Structure-activity relationship of a pyrimidine receptor in the rat isolated superior cervical ganglion

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1 The effects of pyrimidines and purines on the d.c. potential of the rat isolated superior cervical ganglion (SCG) have been examined by a grease-gap technique to determine the structure-activity requirements of the receptor activated by pyrimidines, i.e. a pyrimidinoceptor.

2 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranosyl (ZTP), the pyrimidines, cytidine 5'-triphosphate (CTP), uridine 5'-triphosphate (UTP) and thymidine 5'-triphosphate (TTP) and the purines, adenosine 5'-triphosphate (ATP; in the presence of an A_1-purinoceptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (1 μM)), adenosine 5'-O-(3-thiotriphosphate) (ATPγS), guanosine 5'-triphosphate (GTP), inosine 5'-triphosphate (ITP) depolarized ganglia in a concentration-dependent manner. The relative order of ZTP and purine 5'-triphosphates in depolarizing ganglia was ZTP ≥ ATPγS > > ATP ≥ ITP = GTP, and for the pyrimidine 5'-triphosphates UTP > TTP ≥ CTP. Depolarizations evoked by ATPγS were followed by concentration-dependent hyperpolarizations at 100 and 1000 μM.

3 At concentrations of between 0.1 μM and 1 mM, uridine 5'-diphosphate (UDP), uridine 5'-diphosphoglucose (UDPG) and uridine 5'-diphosphoglucuronic acid (UDPGA) evoked significant and concentration-dependent depolarizations, whereas uridine 5'-monophosphate (UMP), uridine and uracil were inactive or produced small (< 45 μV) depolarizations. The relative order of potency of uridine analogues in depolarizing ganglia was UDP ≥ UTP > UDPG > UDPGA > > uracil ≥ UMP = pseudouridine ≥ uridine. At 3 and 10 mM, uridine produced concentration-dependent hyperpolarizations. Nikkomycin Z, a nucleoside resembling UTP (viz. the triphosphate chain at the 5'-position on the ribose moiety being replaced by a peptide), was inactive between 1 μM and 1 mM. Generally, a concentration of 10 mM was required before thymidine, 6-azathymine, 6-azauracil or 6-azauridine depolarized ganglia.

4 Suramin (300 μM), a P_2-purinoceptor antagonist, significantly depressed depolarizations evoked by α,β-methylene-ATP (α,β-MeATP; 100 μM), ATPγS (100 μM), CTP (1 mM), GTP (1 mM), ZTP (30 μM) and ATP (300 μM) in the presence of DPCPX (1 μM). Suramin reversed a small depolarization evoked by UMP (1 mM) into a small hyperpolarization. In contrast depolarizations evoked by UDP, UTP, UDPG (all at 100 μM) and TTP (300 μM) were unaltered or enhanced by suramin.

5 It is concluded that the rat SCG contains distinct nucleotide receptors including a P_2-purinoceptor (activated by α,β-MeATP, ATP, GTP, ITP and ZTP) and a pyrimidinoceptor (activated by UTP, UDP, UDPG, UDPGA and TTP). The pyrimidinoceptor on rat SCG neurones had specific structure activity requirements with the di- and triphosphates of uridine being the most effective depolarizing agonists examined.

Keywords: Adenosine 5'-triphosphate; nucleotide; P_2-purinoceptor; pyrimidinoceptor; superior cervical ganglion (SCG); uridine 5'-triphosphate

Introduction

In contrast to the extensively studied effects of purines on the central and autonomic nervous systems, remarkably little is known about the effects of pyrimidines on the nervous system. The possibility that purine nucleotides (e.g. adenosine 5'-triphosphate, ATP) and pyrimidine nucleotides (e.g. uridine 5'-triphosphate, UTP) activate separate and distinct receptors on non-neuronal tissues has been reviewed by Seifert & Schultz (1989). We recently reported that the effects of ATP and UTP on the rat superior cervical ganglion (SCG) may be mediated through purinoceptors and pyrimidinoceptors respectively (Connolly & Harrison, 1993). Our hypothesis was based upon the observations that the UTP-evoked depolarization was unaltered or enhanced in the presence of suramin (Connolly *et al.*, 1993b), reactive blue 2 (Connolly & Harrison, 1994), the 2',4'- and 2',5'-disulphonic acid isomers of 6-azophenyl-pyridoxal-phosphate (PPADS and IsoPPADS) (Connolly, 1995) whereas these P_2-purinoceptor antagonists decreased depolarizations evoked by α,β-methylene-ATP (α,β-MeATP) or ATP. In addition it has recently been reported that prolonged application of α,β-MeATP desensitized subsequent depolarizations of the rat SCG evoked by either α,β-MeATP or ATP but not depolarizations evoked by UTP. Conversely, UTP but not α,β-MeATP-evoked depolarizations were depressed by prolonged treatment with UTP (Connolly, 1994).

The objective of the present paper was to examine the actions of pyrimidine nucleotides, nucleosides and bases on the d.c. potential of the rat SCG to characterize the structural requirements of the receptors activated by pyrimidines. As part of this study we have examined the effects of some compounds on the rat SCG that have been associated with various neurological disorders, e.g. ZTP (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranosyl-5'-triphosphate) (Jaeken & Van den Berghe, 1984; Sidi & Mitchell, 1985) and pseudouridine (Gerritis *et al.*, 1991). In addition we also examined the effect of suramin, a P_{2X}- and P_{2Y}-purinoceptor antagonist (Dunn & Blakeley, 1988; Den Hertog *et al.*, 1989) on the depolarizing responses of SCG neurones evoked by pyrimidine and purine 5'-triphosphates to determine if their effects were mediated via the same or different receptors, i.e. via nucleotide, pyrimidinoceptor or P_2-purinoceptors.

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Methods

Ganglia were prepared for recording of d.c. potentials as described before (Connolly & Stone, 1993a). SCG were removed from male Sprague-Dawley rats (200–300 g) killed with a lethal dose of urethane; the SCG were desheathed and submerged between the greased barriers of a three compartment recording bath. The central chamber containing the ganglion body was superfused (approx. 2 ml min^{-1}) with a physiological salt solution (PSS) at pH 7.4 and at a temperature of 25±1°C, containing (mM): NaCl 125, $NaHCO_3$ 25, KCl 1, KH_2PO_4 1, $MgSO_4$ 1, glucose 10, $CaCl_2$ 0.1 and pre-oxygenated (5% CO_2/95% O_2). The potential difference between the earthed central chamber and the internal carotid nerve was recorded with Ag/AgCl electrodes, the signals being filtered through a low pass filter, amplified via d.c. pre-amplifiers and recorded on a pen recorder.

Purines and pyrimidines were dissolved in PSS as 10 or 100 mM stock solutions and frozen (at −20°C) as aliquots. New aliquots of stock solutions were used for each experiment. About 3 mg of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) was dissolved in up to 100 μl of dimethyl sulphoxide (DMSO) and diluted to 1 μM in PSS. To avoid precipitation of insoluble 8-phenyltheophylline (8-PT), 5–10 mg of 8-PT was dissolved in up to 100 μl of 1M NaOH and diluted in PSS to a 10 mM stock solution. This was further diluted with pre-oxygenated PSS to a final concentration of 10 μM and the pH adjusted to 7.4 with dilute HCl acid. Nikkomycin Z was dissolved in PSS as 10 mM stock solution. Just before use, suramin (up to 900 mg) was dissolved in about 20 ml of PSS and diluted to a final concentration of 300 μM in PSS.

Experimental protocols were derived from pilot studies. The rationale for the low calcium/potassium PSS used, was based upon previous observations that responses evoked by ATP and α,β-MeATP were enhanced and were more accurately assessed in this medium (for further details see discussions in Connolly *et al.*, 1993a,b). Application times for agonists were those found previously to be sufficient for potential changes to reach a plateau and allow repeated applications without desensitization. Ganglia were equilibrated with PSS for at least 2 h before use, during which time responses evoked by agonists stabilized and repeated applications produced reproducible responses (e.g. see results and Connolly, 1994). Single concentrations of agonists were applied for 2 min, generally at intervals of more than 10 min between applications. Applications of agonists are shown in the figures as solid lines under each d.c. recording. Responses evoked by agonists were determined by extrapolation of the resting basal d.c. potential at the start of the application of an agonist to the return to the basal level following the response. Peak responses evoked by agonists are expressed as a mean±s.e.mean (n), where n is the number of ganglia.

The effect of suramin on the responses evoked by agonists was determined first without suramin (control responses) and after a minimum of 30 min incubation in suramin (300 μM). For experiments examining the effects of suramin on the depolarizations evoked by UTP (100 μM) in the presence of 8-PT, ganglia were pre-incubated for at least 30 min in 8-PT. To examine the effects of suramin on the depolarizations evoked by ATP it was essential to block the activation of adenosine receptors. To do this ganglia were treated with DPCPX when they were isolated and treatment was maintained for the duration of the experiment. In some experiments the effects of suramin on the responses evoked by UTP and ATP (in the presence of DPCPX) were examined on the same preparation.

The effect of nikkomycin Z on the depolarization evoked by UTP was determined by applying UTP (2 min, 100 μM) 5 min from the start of a 10 min superfusion with nikkomycin Z. Depolarizations evoked by UTP in the presence of nikkomycin Z were compared (Student's paired t test) to control responses (without nikkomycin Z) evoked either 30 min before or after application of nikkomycin Z.

Differences between responses evoked by a given concentration of agonist in the absence and presence of suramin or nikkomycin Z were compared by Student's paired t test (two-tailed) and were considered statistically significant if $P<0.05$.

HCl, NaOH and all salts were of Analar grade and obtained from BDH, Dorset. DPCPX and 8-PT were bought from Research Biochemicals Inc., Semat Technical (UK), Herts. 1-Amino-5-bromouracil (ABU) was a gift from Yamasa Corporation, Chiba, Japan. Nikkomycin Z bis-trifluoroacetate was a gift from Glaxo Group Research Ltd., Greenford, Middlesex. Suramin hexasodium salt was a gift from Bayer plc, Berkshire. Adenosine 5'O-(3-thiotriphosphate) (ATPγS; Sigma) was a gift from Dr P. Leff (Fisons plc, Loughborough, Leicestershire). All other compounds were obtained from Sigma Chemical Co., U.K.

Results

Effect of nucleotide 5'-triphosphates on the d.c. potential

The effects of purine and pyrimidine 5'-triphosphates on the d.c. potentials of ganglia are summarized in Figures 1 and 2. At 100 and 1000 μM the initial rapid depolarization evoked by ATPγS, was followed by a rapid and smaller concentration-dependent hyperpolarization (Figure 1). Because maximum responses were not obtained with all the agonists studied, an arbitrary level of depolarization of 100 μV was used to determine the relative potencies of the agonists studied. The relative order of ZTP and purine 5'-triphosphates in depolarizing ganglia was ZTP≥ATPγS> >ATP≥ITP=GTP, and for the pyrimidine 5'-triphosphates UTP>TTP≥CTP.

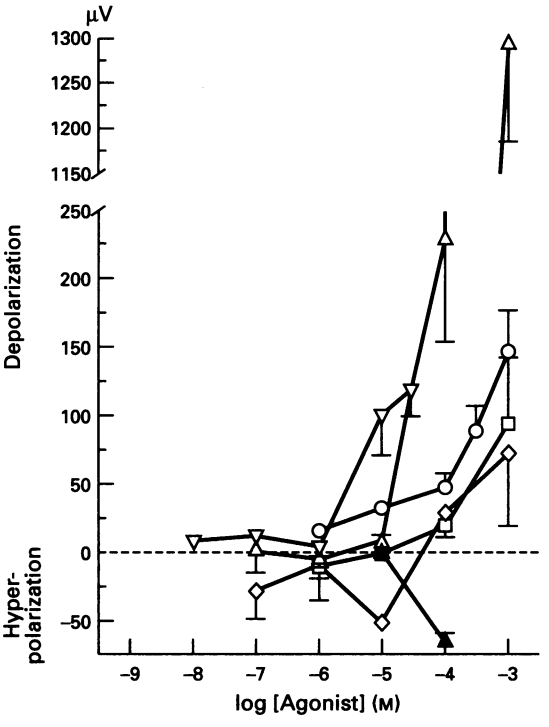

Figure 1 Effect of purine 5'-triphosphates and ZTP on the d.c. potential of the rat SCG. Responses evoked by ZTP (▽), ATPγS (△, ▲), ATP (○), ITP (□) and GTP (◇) obtained from 4 to 12 ganglia. The responses evoked by ATP were recorded in the presence of DPCPX (1 μM). ATPγS at 1 mM in addition to depolarizing ganglia (shown above) also hyperpolarized ganglia by 715±105 μV (n=7) (data not shown). In this and subsequent graphs the mean response and the standard error of the mean response are indicated. Where error bars are not shown, the standard error is so small as to fall within the borders of the symbol itself. For abbreviations see text.

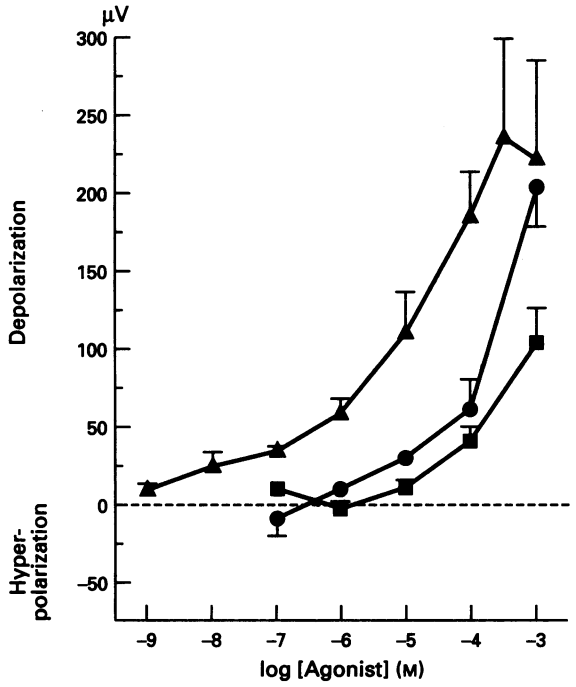

Figure 2 Effect of pyrimidine 5'-triphosphates on the d.c. potential of the rat SCG. Responses evoked by UTP (▲), TTP (●) and CTP (■) obtained from 6 to 12 ganglia. For abbreviations see text.

Effect of uridine and its analogues on the d.c. potential

The effect of uridine and its analogues on the d.c. potentials are summarized in Figures 3 and 4. Uridine was inactive at micromolar concentrations and at 1 and 10 mM produced concentration-dependent hyperpolarizations (Figure 3). In additional studies, 1 mM UMP evoked a small depolarization (35 ± 9 μV, $n=16$) but on five more ganglia, where 100 μM UTP depolarized ganglia (162 ± 46 μV), 100 μM UMP was inactive (16 ± 13 μV). Pseudouridine evoked very small depolarizations at 100 μM (18 ± 5 μV, $n=9$) and at 1 mM (10 ± 6 μV, $n=4$). 1-Amino-5-bromouracil (ABU) was inactive between 0.1 and 100 μM and at 1 mM evoked a small depolarization (26 ± 4 μV, $n=4$).

Although UDP evoked concentration-dependent depolarizations (threshold 1 μM), a maximal response was not obtained at 1 mM. On the same ganglia the depolarizations evoked by UDP and UTP (at 100 μM) were not significantly different ($n=4$; paired t test). The relative order of potency of uridine analogues in depolarizing ganglia (determined at an arbitrary level of 100 μV) was UDP = UTP > > uracil ≥ UMP = pseudouridine ≥ uridine. UDPG and UDPGA depolarized ganglia and at an arbitrary level of 100 μV their relative order of potency was UDP > UDPG > UDPGA (Figure 4).

Effect of nikkomycin Z on the d.c. potential and on the depolarization evoked by UTP

Nikkomycin Z is structurally similar to UTP and therefore might be expected to produce a similar response. However, surprisingly nikkomycin Z did not significantly alter the basal d.c. potential (Figure 4), nor did it significantly alter the depolarization evoked by UTP. Thus, before, during and after the application of 100 μM nikkomycin Z, UTP (100 μM) evoked depolarizations of 422 ± 144 μV, 423 ± 110 μV and 425 ± 118 μV respectively ($n=3$, results not shown).

Effect of 6-azapyrimidines and thymidine on the d.c. potential

Except for 6-azathymine at 1 mM, a concentration of 10 mM was required before thymidine, 6-azathymine, 6-azauracil or 6-azauridine depolarized ganglia (Figure 5).

Effect of suramin on the depolarization evoked by nucleotide 5' triphosphates

Records showing the depolarization evoked by some nucleotide 5'-triphosphates on single ganglia in the absence and

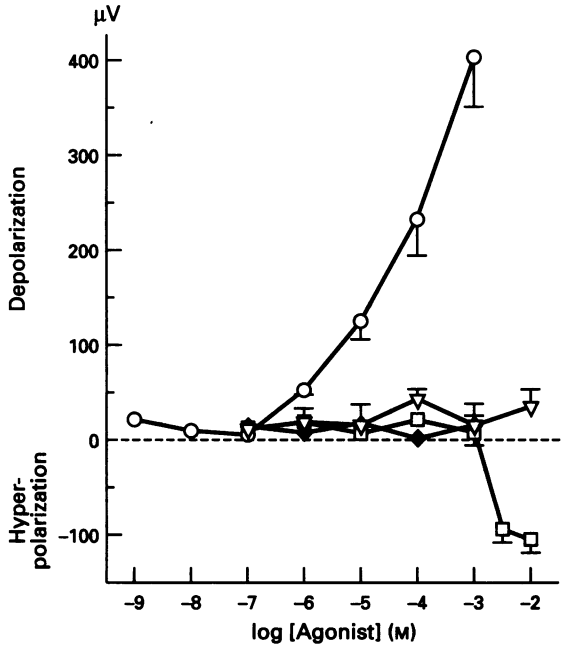

Figure 3 Effect of uracil, uridine and its di- and mono-phosphates on the d.c. potential of the rat SCG. Responses evoked by UDP (○), uracil (▽), UMP (◆) and uridine (□) obtained from 4 to 15 ganglia. For abbreviations see text.

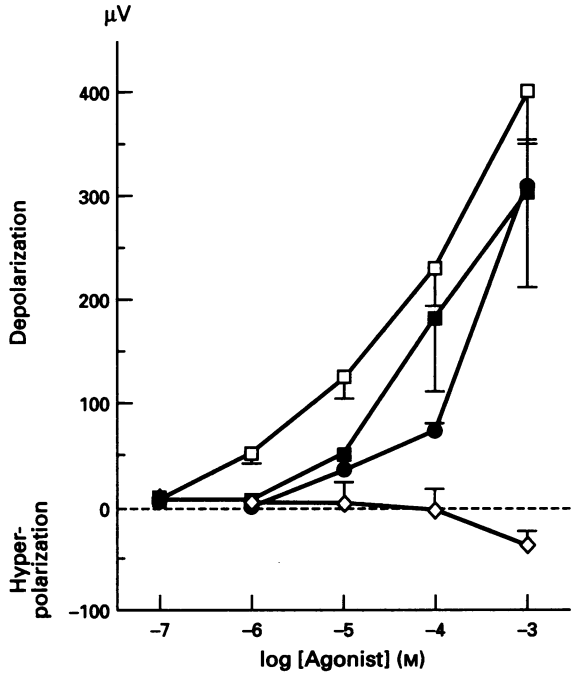

Figure 4 Effect of UDPG, UDPGA and nikkomycin Z on the d.c. potential of the rat SCG. Responses evoked by UDPG (■), UDPGA (●) and nikkomycin Z (◇) obtained from 4 ganglia. Depolarizations evoked by UDP (□) as shown in Figure 3 are provided for comparison. For abbreviations see text.

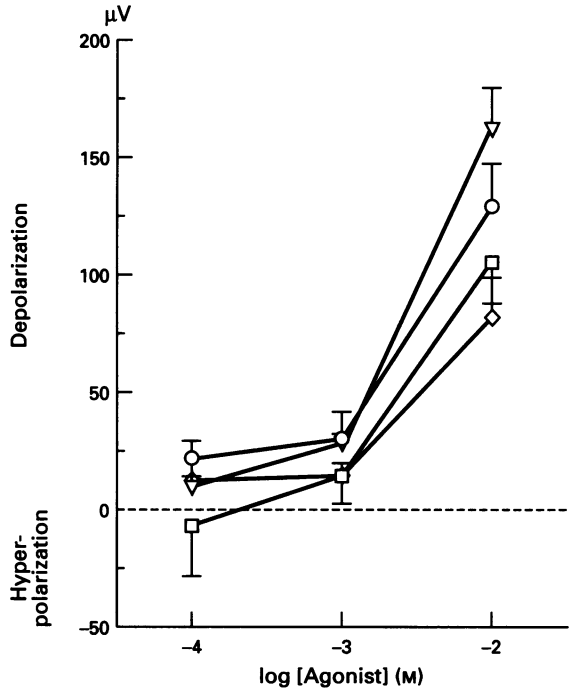

Figure 5 Summary of the depolarizations evoked by 6-azathymine (▽), 6-azauracil (○), 6-azauridine (□) and thymidine (◇) recorded from 4 ganglia. For abbreviations see text.

presence of suramin are shown in Figure 6. ATP (see Table 1), UTP (100 μM), α,β-MeATP (100 μM) and K^+ (5 mM) depolarized ganglia in the presence of 1 μM DPCPX by 217±54, 165±20 and 197±33 μV respectively ($n=3$ each). Suramin did not significantly alter the depolarizations evoked by UDPG whereas on the same ganglia the depolarizations evoked by α,β-MeATP were significantly reduced (Table 1). Suramin (300 μM) significantly depressed depolarizations evoked by ATP, ATPγS, CTP, GTP, ZTP and reversed the small depolarization evoked by UMP (100 μM) into a hyperpolarization (Table 1). Depolarizations evoked by 100 μM ATP, applied under the same conditions as those used to examine the effects of suramin, were not significantly different from each other (control = 56±5 μV cf. time controls 43±6 μV, $n=3$). Suramin did not significantly alter the depolarization evoked by TTP (Figure 6), enhanced depolarizations evoked by 100 μM UDP and 100 μM UTP (Table 1). Depolarizations evoked by 100 μM UTP, applied under the same conditions as those used to examine the effects of suramin, were not significantly different from each other (control = 222±38 μV cf. time controls 206±31 μV, $n=11$). In the presence of 8-PT, suramin did not significantly alter depolarizations evoked by 100 μM UTP.

Discussion

Evidence for pyrimidinoceptors on SCG neurones

The ability of UTP to depolarize SCG neurones in the presence of DPCPX (a selective A_1-purinoceptor antagonist; Bruns *et al.*, 1987) (see also Connolly & Harrison, 1994) or in the presence of 8-PT (a non selective P_1-purinoceptor antagonist that antagonizes both A_1- and A_2-adenosine receptors) suggests that the receptors mediating the actions of UTP are unlikely to be P_1-purinoceptors. Therefore, UTP must act via P_2-purinoceptors, nucleotide or pyrimidinoceptors. At P_2-purinoceptors ATP is more potent than UTP whereas at pyrimidinoceptors UTP is more potent than ATP (Haussinger *et al.*, 1987; Von Kugelgen *et al.*, 1987; Seifert & Schultz, 1989; Von Kugelgen & Stark, 1990; O'Connor *et al.*, 1991). On the rat SCG, UTP was more potent than ATP (although both nucleotides would be expected to be degraded equally; Bischoff *et al.*, 1975) suggesting the presence of pyrimidinoceptors. Moreover, the greater potency of UTP compared to ATP and two ATP analogues that are more resistant to metabolism than ATP, namely α,β-MeATP and ATPγS (Bischoff *et al.*, 1975) suggests that these compounds do not activate a 'nucleotide' receptor (where ATP and UTP are equipotent); rather they support the hypothesis that on the rat SCG the nucleotides ATP and UTP activate distinct receptors, i.e. purinoceptors and pyrimidinoceptors respectively.

A further line of reasoning indicating the presence of pyrimidinoceptors on rat SCG neurones is the observation that suramin, a P_2-purinoceptor antagonist (Dunn & Blakely, 1988; Den Hertog *et al.*, 1989) did not antagonize depolarizations

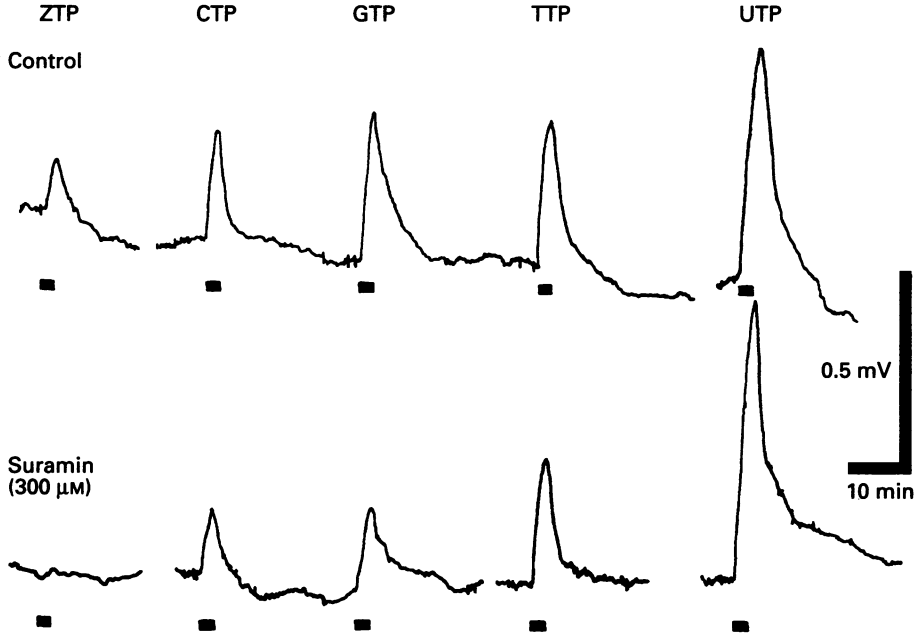

Figure 6 Effect of suramin (300 μM) on the depolarizations evoked by purine and pyrimidine 5'-triphosphates recorded from a single ganglion. The effect of ZTP (30 μM), CTP (1 mM), GTP (1 mM), TTP (300 μM) and UTP (100 μM) on the d.c. potential is shown before (upper trace, Control) and subsequently in the presence of suramin (lower trace). NB Responses evoked by all agonists in this example were larger than the average responses recorded. For abbreviations see text.

Table 1 Effect of suramin on depolarization of the rat isolated superior cervical ganglion evoked by nucleotides

Compound	Conc (μM)	n	Response (μV ± s.e.mean) Control	+ Suramin (300 μM)
ZTP	30	6	103 ± 27	10 ± 5[a]
Purines				
ATP[a]	300	4	89 ± 18	48 ± 18**
ATPγS[d]	100	8	135 ± 39/−75 ± 16	28 ± 17**/−17 ± 8
α,β-MeATP[b]	100	4	104 ± 20	26 ± 17***
GPT	1000	8	197 ± 36	115 ± 23*
Pyrimidines				
CTP	1000	6	202 ± 26	108 ± 19***
UMP	1000	9	42 ± 14	−58 ± 10**
UDP	100	6	395 ± 67	456 ± 86*
UDPG[b]	100	7	133 ± 55	130 ± 15
UTP	10	4	264 ± 63	253 ± 49
UTP	100	8	388 ± 83	436 ± 83*
UTP[c]	100	7	179 ± 39	161 ± 30
TTP	300	7	230 ± 42	184 ± 41

[a]Responses evoked by ATP were recorded in the presence of 1 μM DPCPX. [b]Responses evoked by both agonists recorded on the same ganglia. [c]Responses evoked by UTP recorded in the presence of 10 μM 8-PT. [d]ATPγS produced both a depolarization followed by a hyperpolarization. Statistically significant difference between responses in the absence (control) and presence of suramin (paired *t* test) is indicated by $*P<0.05$, $**P<0.01$ and $***P<0.001$. For abbreviations see text.

evoked by the pyrimidines UTP, UDP, UDPG and TTP. In contrast suramin antagonized depolarizations evoked by ZTP, the purines ATP, ATPγS and GTP, and the pyrimidines CTP and UMP and is therefore consistent with these nucleotides activating P_2-purinoceptors. The ability of suramin to antagonize the hyperpolarization produced after a depolarization evoked by ATPγS suggests that both events were mediated by the activation of P_2-purinoceptors. We have not investigated the mechanism(s) responsible for the ATPγS-evoked hyperpolarization of the rat SCG, but because ATPγS produced responses resembling responses evoked by the nicotinic agonist DMPP (Connolly & Stone, 1993b), we postulate that both compounds act in a similar way. Here it is pertinent to note that Nakazawa (1994) observed that ATP activates the influx of Ca^{2+} and Na^+ in to SCG neurones via non-selective cation channels and interacts with the acetylcholine-activated currents of rat SCG neurones. We too have observed that ATP evokes a concentration-dependent increase in intracellular calcium in SCG neurones (Connolly, unpublished observations) and along with the observations Nakazawa (1994), we suggest that ATP evokes an influx of Na^+ and Ca^{2+} to depolarize ganglia. This depolarization may then be followed by the rapid extrusion of Na^+ due to the activation of a Na^+/K^+ co-transporter, as has been reported for the effects of DMPP (Connolly & Stone, 1993b, and references therein).

Here we report that ZTP was as or more potent than ATPγS as a depolarizing agent on the rat SCG. The ability of suramin to abolish both the ZTP-evoked depolarization and the ATPγS-evoked depolarization and hyperpolarization of SCG suggests that both responses were due to the activation of P_2-purinoceptors. Interestingly it has been observed that patients with a deficiency of adenylosuccinase (one of the enzymes responsible for the *de novo* synthesis of ZTP) exhibit profound neurological complications, e.g. psychomotor retardation, often accompanied by autistic features or mental retardation (Jaeken & Van den Berghe, 1984; Sidi & Mitchell, 1985). The potential of ZTP to activate P_2-purinoceptors on neurones within the human nervous system seems worthwhile investigating, as it may help explain some of the deleterious neurological effects observed in patients with disorders of ZTP metabolism.

Prolonged contact with suramin at higher concentrations than those used here (i.e. 1 and 10 mM) has been reported to produce a slight or notable inhibition of the degradation of ATP by strips of guinea-pig urinary bladder, (Hourani & Chown, 1989). The lack of antagonism by suramin of UTP-evoked depolarizations and the potentiation of both UDP- and UTP-evoked depolarizations of the rat SCG might be explained by an inhibition of their metabolism by ecto-5'-nucleotidase. In fact, Crack *et al.* (1995) have reported that the ability of suramin to antagonize nucleotide-evoked responses of the rabbit ear artery was equal to its ability to inhibit nucleotide metabolism, suggesting these two effects are self-cancelling. An inhibition of ecto-5'-nucleotidase by suramin could also account for the more moderate depression of depolarizations evoked by ATP compared to the greater depression of depolarizations evoked by the metabolically stable nucleotides, α,β-MeATP and ATPγS. We have reported that other P_2-purinoceptor antagonists such as reactive blue 2 and PPADS enhanced the depolarization of the rat SCG evoked by UTP (Connolly & Harrison, 1994; Connolly, 1995). On the guinea-pig urinary bladder, reactive blue 2 at a concentration above that used in our studies (10 mM) did not inhibit ecto-5'-nucleotidase (Hourani & Chown, 1989). Thus the mechanisms by which suramin potentiates pyrimidine-evoked depolarizations of the rat SCG remain unknown. Studies to investigate whether the SCG possesses ecto-5'-nucleotidases and if they are inhibited by P_2-purinoceptor antagonists are now in progress. Given that suramin has a plethora of effects on cellular physiology, many of which could explain our observations, other hypotheses might also be worth studying, e.g. suramin may modulate depolarizations evoked by uridine nucleotides by an allosteric action or it may inhibit a covert hyperpolarization. Turning to the latter hypothesis, it is interesting to note that suramin did not potentiate UTP-evoked depolarizations when examined in the presence of 8-PT and UTP-evoked depolarizations were smaller in the presence of 8-PT than in its absence. These results may indicate that adenosine-receptors are somehow involved in the potentiating effects produced by suramin. Here we note that the objective of our experiments was to examine the effect of suramin (not 8-PT) on UTP-evoked responses and the caveat that the effects of 8-PT on the response evoked by UTP may reflect the variability between responses observed in different preparations.

Structural requirements of a pyrimidinoceptor agonist

The pyrimidine nucleotides producing the largest depolarizations of the SCG were UTP and UDP. Furthermore these compounds were approximately equipotent and this suggests that hydrolysis of the high energy bond between the β- and γ-phosphate groups of UTP is not required for receptor activation. A similar equipotency of UTP and UDP has been observed for the constriction of isolated cerebral arteries of the dog and rabbit (Urquilla & van Dyke, 1978; Von Kugelgen & Starke, 1990 respectively), an increase in portal perfusion

pressure of the rat liver (Haussinger *et al.*, 1987) and depolarization of bullfrog isolated ganglia (Gruol *et al.*, 1981) suggesting this may be a general characteristic of pyrimidinoceptors. In fact, the equipotency of UDP and UTP on the rat SCG supports the idea that these nucleotides activate a pyrimidinoceptor rather than a P_{2U}-purinoceptor, (i.e. a nucleotide receptor) because UDP (at up to 100 μM) was reported to be inactive at P_{2U}-purinoceptors on human airway epithelial cells (Brown *et al.*, 1991) or considerably weaker at P_{2U}-purinoceptors on human fibroblast (Fine *et al.*, 1989).

Further shortening of the phosphate chain to form nucleotide 5'-monophosphates markedly reduced activity not only on rat SCG neurones but on other tissues where it has been investigated (Urquilla *et al.*, 1978; Gruol *et al.*, 1981; Haussinger *et al.*, 1987; Von Kugelgen & Starke, 1990). Moreover, on the SCG the weak activity of UMP, uracil and the pyrimidine bases uridine, pseudouridine and thymidine compared to the potent effects evoked by UTP, UDP and TTP are consistent with the idea that the presence of a polyphosphate group attached to the ribose moiety is required for full activity. Further support for this hypothesis is provided by evidence that replacement of the polyphosphate chain with a peptide at the 5' position of the ribose moiety, as in nikkomycin Z (an analogue of uridine) proved to be inactive either as an agonist itself or as an antagonist of UTP in the present experiments.

In the context of altering the structure of the polyphosphate chain are our interesting observations that UDPG and UDPGA were surprisingly effective in depolarizing SCG neurones, as it might have been predicted that they would be metabolized or inactive on this ganglion. The ability of UDP-sugars to activate the same receptor as UTP and UDP, as shown by the inability of suramin to antagonize UDPG evoked depolarizations suggest that the pyrimidinoceptor can accommodate a large bulky group attached to the polyphosphate chain. Further studies to examine this hypothesis would include an analysis of the relative potency of other uridine nucleotide-sugars and selective desensitization studies.

Structural alterations of the pyrimidine ring such as changing the keto group on the C^4 position of UTP into an amino group (to form CTP) dramatically decreased its activity. In agreement with the observed low potency of CTP, GTP and UMP on the rat SCG, these compounds were almost inactive on the perfused rat liver, a tissue that contains pyrimidinoceptors (Haussinger *et al.*, 1987). Our observations are also in excellent agreement with the reported low potency of cytosine and its nucleotides observed upon application to the dorsal and ventral roots of the toad isolated spinal cord (Phillis & Kirkpatrick, 1978) and the femoral vascular bed of the rat (Sakai *et al.*, 1979). Because of the similar structural features of the pyrimidine ring of cytosine at the C^4 position and the C^6 position of the purine ring of ATP it is conceivable that CTP might activate P_2-purinoceptors. Some evidence in favour of this hypothesis is provided by the observation that both CTP and GTP desensitized the ATP-evoked depolarization of the guinea-pig ileum (Lukacsko & Krell, 1982) and conversely prior treatment with α,β-MeATP desensitized CTP and ATP-evoked vasoconstriction of the rat mesenteric artery (Ralevic & Burnstock, 1991). The ability of suramin to depress α,β-MeATP, CTP and ITP-evoked depolarization of the rat SCG suggests that these nucleotides activate the same receptor, i.e. a P_2-purinoceptor. Because of the similar structural features of the purine ring of inosine at the C^6 position of ITP and that of uridine at the C^4 position of UTP, it is conceivable that ITP could activate both P_2-purinoceptors and pyrimidinoceptors. The similar and low potency of ITP (comparable to GTP and ATP (in the presence of DPCPX)) compared to the greater potency of UTP supports the idea that ITP depolarized the rat SCG by activating P_2-purinoceptors rather than via the activation of pyrimidinoceptors.

Another structural alteration of the pyrimidine ring by adding a methyl group on the C^5 position of UTP to form TTP, reduced the potency but not the maximal depolarization evoked on SCG neurones. This suggests that small substitutions on the C^5 position of the pyrimidine base may be tolerated. The relative order of potency of the pyrimidine 5'-triphosphates reported here is similar to that reported by Gruol *et al.* (1981) on bullfrog isolated ganglia. Similarly, Phillis & Kirkpatrick (1978) found that UTP, UDP and TTP depolarized the dorsal roots of the toad spinal cord with UTP and UDP being the most potent. In addition, on the rabbit central ear artery, a tissue believed to contain pyrimidinoceptors, the relative order of potency for constriction of the artery was UTP > CTP > TTP (Von Kugelgen *et al.*, 1987). Studies on other tissues shown to contain pyrimidinoceptors, e.g., rat femoral vascular responses (Sakai *et al.*, 1979); contraction of dog middle cerebral arteries (Urquilla & van Dyke, 1978); in perfused rat liver (Haussinger *et al.*, 1987) and the activation of NADPH oxidase in human neutrophils and HL-60 leukaemic cells (Seifert *et al.*, 1989a,b) reported that TTP was weak or inactive at up to 1 mM. These observations might indicate that the pyrimidinoceptors on these tissues are somewhat different from those in the rat SCG. The possibility that pyrimidinoceptors are heterogeneous (see also Seifert & Schultz, 1989) and that such differences are reflected in their distribution on neuronal and non-neuronal cells could have important implications for future studies of pyrimidinoceptors including the study of their structure-activity relationships.

At present further characterization of pyrimidine receptors on the rat SCG awaits the development and synthesis of more stable analogues of UTP, e.g. phosphorothionate analogues of uracil nucleotides (see Seifert & Schultz, 1989) which would be expected to eliminate the effects of uptake and metabolism of pyrimidines. Studies designed to antagonize pyrimidinoceptors would also be useful to confirm the distinct nature of these receptors from P_2-purinoceptors or 'nucleotide' receptors. Even so, the present studies have attempted a preliminary characterization of the receptor activated by pyrimidines on rat SCG neurones. This pyrimidinoceptor has specific structure-activity requirements with UTP and UDP being the most potent pyrimidines and is predicted to possess at least two binding domains to accommodate both the pyrimidine ring and phosphate moieties. The physiological significance of receptors for uridine 5'-nucleotides on the rat SCG remains to be explained but a role in neurotransmitter function or neuromodulation is expected.

We are most grateful for gifts of 1-amino-5-bromouracil from Dr S. Miyazaki, Yamasa Corporation, Japan; Nikkomycin Z from Dr T. Chapman, Glaxo Group Research Ltd., Greenford, Middlesex, U.K.; Ms J. Deeks for suramin, Bayer plc, Berkshire, and Dr P. Leff, Fisons plc, Loughborough, Leicestershire for ATPγS. Part of this work was funded by the Wellcome Trust.

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(Received March 29, 1995
Revised July 25, 1995
Accepted July 28, 1995)