Changes in neurotransmitter sensitivity in the mouse neocortical slice following propranolol and theophylline administration

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- 1 The mouse neocortical slice has been used to examine the sensitivity of neurones to isoprenaline, 5-hydroxytryptamine (5-HT) and adenosine acutely and following chronic treatment of animals with propranolol or theophylline.
- 2 While having little effect alone, all three agonists enhanced the d.c. depolarizing potential produced by N-methyl-D-aspartate (NMDA). The effect of (–)-isoprenaline (0.2 μ M) was shared by (+)-isoprenaline at the much higher concentration of 10 μ M.
- 3 Superfusion of slices with theophylline or 8-phenyltheophylline blocked responses to adenosine with evidence of selectivity. A single injection of theophylline 24 h before slice preparation did not alter agonist sensitivity, but when administered daily at 100 mg kg^{–1} for 14 days, the xanthine caused a loss of sensitivity to adenosine and (–)-isoprenaline but not 5-HT. The lower dose of theophylline, 10 mg kg^{–1} daily, also led to a loss of adenosine responses but no change of sensitivity to the amines.
- 4 Following the 14 day treatment with theophylline at 100 mg kg^{–1} daily in two groups of mice, responses to adenosine recovered to control levels after 20 days.
- 5 Propranolol superfusion blocked responses to both isomers of isoprenaline and 5-HT but did not affect sensitivity to adenosine.
- 6 Chronic treatment with propranolol at 25 mg kg^{–1} daily for 14 days induced a loss of sensitivity to (–)-isoprenaline and 5-HT but not adenosine. A lower dose of 5 mg kg^{–1} daily caused no change in responses to adenosine or 5-HT, but yielded an increased sensitivity to (–)-isoprenaline.
- 7 The results are discussed with respect to reports of receptor up-regulation in binding studies; caution is clearly required in extrapolating from such work to receptor activity in a functional system, especially in the case of theophylline and adenosine.

Introduction

Xanthines, including the caffeine and theophylline found in common beverages are among the most widely used drugs in the world. Large intakes of theophylline have been associated with enuresis, headache and seizures (Rall, 1985). Such symptoms may be particularly distressing for patients taking high therapeutic doses of theophylline or related preparations (Defreitas & Schwartz, 1979; Brumback *et al.*, 1984; Furukawa *et al.*, 1984). It is also clear that withdrawal from these compounds following their chronic usage can lead to a well-defined constellation of symptoms which include headache and confusion beginning, on average, 19 h after the last intake (Griffiths *et al.*, 1986). In order to determine the neuronal mechanisms which may underlie some of these phenomena we have become interested in the adaptive changes which may occur in the animal central nervous system during the chronic administration of theophylline.

It is now widely recognised that among the major pharmacological actions of theophylline, responsible for its behavioural stimulant properties, is the antagonism of endogenous adenosine (Daly *et al.*, 1981; Snyder *et al.*, 1981; Sealman, 1988; Choi *et al.*, 1988). This antagonistic action of theophylline was first described by Sattin & Rall (1970) on an adenylylate cyclase system in guinea-pig neocortex but has since been demonstrated at a wide variety of adenosine receptors located presynaptically or postsynaptically (Stone, 1981; 1989; Williams, 1987). The concentrations of xanthines which induce behavioural effects are sufficient to block such adenosine receptors at far lower concentrations than are needed, for example, to inhibit phosphodiesterase or nucleotidase (Fredholm *et al.*, 1978; Rall, 1982; Fredholm & Lindgren, 1983).

One view of receptor regulation would predict that the chronic administration of theophylline would lead to an up-

regulation of adenosine receptor density and function and that increased receptor activity would mediate the adverse effects seen during theophylline withdrawal. The present project was therefore initiated partly to determine whether any change in adenosine receptor function does indeed result from the chronic treatment of mice with theophylline.

In addition low doses of theophylline have recently been found to be of value in the treatment of essential tremor (Mally, 1989) being associated with fewer unwanted effects than the commonly accepted drug for this disorder, propranolol. The latter drug has also found a clinical use in the treatment of migraine (Anthony, 1978; Weber & Reinmuth, 1972) and anxiety states (Heiser & DeFrancisco, 1976; Linken, 1971). It remains unclear, however, whether any of these effects of propranolol are due to actions at β -adrenoceptors or whether other receptor populations are involved with an intermediate situation on pathways involved in the control of tremor and cerebrovascular tone. While it has been proposed that antagonism of β -adrenoceptors is involved in the anti-tremor activity (Young *et al.*, 1975; Taylor *et al.*, 1981; Kuroda *et al.*, 1988) an interference with 5-HT systems may be important (Hallberg, 1987).

The effects of chronic treatment with propranolol and theophylline have therefore been compared in order to ascertain both whether any changes of transmitter sensitivity could be found and whether any such changes of transmitter sensitivity are common to the two agents. Such a demonstration might provide clues as to why both drugs are effective in essential tremor (Mally, 1989).

In order to obtain the large amount of quantitative data required in this study the cortical wedge preparation was used. First described for rat tissue (Harrison & Simmonds, 1985) this preparation was subsequently developed for mouse neocortex by Burton *et al.* (1987), this species yielding a higher proportion of successful slices presumably by virtue of the more densely organised callosal projection. In preliminary experiments few of the compounds of interest to the present

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work had effects by themselves on these slices, but several were found to modify responses to the excitatory amino acid N-methyl-D-aspartate (NMDA). NMDA is a relatively selective agonist for one of three glutamate receptor subtypes (Watkins & Evans, 1981; Stone & Burton, 1988). The pharmacology of depolarizations induced by NMDA in mouse neocortex is comparable with the pharmacology in other species (Burton *et al.*, 1987).

Methods

Male mice were killed by cervical dislocation and the brain removed into cold bicarbonate buffered medium of the following composition (mM): NaCl 115, KCl 2, KH_2PO_4 1.2, CaCl_2 2.5, MgSO_4 1.2, NaHCO_3 25 and glucose 10. Sections of the brain were cut at $500\ \mu\text{m}$ with a vibratome, in a coronal plane to include regions of frontoparietal and cingulate cortex as described in detail previously (Burton *et al.*, 1987). These slices were then trimmed and transferred to two-compartment chambers where they were positioned across a greased slot in the dividing wall so that most of the grey matter lay on one side while the white matter extended into the second chamber.

Silver/silver chloride electrodes in contact with the solutions on the two sides of the dividing wall were used to record d.c. potentials which were displayed on digital oscilloscopes and chart recorders. The amplitude of induced d.c. potentials was used to quantify the drug effects. Both compartments were superfused with the bicarbonate buffer. N-methyl-D-aspartate (NMDA) was added into the fluid superfusing the pial side of the slice for 1 min periods. Other agonists were applied for 2 min periods beginning 1 min before NMDA. After obtaining control responses to agonists, antagonists were superfused for at least 20 min before further applications of agonists. Statistical comparisons were made by Student's *t* test.

For chronic administration, theophylline was dissolved fresh in dilute sodium hydroxide (pH 5.0) and injected immediately. Propranolol was dissolved in saline. Injections were given i.p.

Results

When applied alone to neocortical slices, adenosine $20\ \mu\text{M}$, 5-HT $10\ \mu\text{M}$, noradrenaline $10\ \mu\text{M}$ and ($\pm$)-isoprenaline $10\ \mu\text{M}$ had no discernible effects. We therefore elected to examine the possible modulation of sensitivity to the depolarizing amino acid NMDA as in our previous characterization of the mouse neocortical preparation (Burton *et al.*, 1987).

When superfused over the slices for periods of 1 or 2 min, NMDA induced depolarizations which have been discussed and illustrated extensively (Burton *et al.*, 1987; 1988) and which could be readily quantified. The concentration used throughout the present work was $20\ \mu\text{M}$, an amount that we have previously found to yield a response which is approximately 50% of the maximum attainable in this system.

A concentration-response curve to adenosine was obtained with concentrations between 0.2 and $250\ \mu\text{M}$. The curve was bell-shaped, with a dose-related enhancement of NMDA depolarization being seen up to $50\ \mu\text{M}$ adenosine but with smaller enhancements at concentrations of 100 and $250\ \mu\text{M}$ (Figure 1). A concentration of $20\ \mu\text{M}$ was therefore routinely used, being a sub-maximal concentration on the ascending limb of the curve.

As well as adenosine, the effects of isoprenaline and 5-HT were examined so as to compare the effects of theophylline on other receptors and to determine whether any changes of receptor function were selective. The (–)-isomer of isoprenaline produced an increase in the size of NMDA responses and a concentration of $200\ \text{nM}$ was used in further experiments since it induced an enhancement of NMDA depolarization by $38.5\% \pm 9.1$ ($n = 7$) similar to that of $20\ \mu\text{M}$ adenosine (Figure

Figure 1 Concentration-response curves for adenosine potentiation of N-methyl-D-aspartate (NMDA) induced depolarization of neocortical slices. Results are shown for adenosine in control slices ($\circ$) and after 14 days pretreatment with theophylline $100\ \text{mg kg}^{-1}$ daily (Δ). The points are the mean and vertical bars show 1 s.e. mean ($n = 6$). * $P < 0.05$ for pretreated compared with naive preparations.

2). This effect was shared by the (+)-isomer although a much higher concentration ($10\ \mu\text{M}$) was required in order to produce a similar increase ($33.0\% \pm 11.72$, $n = 4$).

Lower concentrations of (+)-isoprenaline ($200\ \text{nM}$ and $1\ \mu\text{M}$) had no significant effect on the slices.

5-HT at $10\ \mu\text{M}$ but not $1\ \mu\text{M}$ also potentiated significantly responses to NMDA (Figure 2). The potentiation seen at the higher concentration, $48.4\% \pm 8.8$ ($n = 7$) was again comparable with that seen with $20\ \mu\text{M}$ adenosine.

Effect of theophylline superfusion

Superfusion of the slices with theophylline at $10\ \mu\text{M}$ and $100\ \mu\text{M}$ had no effect on the size of control depolarizing

Figure 2 Records of cortical slice depolarization. Each panel shows a control response to $20\ \mu\text{M}$ N-methyl-D-aspartate (NMDA) (a), a response in the presence of test agent (b) and recovery (c). Group A illustrates the enhancement of NMDA-induced depolarization by (–)-isoprenaline ($200\ \text{nM}$), group B by 5-hydroxytryptamine ($10\ \mu\text{M}$) and group C by adenosine ($20\ \mu\text{M}$). Calibrations: 5 min for all records; $500\ \mu\text{V}$ for (A) and (C), $250\ \mu\text{V}$ for (B).

Figure 3 Histogram of the enhancement of N-methyl-D-aspartate (NMDA) depolarization induced by adenosine (20 μM) in the presence of theophylline 10 or 100 μM or 8-phenyltheophylline (8-PT) 0.1 or 1 μM. Columns show the mean with vertical bars indicating 1 s.e.mean (n = 6).

** $P < 0.01$ compared with the xanthine-free controls.

responses to NMDA alone. With both concentrations there was a reduction in the mean level of potentiation by adenosine though this antagonism was only significant at the higher concentration of xanthine. The more selective adenosine antagonist, 8-phenyltheophylline (8-PT), reduced adenosine responses significantly at a concentration of 100 nM and 1 μM (Figure 3).

In contrast, responses to 5-HT were not changed significantly by 100 nM or 1 μM 8-PT (Figure 4). The effect of 200 nM (—)isoprenaline was reduced significantly by 100 μM theophylline; at 10 μM theophylline the response was reduced but not significantly. Since these effects of theophylline were not shared by 8-PT, it is unlikely they were related to any blockade of adenosine receptors (Figure 4).

Subacute and chronic treatment with theophylline

Adenosine In order to examine the persistence of any acute blockade of adenosine receptors by injected theophylline, one group of mice was killed 24 h after a single injection of theophylline 100 mg kg⁻¹, i.p. This procedure did not result in any diminution of adenosine response which now reached 140.8% ± 8.98 (n = 5) of control NMDA responses compared with 150.9 ± 15.9 (n = 13; NS) without xanthine administration.

Daily administration of theophylline for 14 days did result in a reduction of adenosine sensitivity. At a daily dose of 100 mg kg⁻¹ the potentiation of NMDA by adenosine was reduced to only 101.7 ± 6.4 (n = 6) % of control (Figure 5).

Figure 4 Histogram of the enhancement of N-methyl-D-aspartate (NMDA) depolarizations induced by (—)isoprenaline (0.2 μM) and 5-hydroxytryptamine (5-HT, 10 μM) in the presence of theophylline 100 or 10 μM or 8-phenyltheophylline (8-PT) 1 μM. Columns show the mean with vertical bar indicating 1 s.e.mean (n = 6).

* $P < 0.05$ compared with the xanthine-free controls.

Figure 5 Histogram of the enhancement of N-methyl-D-aspartate (NMDA) depolarization induced by adenosine (20 μM) showing the time course of recovery of sensitivity 7 days and 20 days following treatment with theophylline 100 mg kg⁻¹ daily. Columns show the mean with vertical bars indicating 1 s.e.mean with n shown within the columns.

** $P < 0.01$ compared with the xanthine-free controls.

The lower dose of 10 mg kg⁻¹ daily for two weeks also abolished sensitivity to adenosine (Figure 6) with no change in the size of control responses to NMDA itself. In order to assess whether this loss of sensitivity represented a total absence of functional adenosine receptors or a shift in the dose-response curve, higher concentrations of adenosine were also examined in combination with this treatment schedule. As illustrated in Figure 6, responses could be obtained with 50 and 100 μM adenosine which represented significant potentiation of NMDA responses, though they also remained significantly below the amplitude of the respective control responses.

Two groups of mice were treated with theophylline at the high dose of 100 mg kg⁻¹ daily for 14 days and then the xanthine treatment was stopped in order to follow events on

Figure 6 Histogram of the enhancement of N-methyl-D-aspartate (NMDA) depolarization induced by adenosine at 20, 50 and 100 μM in naive slices (open columns) or slices taken from animals pretreated with theophylline 10 mg kg⁻¹ daily for 14 days (solid columns). Columns show the mean with vertical bars indicating 1 s.e.mean (n = 7).

* $P < 0.05$ compared with NMDA alone; † $P < 0.05$ compared with non-pretreated slices.

Figure 7 Histogram of the enhancement of N-methyl-D-aspartate (NMDA) depolarization induced by (-)-isoprenaline (0.2 μM) following pretreatment with propranolol 5 or 25 mg kg⁻¹ daily for 14 days, or theophylline 10 or 100 mg kg⁻¹ daily. Columns show the mean with 1 s.e.mean indicated by vertical bars; n is shown within each column.

* $P < 0.05$ compared with non-pretreated slices.

withdrawal. Seven days after the theophylline injections adenosine responses were again detectable, NMDA responses being potentiated significantly by $23.0\% \pm 12\%$ ($n = 6$) (Figure 5). By 20 days after withdrawal adenosine responses had returned to control levels of enhancement of NMDA responses by $46.1\% \pm 12.4$ ($n = 7$) compared with controls; $P < 0.05$ (Figure 5).

5-HT In contrast neither dose of theophylline had any effect on sensitivity to 5-HT (10 μM); at 100 mg kg⁻¹ daily, 5-HT potentiated NMDA responses by $39\% \pm 13.2$ ($n = 7$, $P < 0.001$) compared with $48.4\% \pm 8.8$ ($n = 6$) in control slices. After chronic theophylline at 10 mg kg⁻¹ daily, 5-HT still enhanced NMDA depolarization by $57.5\% \pm 6.5$ ($n = 6$; NS).

Isoprenaline Treatment with the lower dose of theophylline (10 mg kg⁻¹, daily) did not result in any diminution of responses to (-)-isoprenaline, the potentiated NMDA response reaching $134.6\% \pm 18.5$ ($n = 5$) of control compared with $136.4\% \pm 7.6$ ($n = 6$) in slices from naive animals. The higher dose of 100 mg kg⁻¹ daily did lead to a significant reduction of isoprenaline sensitivity (Figure 7) to control levels.

Figure 8 Histogram of the enhancement of N-methyl-D-aspartate (NMDA) depolarization induced by (-)-isoprenaline 0.2 μM, adenosine 20 μM or 5-hydroxytryptamine (5-HT) 10 μM in control slices (open columns) or in the presence of propranolol 40 μM (solid columns). Columns show the mean with 1 s.e.mean indicated by vertical bars ($n = 8$).

** $P < 0.05$ compared with NMDA alone. † $P < 0.05$ compared with control agonist responses.

Figure 9 Histogram of the enhancement of N-methyl-D-aspartate (NMDA) depolarization induced by (-)-isoprenaline 0.2 μM, 5-hydroxytryptamine (5-HT) 10 μM or adenosine 20 μM in control slices (open columns) or following 14 days treatment with propranolol 25 mg kg⁻¹ daily (solid columns). Columns show the mean with 1 s.e.mean shown by vertical bars ($n = 6$).

** $P < 0.05$ compared with NMDA alone. † $P < 0.05$ compared with agonist in propranolol naive slices.

Effect of propranolol superfusion

When slices were superfused with (±)-propranolol at a concentration of 40 μM, the potentiation of NMDA responses by (-)-isoprenaline (200 nM) or (+)-isoprenaline (10 μM) was abolished. Similarly, the potentiating effect of 5-HT (10 μM) was prevented by propranolol. Responses to adenosine were not changed significantly (Figure 8).

Subacute and chronic treatment with propranolol

Administration of propranolol at a dose of 25 mg kg⁻¹ 24 h before death caused only small changes in the size of responses to (-)-isoprenaline (200 nM), (+)-isoprenaline (10 μM) or adenosine (20 μM), which were not significant. There was a somewhat larger reduction in the mean amplitude of responses to 5-HT (10 μM) but this did not reach statistical significance.

Chronic treatment with the same dose of propranolol, 25 mg kg⁻¹ daily for 14 days, yielded a marked and significant loss of sensitivity to both (-)-isoprenaline and 5-HT (Figure 9). Responses to adenosine, however, were unaffected by chronic administration of propranolol.

A lower dose of propranolol (5 mg kg⁻¹ daily given for 14 days) resulted in a small reduction of sensitivity to 5-HT which did not attain significance, but induced an elevation in the size of responses to (-)-isoprenaline (Figure 10). No statistically significant changes were seen in sensitivity to adenosine.

Discussion

The use of NMDA to provide a depolarization of neocortical neurones upon which the effects of other agents can be examined appears to be validated by the pharmacological confirmation that conventional receptors are involved in the effects of isoprenaline (stereospecific and blocked by propranolol) and adenosine (blocked by theophylline and 8-phenyltheophylline). The observation that adenosine enhanced the NMDA-induced depolarization rather than reducing it was unexpected. Adenosine is generally considered to be inhibitory on cortical neurones (Stone, 1989) increasing potassium conductance (Segal, 1981; Haas & Greene, 1984; Trussel & Jackson, 1985) and suppressing neuronal firing and transmitter release (Stone, 1981; 1982; Phillis, 1982). The enhancement of NMDA sensitivity may result from a disinhibitory action of adenosine within the cortex.

Figure 10 Histogram of the enhancement of N-methyl-D-aspartate (NMDA) depolarization induced by (-)-isoprenaline $0.2 \mu\text{M}$, 5-hydroxytryptamine (5-HT) $10 \mu\text{M}$ or adenosine $20 \mu\text{M}$ in control slices (open columns) or following 14 days treatment with propranolol 5 mg kg^{-1} daily (solid columns). Columns show the mean with 1 s.e. mean shown by vertical bars ($n = 6$ or 7).

** $P < 0.05$ compared with NMDA alone. † $P < 0.05$ compared with agonist in propranolol-naïve slices.

The potentiation of NMDA by isoprenaline is, however, consistent with reports that activation of β -adrenoceptors can have a net excitatory effect on central neurones. Thus noradrenaline can potentiate the excitatory effects of glutamate by an action at β -adrenoceptors (Madison & Nicoll, 1986; Wohlberg *et al.*, 1987; Marshall & Tsai, 1988; Mori-Okamoto & Tatsumo, 1989). This effect can occur without significant changes of spontaneous firing or against a background of reduced firing and hyperpolarization (Mori-Okamoto & Tatsumo, 1989), and probably results from a reduced calcium-activated potassium conductance (Haas & Rose, 1987; Madison & Nicoll, 1986). The nature of the 5-HT receptor involved in potentiating responses to NMDA has not been examined in this study in view of the complexity of 5-HT receptor pharmacology. These responses were, nevertheless, antagonized by propranolol which has become recognised as an antagonist at 5-HT₁ receptors in other neuronal systems (Weinstock & Schechter, 1975; Nahorski & Willcocks, 1983; Middlemiss, 1984; Richards, 1985). The failure of propranolol to affect responses to adenosine strongly suggests that it was not having any significant non-specific neuronal membrane stabilising action which might otherwise account for the antagonism of isoprenaline and 5-HT.

Chronic treatment with the lower dose of propranolol (5 mg kg^{-1} daily) for two weeks resulted in a significant increase in sensitivity to (-)-isoprenaline, consistent with previous work indicating an increase in the density of β -adrenoceptors in the CNS following propranolol treatment (Glaubiger & Lefkowitz, 1977; Wolfe *et al.*, 1978; 1982; Aarons & Molinoff, 1982). However, when animals were treated with propranolol at the higher dose of 25 mg kg^{-1} daily, sensitivity to (-)-isoprenaline was substantially diminished, a change noted also with responses to 5-HT. This may reflect a secondary down-regulation of receptor sensitivity or number resulting from a period of increased receptor sensitivity as obtained with the lower dose of propranolol. Again the absence of any change of adenosine responses excludes the involvement of membrane stabilisation or other non-specific membrane effects in the reduction of amine sensitivity.

Since the net effect of a high dose propranolol was qualitatively similar to the effect of acute superfusion it is important to note that, while little work has been done in mice, propranolol is rapidly metabolised by man, rat and guinea-pig with elimination half life estimates between 47 min and 3 h (Smits & Struyker-Boudier, 1979; Terao & Shen, 1983; 1984; Wooltrey *et al.*, 1989). It is thus unlikely that residual propranolol could accumulate sufficiently to account for the present observations 24 h after the last dose. The effects of low dose chronic propranolol are qualitatively different from acute superfusion so that residual propranolol cannot account for these effects.

Chronic administration of xanthines in contrast, has been shown clearly to increase the density of receptors for adenosine or its radiolabelled analogues. This result seems to follow administration of caffeine (Fredholm, 1982; Boulenger *et al.*, 1983; Marangos *et al.*, 1984; Daval *et al.*, 1989) or theophylline (Murray, 1982; Szot *et al.*, 1987; Zielke & Zielke, 1987; Sanders & Murray, 1988; Fastbom, 1988). In one of these studies a correlation was made between the increased number of adenosine receptors and the observed increase of seizure threshold (Szot *et al.*, 1987). In a different study Fredholm (1982) concluded that there was no relationship between the caffeine-induced increase of binding of phenylisopropyladenosine and the stimulation of adenosine 3':5'-cyclic monophosphate (cyclic AMP) levels in the brain. This may merely reflect a preferential activity of the analogue at A₁ receptors (Stone, 1989), although both A₁ and A₂ receptors, examined separately do seem to up-regulate with caffeine treatment (Hawkins *et al.*, 1988; Daval *et al.*, 1989).

It is unlikely that any of these effects of theophylline can be attributed to the persistence of the xanthine in the brain since the tests performed with single injections of theophylline 24 h before death revealed no loss of adenosine sensitivity.

Furthermore kinetic studies have shown that little theophylline can be detected in brain 24 h after injection (Fredholm *et al.*, 1983) and since theophylline blockade of adenosine receptors is readily reversible *in vitro* the continuous flow of superfusion medium should rapidly relieve adenosine receptors from any residual xanthine. Indeed the half life of theophylline in different species has been measured at between 1 and 5 h (Walle & Walle, 1979; Madsen & Ribel, 1981; Chiou *et al.*, 1982). These various points together with the delay of at least 7 days before agonist responses returned to control levels after ceasing theophylline administration argues strongly against the possibility that theophylline could have accumulated in the brain sufficiently to account for the loss of adenosine sensitivity.

Previous biochemical work has led to reports that chronic xanthines can increase the inhibition of adenylate cyclase (Green & Stiles, 1986) and transmitter release by adenosine (Fredholm *et al.*, 1984) although it has also been emphasised that major regional differences exist in the ability of xanthines to alter adenosine sensitivity (Fastbom, 1988). The present study has attempted to examine a functional response to adenosine in an electropharmacological system. The results have demonstrated that, in contrast to the up-regulation of binding sites described previously, theophylline causes a dramatic loss of sensitivity to adenosine. The present work therefore emphasises the importance of confirming directly any changes of adenosine function postulated to occur on the basis of binding data alone. An additional finding was the reduced sensitivity to isoprenaline after theophylline treatment. This would be more consistent with binding studies which have revealed a down-regulation of β -adrenoceptor number after theophylline treatment (Goldberg *et al.*, 1982; Fredholm *et al.*, 1984). Since adenosine is known to suppress the release of noradrenaline from both peripheral and central neurones this phenomenon could be secondary to a reduction of adenosine sensitivity in the CNS as demonstrated here, with a consequent loss of adenosine inhibitory 'tone' and increased noradrenaline release.

In conclusion, the mouse neocortical slice may be a suitable preparation with which to investigate quantitatively changes of receptor function in the central nervous system following chronic treatment with drugs. The present data suggest that an explanation of some components of the xanthine withdrawal syndrome should be sought in terms of a paradoxical reduction of adenosine sensitivity rather than the increase predicted from binding data. Since alterations in sensitivity to isoprenaline have also been detected, however, β -adrenoceptors may also be involved.

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