Adenosine receptor-induced cyclic AMP generation and inhibition of 5-hydroxytryptamine release in human platelets

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- 1 We have assessed the effects of adenosine receptor agonists and antagonists on collagen-induced 5-hydroxytryptamine (5-HT) release and cyclic AMP generation in human platelets.
- 2 5'-*N*-ethylcarboxamidoadenosine (NECA) and CGS 21680 elicited accumulations of cyclic AMP with mean EC₅₀ values of 2678 and 980 nM, respectively. The maximal response to CGS 21680 was approximately half that of the response to 10 µM NECA.
- 3 NECA and CGS 21680 inhibited collagen-induced 5-hydroxytryptamine release with mean EC₅₀ values of 960 and 210 nM, respectively. The maximal response to CGS 21680 was approximately 25% of the response to 10 µM NECA.
- 4 The A₁/A_{2a}-selective adenosine receptor antagonist PD 115,199 was more potent as an inhibitor of NECA-elicited responses than the A₁-selective antagonist DPCPX with calculated K_i values of 22–32 nM and > 10 µM, respectively.
- 5 In the presence of a cyclic AMP phosphodiesterase inhibitor, the effects of CGS 21680 on cyclic AMP accumulation and 5-HT release were enhanced to levels similar to those elicited by 10 µM NECA. In the absence of phosphodiesterase inhibition, CGS 21680 did not antagonise the effects of NECA. Furthermore, endogenous adenosine did not contribute to the effects of CGS 21680 when phosphodiesterase was inhibited.
- 6 We conclude that an A_{2a} adenosine receptor appears to be involved in the NECA-elicited increases in cyclic AMP levels and inhibition of 5-HT release in human platelets. However, the difference in efficacy between NECA and the A_{2a}-selective agonist CGS 21680, observed in the absence but not the presence of a phosphodiesterase inhibitor, suggests that other mechanisms must underlie this differential effect.

Keywords A_{2a} adenosine receptor NECA CGS 21680 human platelets

Introduction

Adenosine acts as a modulator of cell function in a wide variety of tissues [1, 2]. Continuous release of adenosine from cells takes place physiologically and pathological states giving rise to metabolic stress or ischaemia increase cellular adenosine release [3]. Rapid cellular reuptake of adenosine also occurs constantly [4]. The principal current therapeutic use of adenosine is in the treatment of supraventricular

tachycardia [5]. Therapeutic applications of available adenosine modulators have been limited by their broad spectrum of pharmacological effects and in the case of adenosine itself by its short duration of action. In recent years, however, considerable advances have been made in the characterisation of different types and subtypes of adenosine receptors and it has been shown that there is differential tissue expression of

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such receptors [2]. There is therefore a potential for development of selective adenosine agonists and antagonists targeted to produce specific therapeutic effects.

Responses to adenosine are mediated by two major subclasses of cell surface receptors; A_1 and A_2 [6, 7], which are coupled to adenylate cyclase via inhibitory and stimulatory G proteins, respectively [1, 8, 9]. The role of adenosine as an inhibitor of platelet activation [10–12] and stimulus of platelet cyclic AMP levels [11, 12] is well-established. As the signal transduction effects of adenosine receptor activation have previously been used as a means of classifying adenosine receptors, the platelet receptor has been labelled as an A_2 type [8, 12, 13]. The availability of selective adenosine analogues and antagonists as research tools has facilitated the definition of adenosine receptors on the basis of potency series of agonists and antagonists [14–16]. Studies using such compounds have suggested that A_2 receptors may be divided into further subtypes, a high affinity type found in brain structures such as rat striatum, nucleus accumbens and olfactory tubercle (A_{2a}), and a low affinity site (A_{2b}) found throughout the rat brain and also in peripheral tissues such as guinea pig aorta [2, 16] and a human fibroblast cell line [2, 17]. The agonist 2-*p*-(2-carboxyethyl)phenethylamino-5'-*N*-ethylcarboxamido-adenosine (CGS 21680), which is selective for the high affinity A_{2a} receptor has proved particularly useful in defining adenosine receptor subclasses [18]. We have also previously shown that the xanthine-derived antagonists *N*-[2-(dimethylamino)-*N*-methyl-4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)benzene sulphonamide (PD 115,199); a mixed A_1/A_2 -selective antagonist [19]; and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), an A_1 selective antagonist [20, 21], are useful discriminatory tools for the classification of adenosine receptor subtypes [16, 22, 23].

The aim of the studies reported here was to use CGS 21680 and PD 115,199 to characterise the adenosine receptor on human platelets by studying their effect on platelet activation and cyclic AMP accumulation. Comparison of the effects of CGS 21680 has been made with the non-selective adenosine analogue 5'-*N*-ethylcarboxamido-adenosine (NECA; [2, 17]), and with the A_1 selective agonist *R*-*N*⁶-phenylisopropyladenosine (*R*-PIA; [2, 17]). The effects of PD 115,199 [20] have been compared with the non-selective antagonist 8-phenyltheophylline (8-PT; [2, 17]) and the A_1 -selective antagonist DPCPX [20, 21].

Methods

Platelet preparation

All experiments were carried out using a washed platelet preparation as described by Lagarde [24]. Briefly, blood (60–100 ml) was taken from healthy, drug-free volunteers (18–55 years), who had been asked to refrain from caffeine for 24 h prior to donation and were specifically questioned to exclude aspirin in-

gestion. Blood was immediately placed in plastic tubes containing acid citrate dextrose anticoagulant (1 ml:9 ml blood). For those experiments in which the effect of adenosine analogues and antagonists on platelet dense granule release was assessed, 6 μ l [14 C]-5-HT per ml of ACD was placed in the tube immediately prior to blood collection. Blood was spun at 150 *g* for 10 min and the platelet-rich plasma was carefully aspirated and acidified to pH 6.4 with 0.15 M citric acid. Aliquots of platelet-rich plasma (3 ml) were re-centrifuged at 370 *g* for 20 min and the supernatant plasma decanted. Platelets were re-suspended in modified calcium free Tyrode Hepes buffer pH 7.35 to a standardised count of $300 \times 10^9/l$.

Release reaction

Platelet dense granule release was measured by a [14 C]-5-HT labelling method, based on that described by Heptinstall *et al.* [25]. [14 C]-5-HT-pre-labelled washed platelets were pre-incubated in the absence or presence of adenosine antagonist or vehicle control for 15 min at 37° C. The adenosine agonist (or vehicle control) was then added and incubated with the washed platelets for 2 min at 37° C prior to induction of the release reaction with collagen. Calcium was then replaced to a final concentration of 1.6 mM. Platelet secretion was stimulated using 4.0 μ g ml⁻¹ of collagen. This was found in preliminary studies to give submaximal [14 C]-5-HT release. In some tubes saline was added in place of collagen for estimation of the extent of [14 C]-5-HT uptake by platelets. The release reaction was allowed to proceed for 6 min. At that time 50 μ l of a saturated solution of aspirin (2.5 mg ml⁻¹) was added and the tubes immediately placed on ice to prevent further release from taking place. The tubes were then centrifuged at 4° C at 4000 *g* for 10 min. Data are expressed as [14 C]-5-HT released into the supernatant layer compared with total [14 C]-5-HT taken up into the platelets.

Measurement of platelet cyclic AMP

Platelet cyclic AMP accumulation was assayed by modifications [26] of the method described by Haslam & McClenaghan [27]. Briefly, washed platelet suspensions, prepared as described above, in which the platelet count was adjusted to $300 \times 10^9/l$ were incubated with [3 H]-adenine for 30 min at 37° C. The platelets were then incubated for 15 min at 37° C in the presence or absence of an adenosine antagonist. Aliquots of the washed platelet suspension were then recalcified with 1.6 mM calcium chloride and, whilst stirring at 1000 rev min⁻¹ at 37° C, an adenosine analogue was then added. Pilot time course studies were conducted, but in all subsequent experiments the reaction was stopped at 45 s following the addition of the agonist. This was carried out by adding 15% trichloroacetic acid to which a standard quantity of [14 C]-cyclic AMP was added to monitor recovery of cyclic AMP following subsequent column chromatography. The tubes were then thoroughly mixed and centrifuged at 4000 *g* at 4° C. Supernatants were

then subjected to triple column chromatography on alumina and Dowex columns to isolate cyclic AMP. The final column eluate was added to liquid scintillant and [^3H]- and [^{14}C]-counts measured on a beta counter. After correction for [^3H]-adenine uptake by platelets, and cyclic AMP recovery, results were expressed as percentage conversion [^3H]-adenine to [^3H]-cyclic AMP.

Data analysis

Concentration-response curves were constructed and EC_{50} values were estimated using the computer program InPlot (GraphPad, California, USA). K_i values were estimated as previously described [23] using increasing antagonist concentrations.

Materials

5'-*N*-ethylcarboxamidoadenosine (NECA), 8-phenyltheophylline (8-PT) and R-phenylisopropyladenosine (R-PIA) were purchased from Sigma Chemicals, Dorset, UK; 2-*p*-(2-carboxyethyl)phenethylamino-5'-*N*-ethylcarboxamidoadenosine (CGS 21680) and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) were purchased from Research Biochemicals Inc., Semat, Herts, UK. *N*-[2-(dimethylamino)-*N*-methyl-4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)benzene sulphonamide (PD 115,199) was a gift from Warner Lambert, USA. [^{14}C]-5-hydroxytryptamine ([^{14}C]-5-HT, 2.04 GBq mmol $^{-1}$) was supplied by Amersham. Fibrillar type I collagen from equine Achilles tendon was supplied by Hormon-Chemie, München, Germany, and was suspended in glucose buffer pH 2.9. [^3H]-Adenine (888 GBq mmol $^{-1}$) was purchased from New England Nuclear, Herts, UK and [^{14}C]-cAMP (10.3 GBq mmol $^{-1}$) from Amersham, Bucks, UK. Iloprost was a gift from Schering Health Care. 4-[6-Ethoxy-5-nitro-2-(1-piperazinyl)-pyrimidin-4-yl]-1-oxido-4-thiomorpholine (AH-P719) was a gift from Dr Karl-Thomae GmbH, Germany. All other reagents for the cyclic AMP assay were purchased from Sigma Chemicals, Dorset, UK.

NECA and CGS 21680 were dissolved in dimethylsulphoxide to concentrations of 80 mM and 40 mM respectively. R-PIA was dissolved in an aqueous solution containing 10% glacial acetic acid and 0.05% sodium carbonate to a concentration of 10 mM. PD 115,199 (5 mM) and DPCPX (10 mM) were each dissolved in dimethylsulphoxide and 8-PT (10 mM) was dissolved in a solution of 22% ethanol and 0.01% sodium hydroxide (pH 14). Iloprost and AH-P719 were diluted in 150 mM saline. Solutions of aspirin (2.5 mg ml $^{-1}$) were prepared in distilled water and stored at room temperature for no longer than 1 week.

Results

Time course studies of the platelet cyclic AMP response to the adenosine analogue NECA showed an initial rise to a peak at 30–45 s after the addition of

the agonist, followed by a decline, with a subsequent highly variable further rise after 1 min (data not shown). For purposes of standardisation, all subsequent measurements of cyclic AMP were made 45 s following the addition of the agonist.

A concentration-dependent inhibition of collagen-induced [^{14}C]-5-HT release and cyclic AMP accumulation was observed in the presence of the non-selective adenosine analogue NECA (Figure 1). The EC_{50} values for inhibition of [^{14}C]-5-HT release and cyclic AMP accumulation were 959 ± 356 nM and 2678 ± 471 nM (mean $\pm$ s.e. mean from six subjects each). The effect of 10 μM NECA on collagen-induced platelet 5-HT release was inhibited by the non-selective adenosine receptor antagonist 8-PT with an IC_{50} value of 12 ± 3 μM (allowing the calculation of an apparent K_i value of 1.3 μM). PD 115,199, which has been shown to be useful in discriminating A_{2a} from A_{2b} effects [12, 19, 20], potently antagonised NECA-induced inhibition of the platelet release reaction with an IC_{50} value of 213 ± 62 nM (Figure 2b, allowing the calculation of an apparent K_i value of 22 nM). In the presence of the vehicle for PD 115,199, NECA elicited a stimulation of 2.09 ± 0.14 -fold basal cyclic AMP with an EC_{50} value of 1341 ± 191 nM. In the presence of 3 μM PD 115,199, however, the EC_{50} value for NECA-induced

Figure 1 Concentration-response relationships for a) generation of [^3H]-cyclic AMP and b) inhibition of collagen induced [^{14}C]-5-HT release by NECA (■; data are from single experiments representative of 6 and 10, respectively) and CGS 21680 (●; data are from single experiments representative of 6 and 6, respectively). Basal accumulation of [^3H]-cyclic AMP and the vehicle effect on [^{14}C]-5-HT release have been subtracted.

cyclic AMP generation was greater than 30 μM (as indicated in Figure 2a), indicating a K_i for PD 115,199 of less than 100 nM.

The effects of other, more selective adenosine receptor ligands were also assessed. The A_1 -selective agonist R-PIA (0.3–100 μM) had no effect on collagen-induced 5-HT release or on platelet cyclic AMP levels ($n = 6$). R-PIA (10 μM) was also ineffective in diminishing the platelet cyclic AMP response to 30 ng ml⁻¹ iloprost (Figure 3). Furthermore, the A_1 -selective antagonist DPCPX, at concentrations up to 1 μM , had no significant effect on 10 μM NECA induced inhibition of [¹⁴C]-5-HT release ($n = 9$, data not shown). This agent also had no effect on NECA-induced increases in cyclic AMP. NECA (10 μM) induced a 2.21 ± 0.24 -fold rise in platelet cyclic AMP above basal levels in the presence of 100 nM DPCPX, and a 2.29 ± 0.33 -fold rise in the presence of the vehicle control ($n = 5$). The corresponding EC_{50} values for NECA were 937 ± 311 nM and 850 ± 121 nM ($n = 5$) respectively. Thus, the K_i value for DPCPX at the adenosine receptors mediating these responses is greater than 10 μM .

CGS 21680, which has been shown to have a high affinity for A_{2a} receptors in other tissues [15], proved

to be only a poor inhibitor of collagen-induced [¹⁴C]-5-HT release. Thus, the maximal response to CGS 21680 only gave $26 \pm 3\%$ of the effect observed with 10 μM NECA ($n = 6$), with an EC_{50} value of 210 ± 57 nM. A similarly reduced maximal response was observed with platelet cyclic AMP accumulation where the maximal response to CGS 21680 was $51 \pm 5\%$ ($n = 5$) of the effect observed with 10 μM NECA (Figure 1). The difference in the maximal responses for NECA and CGS 21680 was not explained by a difference in time courses, as these were found to be comparable, with both agents showing an initial peak at 30–45 s followed by a fall in cyclic AMP levels; a further rise was seen after 1 min but this finding was variable (data not shown).

By contrast, in the presence of the type III phosphodiesterase inhibitor, AH-P719 (100 μM), NECA and CGS 21680 elicited cyclic AMP responses of similar magnitudes. Thus, the maximal response to CGS 21680 was a stimulation of 3.02 ± 0.3 -fold basal (EC_{50} value 574 ± 76 nM, $n = 6$) compared with that elicited by NECA of 2.98 ± 0.23 -fold (EC_{50} value 331 ± 51 nM, $n = 5$; Figure 4). In the presence of the phosphodiesterase inhibitor, the cyclic AMP response to concentrations of CGS 21680 up to 10 μM was abolished by 3 μM PD 115,199 (data not shown). The possibility was considered that endogenous adenosine may be contributing to the effects of CGS 21680 in the presence of AH-P719. Concentration-response curves of the cyclic AMP response to CGS 21680 were therefore constructed with data from parallel experiments in the presence and absence of adenosine deaminase (Figure 5). It is evident that there was no difference in platelet cyclic AMP responses under these conditions.

To exclude the possibility that CGS 21680 was acting as a partial agonist, the effect on 5-HT release and on cyclic AMP accumulation of varying concentrations of NECA was investigated in the presence of CGS 21680 (Figure 6). The studies of cyclic AMP generation were carried out in the absence of AH-P719. Concentrations of NECA higher than 10 μM

Figure 2 a) Concentration response relationship for generation of [³H]-cyclic AMP by NECA in the presence (●) of 3 μM PD 115,199 or vehicle control (■). Data are from a single experiment representative of six paired observations. b) Concentration-response relationship for the PD 115,199 reversal of the 10 μM NECA-induced inhibition of [¹⁴C]-5-HT release. Data are from a single experiment representative of 10 observations.

Figure 3 Lack of effect of R-PIA on iloprost induced [³H]-cyclic AMP generation. Histograms represent responses in the absence of agonist (C) or in the presence of 10 μM R-PIA (R) or iloprost (I; 30 ng ml⁻¹). Data are from a single experiment representative of three observations.

were not investigated because of limitations due to vehicle effect in these experiments. The results indicate an additive effect of the two agents at low concentrations of NECA, but provide no evidence for antagonism between the two agents at higher NECA concentrations.

Discussion

Our study has confirmed the previous findings of others [13, 28–30] by showing that the adenosine analogue NECA exerts a concentration-dependent inhibitory effect upon platelet activation, accompanied by an increase in platelet cyclic AMP levels. The effects on cyclic AMP of NECA, and indeed all other

agents studied, correlated closely with inhibition of 5-HT release. Our data indicate that these effects of NECA are mediated via adenosine receptors of the A_2 subtype. No A_1 mediated inhibition of adenylate cyclase could be demonstrated by experiments carried out in the presence of NECA and DPCPX, or by experiments using R-PIA and iloprost.

Which subtype of A_2 adenosine receptor is present on human platelets?

The effects of NECA were potentially antagonised by adenosine receptor antagonists with both narrow (PD 115,199) and broad specificity (8-PT). Whilst PD 115,199 has been shown in radioligand binding studies to interact with both A_1 and A_{2a} receptors, it shows a high degree of selectivity for the high affinity A_{2a} receptor over the low affinity A_{2b} receptor, with K_i values of approximately 20 nM and 400 nM respectively [16, 19, 22]. It is therefore a useful tool for the subclassification of A_2 adenosine receptors. In the present study, PD 115,199 showed high potency as an antagonist of the NECA effect on platelet secretion and cyclic AMP accumulation with a calculated K_i value of 22 nM. This value is therefore

Figure 4 Concentration-response relationship for generation of $[^3H]$ -cyclic AMP by CGS 21680 (●; representative of seven observations) and NECA (■; representative of six observations) in the presence of the phosphodiesterase inhibitor AH-P719 (100 μ M). $[^3H]$ -cyclic AMP accumulations in the presence of the phosphodiesterase inhibitor alone were 0.26% (●) and 0.32% (■), respectively.

Figure 5 Concentration-response relationship for the generation of $[^3H]$ -cyclic AMP by CGS-21680 in the absence (■) and presence of (●) of 1.2 $u\ ml^{-1}$ adenosine deaminase. Histograms represent responses in the absence of agonist (C) or in the presence of 100 μ M AH-P719 alone (I) or in combination with adenosine deaminase (A). Data are from a single experiment representative of three observations.

Figure 6 Concentration-response relationships for NECA-induced a) generation of $[^3H]$ -cyclic AMP and b) inhibition of $[^{14}C]$ -5-HT release in the absence (■) and presence (●) of either a) 10 μ M or b) 1 μ M CGS 21680. C represents the response to CGS 21680 alone. Data are from single experiments representative of six observations. Mean ($\pm$ s.e. mean) responses to CGS 21680 alone were a) $49 \pm 4\%$ and b) $28 \pm 7\%$ of the responses to NECA, respectively.

in good agreement with data generated for A_{2a} receptors in other tissues, strongly suggesting that the effects of NECA on human platelets are mediated through A_{2a} receptors.

Consequently, it was surprising to observe that the selective A_{2a} agonist CGS 21680 [18] showed only a weak effect on cyclic AMP accumulation and inhibition of platelet activation when compared with NECA. This appeared not to derive from diffusion or on rate phenomena since both agonists elicited similar time courses for the cyclic AMP responses. In the presence of AH-P719, a type III phosphodiesterase inhibitor [31], however, the cyclic AMP responses to NECA and CGS 21680 were more similar. When phosphodiesterase activity was inhibited, the cyclic AMP response to CGS 21680 was antagonised by PD 115,199, confirming that A_{2a} receptors were involved in mediating the effects of this agonist. Investigation of the NECA concentration-response relationships for generation of cyclic AMP and inhibition of platelet secretion were carried out in the presence and absence of CGS 21680 (1–10 μ M). No antagonism of NECA by CGS 21680 could be demonstrated, suggesting that CGS 21680 was not acting as a partial agonist at the platelet adenosine receptor.

Are A_{2a} and A_{2b} receptors present on human platelets?

Two very recent studies have also utilised CGS 21680 in an attempt to characterise the platelet adenosine receptor. One study demonstrated binding of [3 H]-CGS 21680 to human platelet membranes, and, in contrast with our findings, implied that CGS 21680 and NECA were of similar potency in stimulation of adenylate cyclase activity [32]. It may be relevant that this work was carried out in membranes whereas all of our studies were carried out in intact cells. Another recently reported study employed CGS 21680 to compare the characteristics of adenosine receptors on human platelets and a human erythroleukaemia cell line which expresses platelet markers [33]. It was shown that CGS 21680 was virtually inactive in human erythroleukaemia cells and it was suggested that this receptor could be tentatively classified as an A_{2b} receptor. A definitive classification for the platelet receptor was not offered, but a cyclic AMP response to CGS 21680 was noted in platelets. In view of our demonstration of marked synergism between CGS 21680 and AH-P719, it is of note that all experiments in the latter study were carried out in the presence of a phosphodiesterase inhibitor.

Our data therefore suggest that A_{2a} receptors are involved in mediating the effects of adenosine analogues, but that NECA may additionally bind to another site, distinct from the A_{2a} receptor, or produce an additional effect independent of A_{2a} receptor activation. The mechanisms involved can only remain speculative, but several hypotheses could be put forward to explain the differences in maximal responses evoked by NECA and CGS 21680 in intact platelets. It is possible that NECA itself possesses some phosphodiesterase inhibitory activity and further studies

specifically investigating phosphodiesterase activity in broken cell preparations in the presence of NECA would be worthwhile. It is equally possible that NECA may activate both a high affinity A_{2a} and a low affinity A_{2b} receptor, similar to that described in mammalian CNS. Binding to both may be necessary for adenosine agonists to achieve full potency in increasing cyclic AMP in platelets. If this were the case, a facilitatory role for the A_{2a} receptor would be necessary, in view of the high potency of PD 115,199 in antagonising the response to NECA. Another possible explanation lies in the previously reported [3 H]-NECA binding sites on platelets whose functional role remains to be established [34]. These may be involved, in a manner yet to be defined, in contributing to the increases in cyclic AMP in platelets following exposure to NECA.

Are there therapeutic implications for the classification of platelet adenosine receptors?

Whilst there is considerable evidence that adenosine agonists inhibit platelet activation *in vitro*, at present the role of adenosine modulators as antithrombotic agents is at best limited. Dipyridamole, whose main action is the inhibition of adenosine uptake into cells [4, 35], has been used as an antiplatelet drug but clinical trials have failed to demonstrate its efficacy as an antithrombotic agent [35]. Effects of dipyridamole on platelet aggregation in platelet rich plasma have also been difficult to demonstrate [36], but inhibition of platelet reactivity in whole blood has been well documented, presumably as a result of adenosine release from red cells [37–39]. This has led to the suggestion that dipyridamole may be worthy of further study in clinical situations where red cell destruction and platelet activation co-exist, for example in vascular grafts [35, 38]. The disappointing clinical results with dipyridamole do not preclude the further investigation of adenosine analogues as potential antiplatelet agents. In contrast with dipyridamole, many studies including the present study have shown that inhibition of platelet activation by A_2 agonists is readily demonstrable *in vitro* [10–12, 28]. Furthermore, there is some evidence that physiologically relevant concentrations of adenosine can contribute to regulation of platelet activity, probably at a local level [40]. There may therefore be a future role for specific and potent adenosine agonists as antithrombotic agents. Knowledge of the subtype of adenosine receptor in platelets would be useful in the design of such compounds, and is also relevant to the prediction of spectrum of activity of adenosine agonists and antagonists developed for other therapeutic purposes. Other potential applications for adenosine agonists include the treatment of hypertension, cardiac tachyarrhythmias, and epilepsy or as antipsychotic agents [2, 17]. Adenosine antagonists may have a role in renal disease, various ischaemic conditions, and asthma [2, 17].

In conclusion, an A_{2a} receptor appears to be involved in mediating the increase in platelet cyclic AMP and inhibition of 5-HT release in response to

NECA. However, inhibition of phosphodiesterase markedly increases the relative efficacy of CGS 21680, compared with NECA. Other mechanisms, which remain to be established, must therefore underpin this differential effect. The future development of specific A_{2b} analogues and antagonists would be a useful step in the further characterisation of the platelet adenosine receptor. As both human A_{2a} and

A_{2b} adenosine receptors have recently been cloned [41, 42] a molecular biological approach may also be useful in the further investigation of adenosine receptor types on human platelets. The further investigation of differential tissue expression of adenosine receptor subtypes is of relevance to future drug development and will facilitate specific targeting of therapeutic effects whilst limiting adverse effects.

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