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Dihydropyridines alter adenosine sensitivity in the rat hippocampal slice

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1 The effects of adenosine and a range of adenosine analogues, which are resistant to uptake processes, were studied in the presence of dihydropyridines and verapamil on the population spike potential recorded from the CA1 area of the hippocampal slice.

2 Nifedipine and Bay K 8644, a calcium channel antagonist and activator respectively, enhanced the inhibitory action of adenosine in a concentration-dependent manner. This was in contrast to their effect on adenosine analogues where the inhibition of the population potential was significantly attenuated. Similiar interactions between the adenosine compounds and the dihydropyridines were also displayed in studies on spontaneous epileptiform activity in the CA3 region.

3 This effect of nifedipine and Bay K 8644 was not shown by the dihydropyridines, nimodipine or nitrendipine, or by the phenylalkylamine, verapamil.

4 Addition of the adenosine uptake blocker dipyridamole reversed the action of nifedipine on adenosine, so that inhibition by adenosine was now attenuated by nifedipine in a similar manner to that observed with the adenosine analogues.

5 These results can be explained with reference to binding studies that show displacement of adenosine analogues from the adenosine receptor by dihydropyridines. An action at the adenosine uptake site by the dihydropyridines explains the enhancement of adenosine inhibition.

6 The possible sites for this interaction are discussed.

Introduction

Binding sites exist in the central nervous system for several members of the dihydropyridine series of calcium channel activators, such as Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate), and blockers such as nifedipine, nimodipine and nitrendipine (Gould *et al.*, 1982; Bellemann *et al.*, 1983). This binding exhibits well defined regional distributions within the rat brain (Skatteböl & Triggle, 1987), the highest density of receptors being found in the hippocampus and olfactory bulb. However, a functional effect of dihydropyridines on calcium channels in neuronal tissue has so far proved difficult to demonstrate. Middlemiss & Spedding (1985), when studying potassium-stimulated, calcium-dependent [^{3}H]-5-hydroxytryptamine release from rat cortical slices, showed that Bay K 8644 could augment 5-hydroxytryptamine release. Nifedipine reversed the augmentation but had no effect on potassium-stimulated release. A partial inhibition of potassium-stimulated ^{45}Ca uptake into cultured cerebellar granule cells by dihydropyridines has been demonstrated by Carboni *et al.* (1985), while Thayer *et al.* (1986) were able to show that nitrendipine could reduce, but not abolish, calcium influx into cultured neuronal cells from various regions of the brain, determined by use of fura 2. In electrophysiological studies, Docherty & Brown (1986), using voltage clamp techniques on hippocampal pyramidal cells in slices, have shown a partial inhibition of a persistent L-type calcium channel by nimodipine. However, the dihydropyridines appear to have no effect on synaptic transmission. Studies of ^{45}Ca uptake into rat brain synaptosomes have failed to show any significant action of the dihydropyridines on calcium uptake (Daniell *et al.*, 1983; Rampe *et al.*, 1984) and there are few accounts of dihydropyridine action on electrophysiological studies of synaptic transmission in the CNS.

Adenosine, on the other hand, is an endogenous neuromodulator that is capable of inhibiting synaptic transmission, particularly in the hippocampus (Dunwiddie & Hoffer, 1980). This effect appears to be mediated presynaptically and is probably due to a reduction of calcium influx into presynaptic terminals causing a reduced release of neurotransmitter (Stone, 1981; Schubert & Kreutzberg, 1987; Bartrup *et al.*, 1988).

Ligand binding studies have demonstrated that some dihydropyridines, most notably nifedipine and Bay K 8644, are able to reduce adenosine analogue binding to brain membranes, while having no effect on other receptors such as α- and β-adrenoceptors, muscarinic cholinoreceptors, opiate or γ-aminobutyric acid (GABA) receptors (Morgan *et al.*, 1987). These findings raise the possibility of a close functional relationship between adenosine receptors and dihydropyridine binding sites.

In contrast, the inhibitory action of adenosine on acetylcholine excited cortical neurones is enhanced by the addition of nifedipine (Phillis *et al.*, 1984). Binding studies have also revealed that the dihydropyridines can interact with the adenosine uptake system, indicating that the dihydropyridines may have a marked effect on adenosine metabolism which could explain the potentiation of adenosine.

So far, the existence of these interactions between the dihydropyridines and adenosine compounds, suggested by binding and uptake studies, has not been studied in detail in a functional model. As the hippocampus is rich in both dihydropyridine (Skatteböl & Triggle, 1987) and adenosine (Fastbom *et al.*, 1987) binding sites, the hippocampal slice was used in the present work to study the effect of adenosine and adenosine analogues on CA1-evoked field potentials, and the effect of the dihydropyridines on this action.

Methods

Male Wistar rats (150–250 g) were killed by stunning and cervical dislocation and the hippocampus was dissected out into ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (mM): KH_2PO_4 2.2, $MgSO_4$ 1.2, KCl 2, glucose 10, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5, gassed with 95% O_2/5% CO_2. Five hundred μm transverse slices were prepared and incubated at room temperature for 1 h before trans-

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fer to the recording chamber. Individual slices were then transferred to a recording chamber where they were submerged in the ACSF and superfused at a rate of 4 ml min^{-1} at 30°C. A concentric bipolar stimulating electrode was placed in the stratum radiatum in the Schaffer collateral-commissural pathway. Evoked field potentials were recorded from the CA1 pyramidal cell body layer. Stimulation was at 250–350 μA, 0.1 ms, 0.1 Hz. The resulting field potential was recorded via a Grass amplifier on a digital storage oscilloscope, from which the population potential was plotted on a Grass chart recorder. The size of the negative going population potential was measured from the chart records. The effects of the drugs on the potential were determined as a percentage change with respect to potential size immediately before drug application.

All experiments were carried out under sodium lamp lighting to protect the light-sensitive dihydropyridines. The dihydropyridines were dissolved in ethanol as a 10 mM stock solution. The maximum final ethanol concentration added to the slice was never more than 0.1% and control ethanol concentrations of up to 1% had no effect on the population potential. Control responses to adenosine compounds were performed in triplicate before the addition of the dihydropyridines, which were then perfused for 10 min before the adenosine compounds were again added. A washout period of 20 min was allowed between each dihydropyridine concentration during preparation of the concentration-response curves.

Spontaneous epileptiform activity was induced by omitting magnesium from the ACSF or by increasing the potassium concentration to give a final level of 8.5 mM. The bursts were recorded via an electrode placed in the pyramidal cell layer of the CA3 region and recorded direct from the amplifier on a Grass chart recorder. The action of the drugs was measured as a percentage change in burst frequency, measured over a ten min period when the burst frequency had stabilized at its new level with respect to burst frequency prior to drug application.

All values were calculated as mean ± standard error of the mean (s.e.mean). Statistical significance was determined by a paired t test.

Results

The well documented action of adenosine compounds on hippocampal-evoked field potentials is to reduce synaptic transmission, as assessed by a reduction in the size of the population potential. The potency of adenosine in this system has been determined in previous experiments carried out in this laboratory (Bartrup & Stone, 1988). It was decided to use a single adenosine concentration of 25 μM throughout these experiments, as this gave a significant decrease in potential size that was not totally abolished in the presence of nifedipine. The addition of 25 μM adenosine inhibited population potential by 59 ± 3.4%, $n = 21$. The decrease in potential size was apparent within 1 min of the addition of adenosine and a maximum decrease was obtained within 2–3 min.

The addition of nifedipine alone to the perfusion medium had no detectable effect on the size of the population potential. In trial experiments, a range of nifedipine concentrations from 1 to 50 μM were perfused for up to 15 min with no change in potential size. When adenosine was added after nifedipine had been perfused for a minimum of 10 min, the inhibitory effect of adenosine on the population potential was increased. This effect was concentration-dependent (Figure 1).

The adenosine analogue 2-chloroadenosine also reduced the potential size, a stable depression of the potential being obtained within 10 min of application. However, in contrast to adenosine, the presence of nifedipine resulted in a significant reduction in the inhibitory action of 2-chloroadenosine (Figure 1).

These experiments were repeated with the dihydropyridine calcium channel activator Bay K 8644. This exerted a similar effect on the adenosine compounds to that observed with

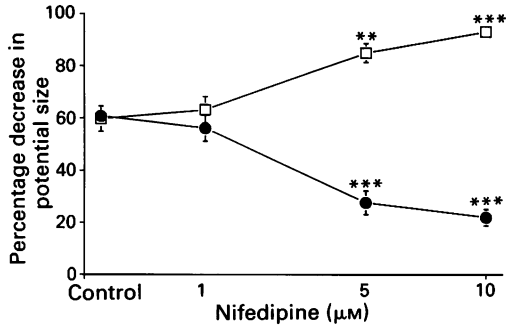


Figure 1 Percentage decrease in the CA1 evoked population potential size by adenosine and 2-chloroadenosine in the presence of increasing concentrations of nifedipine. Points are mean and vertical lines indicate s.e.mean of 8–14 experiments. (□) Adenosine 25 μM, (●) 2-chloroadenosine 0.5 μM. ** $P < 0.05$, *** $P < 0.01$, paired t test.

nifedipine, the inhibitory effect of adenosine being enhanced while that of 2-chloroadenosine was diminished at concentrations of 1–10 μM Bay K 8644 (Figure 2). With both nifedipine and Bay K 8644 the response to adenosine or 2-chloroadenosine was not restored to control levels even if the dihydropyridines were washed out for up to an hour. If, however, increasing concentrations of 2-chloroadenosine were added during this washout period, the action of nifedipine could be overcome. Once this potential size was restored, subsequent additions of 2-chloroadenosine at the original concentration (0.5 μM) elicited responses comparable to control values. Increasing the concentration of 2-chloroadenosine while nifedipine was present also increased the percentage inhibition of the population potential (Figure 3).

The adenosine analogues cyclohexyladenosine (CHA), N-ethylcarboxamidoadenosine (NECA) and R-phenylisopropyladenosine (R-PIA) were also affected by the addition of nifedipine in a similar manner to that observed with 2-chloroadenosine. Concentrations of the analogues were selected which produced an approximately 100% depression of the CA1 population potential. In the presence of 10 μM nifedipine these responses were then reduced by at least 50% (Figure 4).

In some experiments, a steady depression of the population potential by 25 μM adenosine was obtained, after which 10 μM nifedipine was added with the adenosine present. The inhibition of the potential then progressively increased to reach a new plateau in 28 ± 1.9 min ($n = 5$). At this time the potential had declined to 25 ± 4.9% of the size in adenosine alone. In similar experiments, nifedipine was added in the presence of 2-chloroadenosine. The inhibition of the potential gradually decreased to a new plateau over 43 ± 2.8 min ($n = 5$), at which time the response size was within 5% of the potential size prior to 2-chloroadenosine addition (Figure 5).

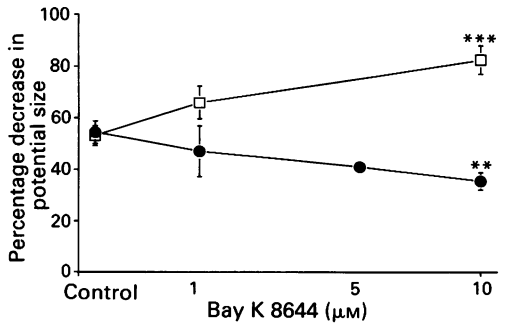


Figure 2 The action of adenosine and 2-chloroadenosine on the population potential in the presence of increasing concentrations of Bay K 8644. Points are mean and vertical lines indicate s.e.mean of 8–14 experiments. (□) Adenosine 25 μM, (●) 2-chloroadenosine 0.5 μM. ** $P < 0.05$, *** $P < 0.01$, paired t test.

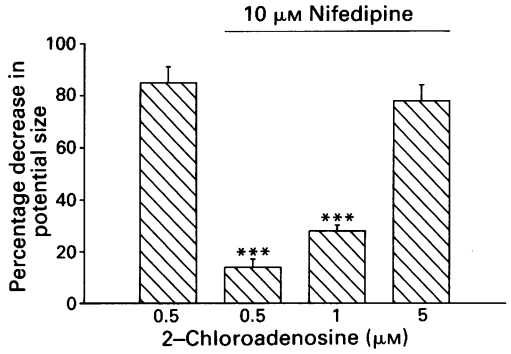


Figure 3 Percentage decrease in the CA1-evoked population potential size by 2-chloroadenosine in the presence of 10 μM nifedipine. Increasing the 2-chloroadenosine concentration overcame the block by nifedipine. Columns represent mean and bars show s.e.mean of 5 experiments. *** $P < 0.001$, paired t test.

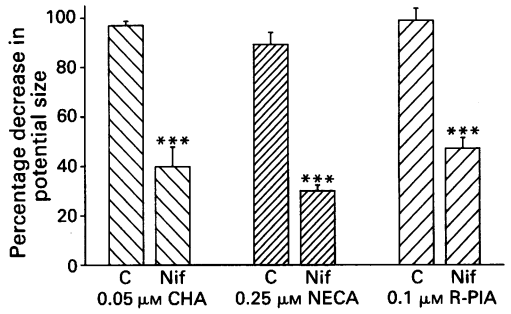


Figure 4 Columns represent the percentage inhibition of the population potential by three adenosine analogues, cyclohexyladenosine (CHA), N-ethylcarboxamidoadenosine (NECA) and R-phenylisopropyladenosine (R-PIA). C = control, Nif = 10 μM nifedipine. The addition of nifedipine significantly reduced the inhibition. Columns represent the mean and bars s.e.mean of experiments. *** $P < 0.01$, paired t test.

The dihydropyridines, nimodipine and nitrendipine and the phenylalkylamine, verapamil were also added to the hippocampal slices. These had little effect on the adenosine compounds at concentrations of 1–10 μM, with only nitrendipine showing a small but significant increase in the action of adenosine at a concentration of 50 μM (Table 1).

Dipyridamole

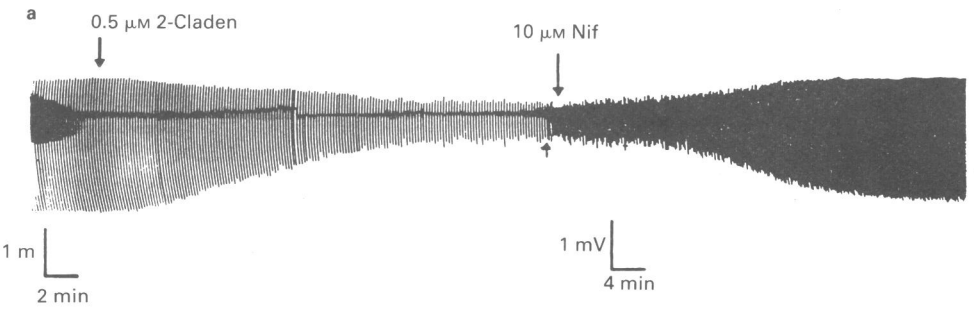
A final set of experiments was carried out to determine the contribution of the nucleoside transport system to the action of adenosine. Adenosine (10 μM) was added to the slice to give a stable depression of the population potential of around 10–20%. The adenosine uptake inhibitor, dipyridamole was then added at a concentration of 10 μM. This increased the level of inhibition by adenosine to approximately 50%. When nifedipine (10 μM) was added together with the adenosine and dipyridamole, the inhibition of the potential was attenuated, although the level of inhibition was not reduced to control levels (Figure 6).

Antiepileptiform activity

In addition to studies on the evoked population potentials, the effect of nifedipine on the antiepileptiform action of adenosine and 2-chloroadenosine was determined. Adenosine compounds are known to exhibit potent antiepileptiform properties in *in vitro* models of epileptiform activity (Ault & Wang, 1986). The action of adenosine and 2-chloroadenosine on spontaneous activity arising in the CA3 region as a result of raised potassium concentration (8.5 mM) or omission of magnesium from the ACSF, was studied in the absence or presence of nifedipine (Table 2). Nifedipine (10 μM) enhanced the antiepileptiform action of adenosine in both models of epileptiform activity, while reducing the action of 2-chloroadenosine. Nifedipine on its own had no effect on spontaneous activity at 10 μM.

Discussion

Nifedipine and Bay K 8644 appear to have opposite effects on adenosine and the uptake resistant adenosine analogues,



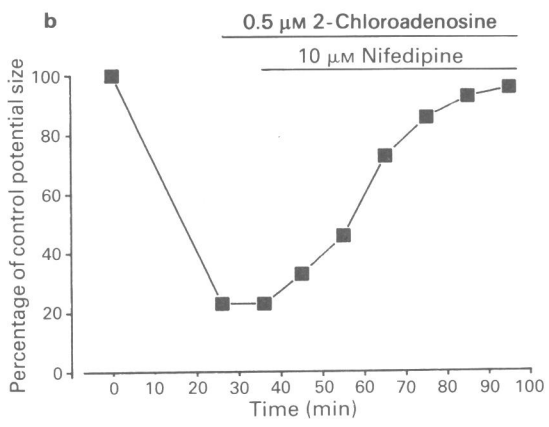


Figure 5 Inhibition of the CA1 population potential by 0.5 μM 2-chloroadenosine (2-Claden) could be overcome by the addition of 10 μM nifedipine (Nif). (a) Sample chart record. Each deflection represents the size of the evoked population potential at 0.1 Hz stimulation rate. 2-Chloroadenosine (0.5 μM) was present throughout the experiment. The time base has been reduced at the point indicated (↑). (b) Graphical representation of (a) showing the inhibition by 2-chloroadenosine over time in the presence of nifedipine. The points represent the size of the population potential as a percentage of the control response (100%).

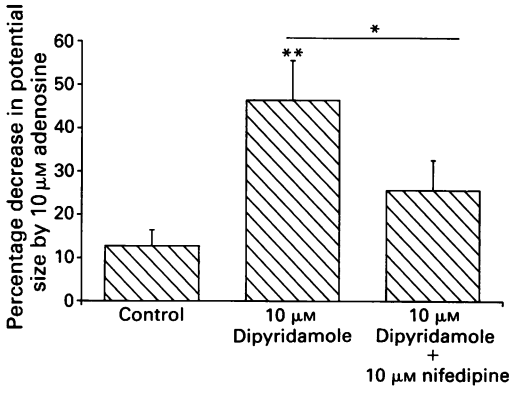


Figure 6 The addition of dipyridamole enhanced the percentage inhibition of the population potential by 10 μM adenosine. In the presence of dipyridamole, 10 μM nifedipine reduced the level of inhibition by adenosine in a similar manner to its effect on the adenosine analogues. Columns represent the mean and bars shown s.e.mean of 6 experiments. * $P < 0.1$, ** $P < 0.05$, paired t test.

enhancing the inhibitory action of adenosine while reducing that of the adenosine analogues. This effect was apparent on spontaneous activity induced by two sets of conditions as well as evoked potentials and appears to be specific to nifedipine and Bay K 8644, as the other calcium channel antagonists used, nitrendipine, nimodipine and verapamil, had no comparable activity.

The results obtained in these experiments may be explained by referring to binding studies which show the displacement of adenosine analogues by the dihydropyridines. Murphy & Snyder (1983) demonstrated that nifedipine could displace [^{3}H]-CHA from adenosine receptors in rat and bovine brain. Nisoldipine and nitrendipine exerted a partial effect while nimodipine and verapamil were virtually ineffective. Subsequently, more extensive studies have confirmed these initial findings (Morgan *et al.*, 1987; Hu *et al.*, 1987; Cheung *et al.*, 1987). Morgan *et al.* (1987) also found that Bay K 8644 was as potent as nifedipine in displacing [^{3}H]-CHA. An interaction of this nature would readily explain the effect of nifedipine and Bay K 8644 on the adenosine analogues, with the dihydropyridines directly displacing the analogues from the adenosine receptor, or possibly acting via an allosteric interaction to reduce the affinity of the receptor for the adenosine compounds.

In contrast, the enhancement of adenosine action by the dihydropyridines may involve an interaction at the nucleoside transport system. Marangos *et al.* (1984) showed that the dihydropyridines could potently displace [^{3}H]-NBI (nitrobenzylthioinosine), a nucleoside transport inhibitor, from binding sites in dog brain and heart. Similar results were obtained by Morgan *et al.* (1987) in rat brain. In this case, the dihydropyridines were all roughly equipotent in displacing [^{3}H]-NBI and Scatchard analysis indicated that binding was strictly competitive. In functional studies, the uptake of [^{3}H]-adenosine into rat brain synaptosomes has been shown directly to be reduced in the presence of the dihydropyridines (Phillis *et al.*, 1984; Morgan *et al.*, 1987). As with the adenosine receptor binding studies, verapamil and diltiazem had no significant effect on [^{3}H]-adenosine uptake or [^{3}H]-NBI binding.

The present studies also provide evidence that an action on adenosine uptake by nifedipine is involved in enhancing adenosine action. The addition of dipyridamole enhanced the inhibitory action of a low concentration of adenosine, as might be expected. However, the addition of nifedipine under these conditions reversed the inhibition of the population potential by adenosine, suggesting that nifedipine, unable to depress uptake further, was now acting to reduce the effects of adenosine.

Sites of interaction

The exact site at which the dihydropyridines bind to interact with adenosine receptors or uptake sites is not clear. As adenosine may act on presynaptic calcium channels to reduce neurotransmitter release (Stone, 1981), it is possible that the adenosine receptor and dihydropyridine binding site occur at a shared calcium channel. However, dihydropyridines have little or no effect on synaptic transmission, as is apparent in this study and by the inability of dihydropyridines to reduce calcium uptake into synaptosomes (Daniell *et al.*, 1983), while adenosine can completely inhibit synaptic transmission. It would therefore seem unlikely that there is a common site of action on synaptic calcium channels.

It is also possible that adenosine receptors exist on postsynaptic dendrites, acting to reduce synaptic transmission by activating potassium channels and hyperpolarizing the postsynaptic membrane (Gerber *et al.*, 1989). The interaction between adenosine compounds and nifedipine was also apparent on spontaneous epileptiform activity induced by both magnesium-free and high potassium ACSF. Adenosine inhi-

Table 1 Percentage inhibition of the CA1-evoked population potential by adenosine and 2-chloroadenosine (2-Claden) in the presence or absence of a calcium channel antagonist

	Nimodipine (50 μM)		*Nitrendipine* (50 μM)		*Verapamil* (10 μM)	
	Control	*Test*	*Control*	*Test*	*Control*	*Test*
Adenosine 25 μM	27.5 ± 4.3	37.5 ± 3.3	52.0 ± 3.1	60.5 ± 1.1*	ND	ND
2-Claden 0.5 μM	55.4 ± 2.2	64.8 ± 4.7	62.1 ± 4.3	72.3 ± 2.7	54.7 ± 4.7	63.0 ± 5.3

Each value is the mean ± s.e.mean of 4 experiments.
* $P < 0.1$, paired t test.
ND = not determined.

Table 2 Effect of 10 μM nifedipine on the antiepileptiform action of adenosine and 2-chloroadenosine (2-Claden)

	Magnesium-free ACSF		*High potassium ACSF*	
	Control	*Nifedipine*	*Control*	*Nifedipine*
Adenosine 5 μM	54.3 ± 3.8	83.3 ± 4.7***	40.0 ± 4.4	72.6 ± 3.0***
2-Claden 0.5 μM	66.3 ± 7.6	23.0 ± 2.1***	72.0 ± 4.8	28.0 ± 2.1***

Values are the percentage decrease in the burst frequency of spontaneous activity induced in the CA3 area by magnesium-free or high potassium (8.5 μM) artificial cerebrospinal fluid (ACSF)
Values are mean ± s.e.mean of 6 experiments.
*** $P < 0.01$, paired t test.

bits spontaneous activity at significantly lower concentrations than are required to reduce synaptic transmission in the hippocampus (Ault & Wang, 1986). It is therefore possible that the antiepileptiform action of adenosine is mediated by adenosine action at postsynaptic receptors linked to potassium channels. This is supported by data showing that adenosine can inhibit spontaneous depolarizations in low calcium ACSF, an effect that is independent of synaptic transmission (Lee *et al.*, 1984). The fact that nifedipine affected the antiepileptiform action of adenosine and 2-chloroadenosine suggests that the dihydropyridines may interact with the postsynaptic adenosine receptor.

The high density, high affinity dihydropyridine receptor is also unlikely to be the binding site for this particular interaction for a number of reasons. Nitrendipine and nimodipine have been shown to bind with high affintiy in the brain but have only weak effects on adenosine action, in comparison to Bay K 8644 and nifedipine. Secondly, the concentration of dihydropyridines required to have an effect on adenosine is several orders of magnitude greater than that required to saturate high affinity dihydropyridine binding sites (Belleman *et al.*, 1983) or to exert an action on calcium channels in smooth muscle. Finally, Morgan *et al.* (1987) showed that CHA or 2-chloroadenosine had no effect on [^{3}H]-nitrendipine binding, indicating that there was no common binding site for adenosine analogues on the high affinity nitrendipine binding site.

However, a separate low affinity dihydropyridine binding site may exist that interacts allosterically with the adenosine receptor. Bay K 8644 and nifedipine both exhibited a mixed competitive/non-competitive effect on ligand binding to the adenosine receptor (Morgan *et al.*, 1987). This suggests that Bay K 8644 and nifedipine are able to act at an additional binding site not available to other dihydropyridines. It is interesting that both nifedipine and Bay K 8644, a calcium channel antagonist and activator respectively, both exert the same effect on the adenosine compounds with a similar potency. There are only small structural differences between the two compounds and dihydropyridine activators can act as antagonists under certain conditions (Triggle & Rampe, 1989). For these compounds to act at a separate binding site, a structural characteristic between them might be expected that distinguishes them from the other dihydropyridines. Indeed, nifedipine and Bay K 8644, and also nisoldipine which can displace [^{3}H]-CHA from its binding site (Murphy & Snyder, 1983), possess 2 substituted phenyl moieties, while nimodipine and nitrendipine, that have little or no effect on binding, possess 3-nitrophenyl groups (Morgan *et al.*, 1987). It is possible, therefore, that a distinct low-affinity dihydropyridine binding site exists that preferentially binds the former molecules and is associated with the adenosine receptor. A low-affinity dihydropyridine binding site has been demonstrated in cardiac tissue (Sarmiento *et al.*, 1987). A study of [^{3}H]-nifedipine binding in brain, as opposed to the [^{3}H]-nitrendipine used by Morgan *et al.* (1987), may demonstrate the existence of a low-affinity receptor site from which ligands may be displaced by the presence of adenosine ligands.

The most straightforward interaction between the dihydropyridines and adenosine would be a simple competitive interaction at the adenosine receptor. In the present experiments, the action of nifedipine on 2-chloroadenosine could be overcome by increasing the concentration of 2-chloroadenosine suggesting that this may be the case.

The action of nifedipine and Bay K 8644 at the nucleotide transport system is also likely to be competitive (Morgan *et al.*, 1987), although in the present study nimodipine and nitrendipine were significantly less potent in enhancing the action of adenosine. This is in contrast to the binding studies where all the dihydropyridines studied were approximately equipotent in displacing [^{3}H]-NBI (Morgan *et al.*, 1987). The reason for this is unclear.

These results provide evidence from a functional approach to support the extensive ligand binding studies, highlighting the action of selective dihydropyridines on both the adenosine receptor and the nucleoside transport system. Because of the relatively high concentration of dihydropyridines required, it is unlikely that the clinical use of these compounds involves any action on endogenous adenosine. However, some of the side-effects, particularly of nifedipine, that have been observed during clinical use, may involve an interaction with adenosine (Swanson & Green, 1986). Similarly, some of the behavioural effects of the dihydropyridines, such as mild sedation and interference with sleep patterns (Hoffmeister *et al.*, 1982), or anticonvulsant effects (Meyer *et al.*, 1986), may be explained by an interaction with the endogenous adenosine system, since adenosine or its receptors may be involved in each of these behaviours (Stone, 1989).

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