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Adenosine receptor-mediated modulation of acetylcholine release from rat striatal synaptosomes

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1 The effects of A_1 and A_{2a} adenosine receptor agonists on the veratridine-evoked release of [^{3}H]-acetylcholine ([^{3}H]-ACh) from rat striatal synaptosomes was investigated by use of the A_1-selective agonist, **R**-PIA and the 185 fold selective A_{2a} agonist, CGS 21680. The effects of NECA, which is equipotent at both receptor subtypes, were also studied.

2 The evoked release of [^{3}H]-ACh was significantly enhanced by the A_{2a} agonist CGS 21680 but decreased by the A_1 agonist, **R**-PIA. The effects of NECA were dependent on the concentration used, with high concentrations inhibiting and low concentrations enhancing the evoked release of [^{3}H]-ACh. In the absence of any antagonists, the rank order of potency for these three drugs on increasing [^{3}H]-ACh release was CGS 21680 > NECA > **R**-PIA.

3 The stimulatory effects of CGS 21680 and low NECA concentrations on evoked [^{3}H]-ACh release were antagonized by the A_{2a} receptor antagonists, CP66,713 (300 nM) and CGS 15943A (50 nM) whilst the inhibitory effects of **R**-PIA were reversed by the selective A_1 antagonist, DPCPX (4 nM). In the presence of DPCPX, NECA greatly enhanced the evoked release of [^{3}H]-ACh at all concentrations studied when, during such A_1 receptor blockade, the rank order of potency was NECA $\gg$ CGS 21680 > **R**-PIA.

4 These results demonstrate that both A_1 and A_{2a} adenosine receptors modulate the veratridine-evoked release of [^{3}H]-ACh from rat striatal synaptosomes.

Keywords: Acetylcholine release; A_{2a} adenosine receptors; CGS 21680; CP66,713; CGS 15943A; rat striatum; neuromodulation

Introduction

Adenosine is a potent modulator of neurotransmitter release in both the peripheral and central nervous systems (Dunwiddie, 1985; Snyder, 1985; Fredholm & Dunwiddie, 1988; Williams, 1989). Adenosine exerts its neuromodulatory actions via four major receptor subtypes, A_1, A_{2a}, A_{2b} and A_3 which have been characterized according to their pharmacological profile, their effects on adenylate cyclase and their organ or tissue distribution (see Abbracchio *et al.*, 1993).

In the rat, mouse, guinea-pig and human brain the localization of high affinity A_{2a} receptors has been confined to the striatum, nucleus accumbens, olfactory tubercle and lateral segment of the globus pallidus (Jarvis *et al.*, 1989; Parkinson & Fredholm, 1990; Wan *et al.*, 1990; Martinez-Mir *et al.*, 1991; James *et al.*, 1992). These excitatory A_{2a} receptors have been shown to stimulate adenosine 3':5'-cyclic monophosphate (cyclic AMP) production in both striatal membranes and PC12 cells (Brown *et al.*, 1990; Hide *et al.*, 1992) and enhance the release of [^{3}H]-acetylcholine ([^{3}H]-ACh) from striatal nerve terminals and the skeletal neuromuscular junction of the rat (Brown *et al.*, 1990; Correra de Sá *et al.*, 1991). A_{2a} adenosine receptors also increase hippocampal excitability (Sebastião & Ribeiro, 1992), enhance the release of excitatory amino acids from ischaemic cerebral cortex of the rat (Simpson *et al.*, 1992), and regulate cardiovascular function via actions in the nucleus tractus solitarius (Barraco & Phillis, 1991).

However, recent *in situ* hybridisation studies in the dog, rat and human brain have revealed, that while the cloned A_{2a} adenosine receptor (RDC8) can be localized to the caudate, putamen and nucleus accumbens, this receptor is only present on the medium sized neurones (Schiffman *et al.*, 1990; 1991a) and not on either substance P containing nor ACh containing nerves (Schiffman *et al.*, 1991b). In an independent study, Fink *et al.* (1992) suggested that the cloned rat A_{2a} receptor (DT-35), which has considerable sequence homology with RDC8, is expressed exclusively in a subpopulation of striatal neurones that also express dopamine D_2 receptors.

In the light of these findings, it was necessary to characterize the A_{2a} receptor responsible for the stimulation of acetylcholine release in the striatum. The 180 fold selective (over the A_1 receptor) A_{2a} agonist, CGS 21680, was used since it has also been reported to have no effect on A_{2b} receptors (Hutchinson *et al.*, 1989; Lupica *et al.*, 1990). If the presence of such a receptor on cholinergic nerves could be confirmed, it would suggest that more than one A_{2a} subtype exists in the striatum.

Methods

Preparation of crude synaptosomal fraction (P_2)

Wistar rats were killed by a blow to the head, decapitated and the brains quickly removed into ice-cold 0.32 M sucrose. The striata were dissected free and crude synaptosomal fractions (P_2) prepared according to the method of Gray & Whittaker (1962). The P_2 pellet was then resuspended in ice-cold standard reaction mixture (SRM) of the following composition (mM): NaCl 125, KCl 4.75, $MgCl_2$ 1.4, $CaCl_2$ 2.0, HEPES 20.0 (pH 7.4) glucose 10.0. The SRM contained physostigmine 100 μM throughout. The synaptosomes were incubated with 1 μM [methyl-^{3}H]-choline chloride (2.5 μCi ml^{-1}) for 30 min at 37°C. The concentration of labelled choline used is well below the K_m for the low affinity uptake and has been shown to be sodium and hemicholinium dependent (Pittel *et al.*, 1990). After radiolabelling, the synaptosomes were washed three times with ice-cold SRM, centrifuging (10,000 *g*, 2 min, 4°C) between washes. Adenosine deaminase (4 u ml^{-1}) was added after the final resuspension to reduce the effects of endogenous adenosine. The resuspended synaptosomes were kept on ice and 200–250 μl samples pipetted out when required.

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Immunoaffinity purification of cholinergic nerve terminals

Cholinergic nerve terminals were affinity purified from rat striatum using sheep anti-(Chol-1) serum and a mouse anti-(sheep IgG) immunoadsorbent as previously described (Richardson *et al.*, 1984; Richardson, 1986). The yield of cholinergic nerve terminals is expressed as units (1 nmol h^{-1} at 37°C) of choline acetyltransferase (EC 2.3.1.6) activity and amounted to approximately 10% of the initial activity present. The purity of terminals used in these experiments was routinely assessed by measuring the % recovery of choline acetyltransferase and lactate dehydrogenase (EC 1.1.1.27). The preparations used showed a 7.0 ± 1.0 ($n = 3$) fold greater yield of choline acetyltransferase over lactate dehydrogenase, which corresponds to cholinergic nerve terminals of very high purity (Richardson, 1981). All the experiments were performed with nerve terminals still attached to the solid phase immunoadsorbent.

Release of radiolabelled ACh and choline

A_1- and A_2-adenosine receptor agonists and/or antagonists were added to microcentrifuge tubes immediately prior to each reaction, while SRM only was added to control (veratridine-only) and basal samples.

Portions (220–250 µl) of the P_2 fraction were added to each tube at the onset of each reaction and the samples incubated at 37°C for 2 min, the final volume being 250 µl. For basal efflux, ethanol, the vehicle for veratridine, was then added to each sample while in evoked-release samples, veratridine (75 µM) was added. The microcentrifuge tubes were then gently inverted and the sample returned to the water bath for a further 2 min. The reaction was stopped by centrifugation at 10,000 *g* for 2 min at 0°C, 75 µl samples (representing the total release of radiolabelled compounds) of the supernatant were removed for scintillation counting. [^{3}H]-ACh was extracted from the remaining supernatant as described below. Total radioactivity remaining in the pellet was measured by solubilizing the pellets overnight in 0.2 ml of 20% Triton X-100. The pellets were then resuspended and counted on a Packard Tricarb liquid scintillation counter.

In desensitization experiments, half of the immunoaffinity purified cholinergic nerve terminals derived from 600 mg tissue were preincubated with 5'-N-ethylcarboxamide adenosine (NECA, 100 pM) and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 4 nM) for 2 min prior to veratridine stimulation as above. The other half of the preparation was incubated with NECA (100 pM) and DPCPX (4 mM) for 10 min prior to stimulation. After their 10 min incubation with NECA, the terminals were washed and resuspended in fresh SRM containing the same concentrations of NECA and DPCPX as before and then incubated with NECA for a further 2 min before stimulation with veratridine.

Extraction of [^{3}H]-ACh

Radiolabelled ACh was extracted from the supernatant by a modified version of the choline kinase method described by Pittel *et al.* (1990). In these experiments, 75 µl of the reaction supernatant was removed to fresh microcentrifuge tubes and 300 µl of 50 mM glycylglycine buffer (pH 8.5) containing (mM): adenosine 5' triphosphate (ATP) 50.0, $MgCl_2$ 1.2 and 0.08 u of choline kinase ml^{-1} added. The final volume was 375 µl. The microcentrifuge tubes were then gently vortexed and incubated at 37°C for 30 min. The phosphorylation process was stopped immediately by placing the tubes on ice and then adding 700 µl of heptanone containing tetraphenylboron (10 mg ml^{-1}). To extract the unwanted phosphorylated choline into the aqueous layer and to separate the two phases that were produced, the samples were thoroughly shaken and vortexed for 5 s before being centrifuged at 10,000 *g* for 3 min; 600 µl of the organic layer, containing the [^{3}H]-ACh, was removed and added to 600 µl 1 M hydrochloric acid to back extract the [^{3}H]-ACh. Then 500 µl of the acid containing [^{3}H]-ACh was counted in 8 ml of scintillant (Emulsifier Safe, this represents total [^{3}H]-ACh release). [^{3}H]-ACh release in each tube was expressed as a fraction of the total radioactivity of each sample (i.e. [^{3}H]-ACh released divided by total label released + total label remaining in pellet). The amount of veratridine-evoked release was obtained after subtracting the appropriate basal values (i.e. without veratridine). The effects of increasing concentrations of A_1 and A_{2a} receptor agonists were assessed by comparing the fraction release of [^{3}H]-ACh evoked by veratridine in their presence, with the fractional release of [^{3}H]-ACh evoked by veratridine in their absence. In those experiments involving antagonists, the control tubes also included the antagonists.

Drugs and chemicals

5'-N-ethylcarboxamide adenosine (NECA), **R**-N^6-phenylisopropyladenosine (**R**-PIA), adenosine deaminase, physostigmine veratridine, ATP, glycylglycine, heptanone and tetraphenylboron were all obtained from Sigma. (2-[*p*-(2-Carboxyethyl) phenylethylamino]-5'-N-ethylcarboxamidine adenosine) (CGS 21680) and 1,3-dipropyl-8-cyclo-pentylxanthine (DPCPX) were obtained from Research Biochemicals Incorporated. 4-Amino-1-phenyl-[1,2,4] triazolo [4,3-a] quinoxaline (CP66,713) was a generous gift from Dr R. Sarges, Pfizer Central Research, CT, U.S.A. Stock solutions of CP66,713 and DPCPX were dissolved in dimethylsulphoxide (DMSO, Sigma), the final DMSO concentration in reaction tubes being 0.02%. 9-Chloro-2-(2-furanyl)-5, 6-dihydro-1,2,4-triazolo [1,5-c] quinazoline-5-imine (CGS 15493A) was a generous gift from Ciba Geigy; stock solutions were dissolved in ethanol. Salts used in SRM and other buffers were obtained from Fisons Laboratory Supplies and [methyl-^{3}H]-choline chloride (specific activity 79.3 Ci $mmol^{-1}$) was purchased from Amersham.

Statistics

All results are given as mean ± s.e.mean and n equals the number of individual experiments. Results were compared by a two-tailed Student's t test.

Results

In control experiments, addition of veratridine (75 µM) to synaptosomes significantly enhanced the efflux of [^{3}H]-ACh. In the presence of veratridine the fractional release of [^{3}H]-ACh increased from a basal level of 0.099 ± 0.008 to 0.150 ± 0.007 ($P < 0.001$, $n = 7$). The effects of increasing concentrations of the A_1 and A_2 adenosine receptor agonists, **R**-PIA, NECA and CGS 21680 on veratridine-evoked [^{3}H]-ACh release from rat striatal synaptosomes is shown in Figure 1. The highly selective A_{2a}-adenosine receptor agonists, CGS 21680, produced a dose-dependent increase in the evoked efflux of [^{3}H]-ACh, its effects were maximal at 10^{-10} M and statistically significant at concentrations as low as 10^{-11} M ($n = 3$, $P < 0.01$). The potentiation of [^{3}H]-ACh release was less marked with higher concentrations ($\geq 10^{-7}$ M) of CGS 21680. Similarly, NECA, which is equipotent at A_1 and A_2 adenosine receptors, produced a significant increase in the veratridine-evoked release of [^{3}H]-ACh; this enhancement was statistically significant at concentrations of 10^{-10} M or less ($n = 4$, $P < 0.01$). Unlike CGS 21680, which is 185 fold selective for A_{2a} adenosine receptors, NECA (10^{-5} M) decreased the release of [^{3}H]-ACh (not shown).

Conversely, the A_1-adenosine receptor agonist, **R**-PIA produced a decrease in the release of [^{3}H]-ACh which was statistically significant at all the concentrations studied; its effects were maximal at 10^{-5} M where the release of [^{3}H]-ACh was reduced by 66 ± 8.6% ($n = 3$, $P < 0.001$).

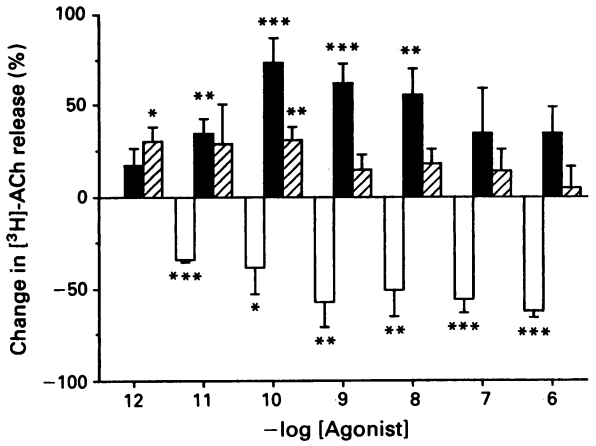

Figure 1 Modulation of veratridine-evoked [^{3}H]-ACh release by adenosine agonists. The results are expressed as % change in [^{3}H]-ACh release when compared to control incubations without agonists, and are means ± s.e.mean. CGS 21680 (solid columns, $n = 3$), R-PIA (open columns, $n = 3$), NECA (hatched columns, $n = 5$). Significant difference from control: $*P<0.05$, $**P<0.01$, $***P<0.001$. For abbreviations, see text.

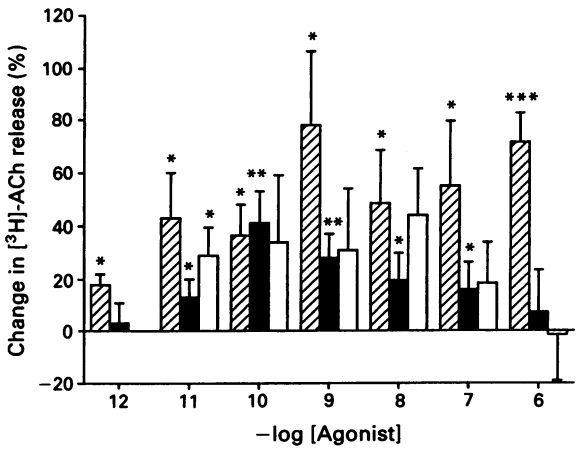

Figure 2 Modulation of veratridine-evoked [^{3}H]-ACh release by adenosine agonists in the presence of DPCPX. DPCPX (4 nM) was included in all incubations and the ability of CGS 21680 (solid columns, $n = 6$), R-PIA (open columns, $n = 4$) and NECA (hatched columns, $n = 3$) to modulate the release determined as described. Significant difference from control as in Figure 1. For abbreviations, see text.

A_1 adenosine receptor antagonism

The results from similar experiments, repeated in the presence of the 750 fold selective A_1 adenosine receptor antagonist, DPCPX are shown in Figure 2. Preincubation of the synaptosomes with DPCPX (4 nM) for 2 min prior to veratridine stimulation had little effect on the enhancement of [^{3}H]-ACh release by CGS 21680 but the stimulatory effect of NECA, which is equipotent at A_1 receptors under control conditions, was greatly augmented; under these circumstances NECA significantly enhanced the release of labelled ACh at all the concentrations studied (see Figure 2). In the presence of DPCPX, R-PIA actually produced an increase in the veratridine-evoked release of [^{3}H]-ACh. Indeed, only when the concentration of R-PIA was greater than 10^{-6} M was there any decrease in the release of [^{3}H]-ACh.

A_2 adenosine receptor antagonism

The effects of 2 min preincubation with 300 nM CP66,713 on A_1 and A_{2a} mediated adenosine responses are shown in Figure 3. CP66,713 significantly impaired the ability of CGS 21680 to enhance [^{3}H]-ACh release. Indeed, when low concentrations of the A_{2a} agonist were used some inhibition, which was not statistically significant, was observed. However, CGS 21680 was able to enhance the release of [^{3}H]-ACh at concentrations greater than 10^{-8} M. In these experiments, the maximal increase in [^{3}H]-ACh release (27.4 ± 6.0%) was obtained when 10^{-7} M CGS 21680 was used. Similar results were obtained with the more potent but less selective A_2-adenosine receptor antagonist, CGS 15943A. As shown in Figure 4, CGS 15943A (50 nM) inhibited the excitatory effect of CGS 21680 on [^{3}H]-ACh release. In fact, low concentrations of CGS 21680 actually inhibited the release of [^{3}H]-ACh; however, higher concentrations increased the evoked release of [^{3}H]-ACh although the enhancement was less marked than with CGS 21680 alone.

In the presence of 300 nM CP66,713, NECA was unable to enhance the veratridine-evoked release of [^{3}H]-ACh; in fact, under these experimental conditions, NECA inhibited the efflux of [^{3}H]-ACh at all the concentrations studied although this inhibition was not statistically significant (Figure 3). The inhibitory effects of R-PIA on [^{3}H]-ACh release were little affected by A_{2a} receptor blockade, until the concentration R-PIA reached 10^{-6} M, then the inhibition by R-PIA in the presence of CP66,713 was greater than observed with R-PIA

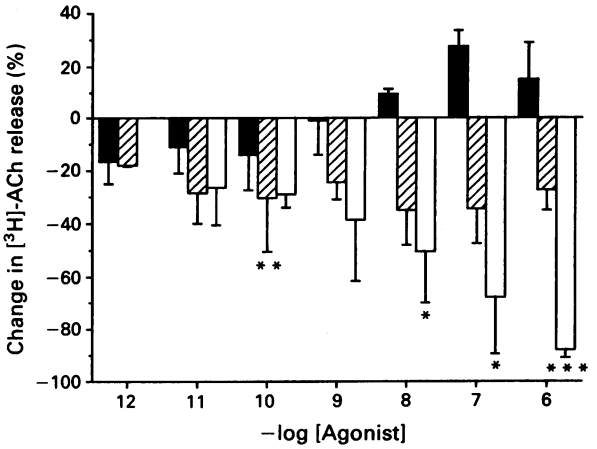

Figure 3 Modulation of veratridine-evoked [^{3}H]-ACh release by adenosine agonists in the presence of CP66,713. CP66,713 (300 nM) was included in all incubations and the ability of CGS 21680 (solid columns, $n = 3$), R-PIA (open columns, $n = 3$) and NECA (hatched columns, $n = 3$) to modulate the release determined as described. Significant difference from control as in Figure 1. For abbreviations, see text.

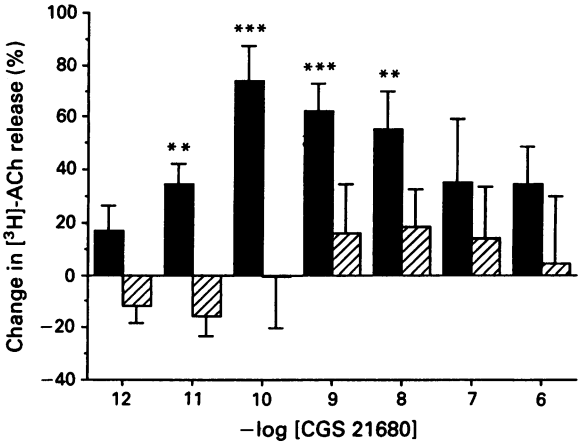

Figure 4 Modulation of veratridine-evoked [^{3}H]-ACh release by CGS 21680 in the presence of CGS 15943. The ability of CGS 21680 to modulate [^{3}H]-ACh release was determined as described, in the presence (hatched columns) or absence (solid columns) of 50 nM CGS 15943. The results are means ± s.e.mean of 3 experiments. Significant difference from control as in Figure 1. For abbreviations, see text.

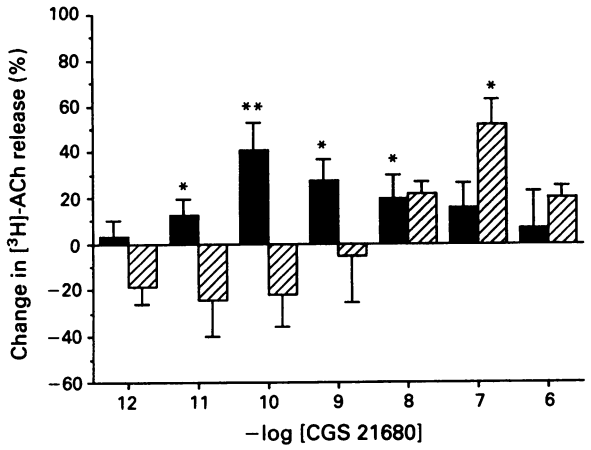

Figure 5 Modulation of veratridine-evoked [^{3}H]-ACh release by CGS 21680 in the presence of DPCPX and CP66,713. The ability of CGS 21680 to modulate [^{3}H]-ACh release was determined as described, in the presence of 4 nM DPCPX (solid columns) and with both 4 nM DPCPX and 300 nM CP66,713 (hatched columns). The results are means ± s.e.mean of 3 experiments. Significant difference between the two conditions: $*P<0.05$, $**P<0.01$. For abbreviations, see text.

alone (c.f. Figure 1). In these experiments, maximal inhibition of [^{3}H]-ACh release occurred at 10^{-6} M **R**-PIA where inhibition was 87 ± 3.4%.

A_1 and A_{2a} adenosine receptor antagonism

In order to assess the effects of CGS 21680 on A_{2a} receptors during A_1 receptor blockade, the effects of CGS 21680 on [^{3}H]-ACh release in the presence of DPCPX (4 nM) and CP66,713 (300 nM) were studied. Under these conditions CGS 21680 was unable to potentiate the evoked efflux of [^{3}H]-ACh until concentrations exceeded 10^{-9} M. Unlike the control experiments where the effects of CGS 21680 were maximal at 10^{-10} M, under these conditions the maximum increase in [^{3}H]-ACh release was obtained at 10^{-7} M CGS 21680 and the maximal enhancement of [^{3}H]-ACh overflow was 51.9 ± 10.7%. At low concentrations of CGS 21680 the release of labelled acetylcholine was actually reduced although this reduction was not statistically significant (see Figure 5).

Desensitization experiments

In the presence of the A_1 antagonist DPCPX (4 nM), the release of [^{3}H]-ACh from immunoaffinity purified cholinergic nerve terminals was enhanced by 23.8 ± 12.4% when NECA (10^{-10} M) was added 2 min prior to addition of veratridine (75 μM). However, preincubation with the same concentration of NECA for 10 min prior to exposure with veratridine and NECA (10^{-10} M) produced a statistically significant decrease in the evoked release of [^{3}H]-ACh. This decrease in the evoked release of [^{3}H]-ACh after 10 min pre-exposure to NECA was seen in all experiments ($n = 4$) despite the fact that DPCPX was present; the mean inhibition was 30.2 ± 13.9%.

Discussion

The data presented in this paper demonstrate that there are A_1 and A_{2a} adenosine receptors, present on the synaptosomal membrane, that can modulate the release of [^{3}H]-ACh from rat striatal synaptosomes. Figure 1 shows quite clearly that while the A_1 selective agent **R**-PIA inhibited [^{3}H]-ACh efflux, the release was stimulated by the A_{2a} selective agonist, CGS 21680. The equipotent A_1/A_2 agonist, NECA, had an intermediate effect which appeared as a low level stimulation of [^{3}H]-ACh release.

In an attempt to analyse this system in greater detail the 740 fold A_1 selective antagonist DPCPX (K_D for A_1 0.5 nM, A_2 340 nM, Bruns *et al.*, 1986) was used. This concentration of DPCPX had little effect on the stimulation by CGS 21680, other than a slight reduction in the maximal stimulation of release. This reduction may reflect a reduced ability of the A_{2a} receptor to stimulate ACh release in the absence of inhibitory A_1 receptor function; however further work would be required to justify such a hypothesis. The most dramatic effect of DPCPX was seen in its abolition of the inhibitory effect of **R**-PIA. Thus the inhibition of ACh release observed with **R**-PIA was reversed. Indeed a variable potentiation of release was seen. DPCPX also augmented the stimulatory effect of NECA, particularly at agonist concentrations greater than 10^{-10} M.

The presence of DPCPX allowed us to determine an apparent relative order of potency of the three agonists at the A_{2a} receptor. From Figure 2 it appears that the order was NECA>>CGS 21680>**R**-PIA. It remains to be seen whether this is significantly different from the order observed when studying A_{2a} receptor modulation of striatal GABA release where the rank order of potency of these agonists was CGS 21680> NECA>**R**-PIA (Kirk & Richardson, 1993).

In the presence of the poorly selective A_{2a} antagonists CP 66,173 (13 fold, Sarges *et al.*, 1990) and CGS 15943A (7 fold, Williams, 1991), the inhibitory effect of **R**-PIA was slightly augmented at high concentrations. This would be consistent with **R**-PIA acting on A_2 receptors at concentrations greater than 10^{-7} M. Similarly, the inhibitory effect of NECA predominated in the presence of such A_2 antagonists, while the stimulatory effect of CGS 21680 was considerably reduced. CGS 21680 was eventually, at much higher concentrations, able to overcome the A_{2a} blockade and enhance the release of [^{3}H]-ACh but the maximum enhancement was much less than that seen when CGS 21680 was used on its own. This is probably because at the higher concentrations required to overcome A_{2a} blockade, the CGS 21680 was also acting at A_1 adenosine receptors. This view is supported by the fact that, in the presence of DPCPX and CP66,713 the maximum enhancement of [^{3}H]-ACh release produced by CGS 21680 was around 52%, substantially more than that seen in the presence of CP66,713 (about 27%) or CGS 15943A (around 20%) alone.

In the presence of DPCPX, CP66,713 was able to abolish the stimulatory effect of CGS 21680 at agonist concentrations less than 10^{-7} M. The apparent shift in EC_{50} values for CGS 21680 (approximately 500 fold in the presence of 4 nM DPCPX and 300 nM CP66,713) implied an affinity of CP66,713 of approximately 10^9 M^{-1}. This is seven fold higher than that estimated from binding studies (James & Richardson, 1993), but the current estimation may have been complicated by the presence of the A_1 receptor.

Since the EC_{50} of CGS 21680 mediated stimulation of [^{3}H]-ACh release was low (50 pM) compared to other studies (0.1–10 nM, Hutchinson *et al.*, 1989; Jarvis *et al.*, 1989; Correra de Sá *et al.*, 1991; Sebastião & Ribeiro, 1992) we investigated whether this A_{2a} receptor showed desensitization (Porter *et al.*, 1988; Ramkumar *et al.*, 1991). Our results support the view that desensitization of this receptor readily occurs, since in simple *in vitro* experiments, preincubation of the synaptosomes with low concentrations of NECA (10^{-10} M) for 10 min, prevented NECA from enhancing the release of [^{3}H]-ACh from immunoaffinity purified nerve terminals. Indeed in desensitized immunoaffinity purified terminals we found that NECA inhibited the release of [^{3}H]-ACh despite the fact that DPCPX (4 nM) was present. While this may be due to the fact that, at this concentration, DPCPX occupies only around 88% of the A_1 receptors and thus NECA may be able to mediate its inhibitory effects via the unoccupied

remainder; it is also possible that NECA mediates these DPCPX-resistant inhibitory actions via the newly cloned and characterized A_3 receptor (Zhou *et al.*, 1993). The role of this novel receptor, which is found in greater numbers in the peripheral nervous system than in the striatum requires further study. This inhibition of [^{3}H]-ACh release is unlikely to be the result of uncoupling of the A_{2a} receptor from G_s since neither a change in receptor number nor an alteration in the coupling of the receptor to its excitatory G-protein are believed to underly the mechanism involved during desensitization (Porter *et al.*, 1988; Ramkumar *et al.*, 1991).

A_{2a} receptor desensitization would occur much more readily when endogenous adenosine levels were allowed to rise (Linden, 1989) and may have occurred in studies where no adenosine deaminase was present (Correra de Sá *et al.*, 1991; Sebastião & Ribeiro, 1992). Receptor desensitization may also be one explanation why Brown *et al.* (1990) were able to detect an increase in the release of ACh from purified terminals only with much higher concentrations of NECA (10^{-7} M). Of course it may also be possible that Brown *et al.* (1990) having already desensitized the high affinity A_{2a} receptor were investigating the effects of NECA on an A_2 receptor with much lower affinity.

In any case, all the data presented are consistent with the presence of both A_1 and A_{2a} receptors on striatal cholinergic nerve terminals. The present findings are in close agreement with previous studies from our laboratory which demonstrated that A_{2a} activation by NECA (in the presence of the A_1-antagonist DPCPX) could enhance the evoked release of ACh by around 50%. These current findings therefore strengthen the view that A_{2a} receptors are present on cholinergic nerve terminals where they increase cyclic AMP production; an effect which has been shown to copurify with immunoaffinity purified cholinergic nerve endings (Brown *et al.*, 1990). In addition [^{3}H]-CGS 21680 binding sites have been shown to be present on immunoaffinity purified striatal cholinergic membranes (James & Richardson, 1993). Further evidence to suggest that CGS 21680 acts on cholinergic nerve endings comes from behavioural studies where CGS 21680 has been shown to inhibit the apomorphine-induced turning of unilaterally 6-hydroxydopamine-lesioned rats in an atropine-dependent manner (Vellucci *et al.*, 1993).

It therefore appears that there are two A_{2a}-like striatal receptors, both of which bind CGS 21680. One has been cloned and shown to be present in the GABA-metenkephalin neurones (Schiffman *et al.*, 1991b), where it is able to inhibit [^{3}H]-GABA release (Kirk & Richardson, 1993). The other is present in cholinergic neurones, was not detected by *in situ* hybridization using probes derived from the cloned receptor, and is able to stimulate [^{3}H]-ACh release. Given the increasing number of reports of CGS 21680 effects in non-striatal neurones (Correra de Sá *et al.*, 1991; Barraco & Phillis, 1991; Sebastião & Ribeiro, 1992; Simpson *et al.*, 1992), it may be that one A_{2a}-like receptor is widely distributed, whereas the other is localized to the striatum. Such a situation has been described in human brain (James *et al.*, 1992).

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