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Comparative potencies of calcium channel antagonists and antischizophrenic drugs on central and peripheral calcium channel binding sites

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Dihydropyridines are potent agents on [^{3}H]nitrendipine binding sites in heart and brain membranes. Like the phenylalkylamines, they are slightly more active on heart than on brain [^{3}H]nitrendipine binding sites. On the other hand, the diphenylalkylamines, the diphenylpiperazines and the antischizophrenic drugs of the diphenylbutylpiperidine type are more potent on brain [^{3}H]nitrendipine binding sites. The findings suggest tissue heterogeneity of [^{3}H]nitrendipine binding sites and the possible development of diphenylbutylpiperidine-diphenylbutylpiperazine analogues that could selectively act on brain calcium channel antagonist binding sites.

Organic calcium channel antagonists have emerged as major therapeutic agents in the treatment of various cardiovascular disorders (Antman et al 1980; Stone et al 1980). Various classes of calcium antagonists have been developed including the 1,4-dihydropyridines (e.g. nifedipine, nitrendipine), the phenylalkylamines (e.g. verapamil), the diphenylbutylpiperazines (e.g. lidoflazine) and the diphenylalkylamines (e.g. phenylamine) (Janis & Triggle 1983: Janis & Scriabine 1983).

Recently, some of these drugs have been radioactively labelled and the existence of specific binding sites for the 1,4-dihydropyridines (Bellemann et al 1981; Bolger et al 1983; Ehlert et al 1982; Ferry & Glossmann 1982a; Janis et al 1982; Murphy & Snyder 1982; Ferry et al 1983; Gould et al 1984; Janis & Triggle 1984), verapamil and derivatives (Reynolds et al 1983; Ferry et al 1984; Galizzi et al 1984; Goll et al 1984) and diltiazem (Glossmann et al 1983) has been described in various tissues. It is currently believed that non-dihydropyridine calcium channel antagonists act at one or more sites allosterically linked to the 1,4-dihydropyridine sites (Ehlert et al 1982; Ferry & Glossmann 1982b; Glossman et al 1982; Janis et al 1982; Yamamura et al 1982; Murphy et al 1983; Miller & Freedman 1984). Autoradiographic studies have shown that [^{3}H]1,4-dihydropyridines (Murphy et al 1982; Cortes et al 1983; Quirion 1983) and [^{3}H]desmethoxyverapamil (Ferry et al 1984) binding sites are similarly distributed in various regions of the brain. These sites are mainly concentrated in the dentate gyrus, superficial layers of the cortex and external plexiform layers of the olfactory bulb (Quirion 1983).

It has been reported that antischizophrenic drugs of the diphenylbutylpiperidine type can inhibit smooth muscle contraction, possibly by an action on calcium channels (Quintana 1978; Spedding 1982). Moreover, Gould et al (1983) have recently demonstrated that these drugs are potent inhibitors of [^{3}H]nitrendipine binding sites in rat brain membranes. It suggests that certain actions of these antischizophrenic drugs could be related to a blockade of calcium channels. We report here that these drugs appear to interact preferentially with brain [^{3}H]nitrendipine binding sites. They are less potent in cardiac membrane preparations. It suggests that sites labelled by [^{3}H]nitrendipine are slightly different sites in these two tissues. Moreover, calcium channel antagonists structurally related to the diphenyl-

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butylpiperidines show slight selectivity toward brain calcium channel antagonist binding sites.

Materials and methods

Cerebral cortices and hearts were obtained from 300 g male Sprague-Dawley rats (Charles River, Canada). After both had been rinsed in 0·9% NaCl at 4 °C, membranes were prepared as follows: minced cortices and hearts were homogenized in 20 volumes of 50 mM Tris HCl buffer, pH 7·4 at 4 °C using a Brinkmann polytron (setting 5–6, 15 s for cortices; 20 s for hearts). Homogenates were then centrifuged for 15 min at 30 000*g*. Supernatants were discarded and pellets washed three times and then homogenized as above. Final pellets were washed twice and resuspended in buffer to appropriate protein concentrations.

For binding assays, 200 µl of membrane preparation were added to 250 µl of 50 mM Tris. HCl buffer, pH 7·4 at 22 °C and 0·25 nM of [^{3}H]nitrendipine (87·4 Ci mmol^{-1}, New England Nuclear) and various concentrations of drugs (8–10 doses) for a total incubation volume of 2 ml. Incubations were for 60 min at 22 °C and terminated by rapid filtration through Whatman GF/B filters (3 washes of 3 ml cold buffer). Specific binding was calculated as the difference in radioactivity bound in the presence and absence of 10 µM pimozide. Binding of the tritiated ligand to filters was quantified by counting filters in 5·5 ml Scinti-Verse II (Fisher Scientific Ltd, Canada) scintillation cocktail. All data were analysed using a computerized curve fitting system to derive the K_I and n_H values.

Results and discussion

As shown in Table 1, the dihydropyridines are the most potent inhibitors of [^{3}H]nitrendipine binding in rat brain and heart membrane preparation. Among the various calcium channel antagonists tested, the dihydropyridines are at least 200 times more active than the phenylalkylamine, verapamil, in both assays. Other calcium channel blockers such as lidoflazine, flunarizine, cinnarizine and prenylamine are weak agents on [^{3}H]nitrendipine binding in both preparations (Table 1).

The ratio of the relative potency in brain and heart membranes is different between the various groups of calcium channel antagonists (Table 2). While the dihydropyridines and verapamil are slightly more active in the heart (ratio lower than 1), the diphenylbutylpiperazines and the diphenylalkylamines are more potent in the brain (ratio higher than 1). These data

Table 1. Relative affinities of calcium channel antagonists and various antischizophrenic drugs on [^{3}H]nitrendipine binding in rat brain and heart membrane preparations.

	Brain		Heart	
Drug	K_i (nM)[a]	n_H	K_i (nM)	n_H
Calcium channel antagonists				
Dihydropyridines				
Nimodipine	0·41 ± 0·09	0·91 ± 0·08	1·08 ± 0·29	0·87 ± 0·10
Nitrendipine	0·30 ± 0·08	0·96 ± 0·03	0·83 ± 0·16	0·99 ± 0·09
Nisoldipine	0·52 ± 0·09	0·98 ± 0·06	0·23 ± 0·02	0·88 ± 0·04
Phenylalkylamine				
Verapamil	218·7 ± 37·6	—	248·2 ± 49·1	—
Diphenylbutylpiperazines				
Lidoflazine	750·0 ± 112·4	0·92 ± 0·16	3970 ± 429	1·09 ± 0·14
Flunarizine	1500 ± 157	0·80 ± 0·14	4255 ± 638	0·91 ± 0 10
Cinnarizine	906 ± 121	1·07 ± 0·13	4609 ± 552	0·84 ± 0·12
Diphenylalkylamine				
Prenylamine	625 ± 94·3	0·82 ± 0·08	2837 ± 462	0·96 ± 0·09
Antischizophrenic-neuroleptics				
Butyrophenone				
Haloperidol	2250 ± 747	—	4250 ± 929	—
Diphenylbutylpiperidines				
Fluspirilene	18·7 ± 2·60	1·02 ± 0·09	127·6 ± 27·4	0·91 ± 0·11
Pimozide	46·2 ± 9·87	0·84 ± 0·06	255·3 ± 36·1	0·99 ± 0·06
Clopimozide	112·5 ± 16·5	0·90 ± 0·18	780·1 ± 100·3	0·84 ± 0·07
Penfluridol	150·0 ± 29·5	1·00 ± 0·08	2127 ± 327	0·98 ± 0·09
Others				
Chlorpromazine	>10 000	—	>10 000	—
Buspirone	>10 000	—	>10 000	—
Imipramine	>10 000	—	>10 000	—

[a] K_i is calculated from the formula $K_i = IC50/1 + F/K_d$ where IC50 is the concentration of drug that inhibited 50% of specifically bound [^{3}H]nitrendipine, F represents the free concentration of ligand and K_d is the apparent affinity of the binding site. Each value is mean ± s.e.m. of at least three determinations, each in triplicate. n_H represent the pseudo-Hill coefficients.

Table 2. Ratio of the relative potencies of calcium channel antagonists and antischizophrenic drugs on [^{3}H]nitrendipine binding in rat brain and heart membrane preparations.

Drug	Brain/heart relative potency[a]
Calcium channel antagonists	
Dihydropyridines	
Nitrendipine	1
Nimodipine	0·95
Nisoldipine	0·15
Phenylalkylamine Verapamil	0·3
Diphenylbutylpiperazines	
Lidoflazine	2
Flunarizine	1
Cinnarizine	1·5
Diphenylalkylamine Prenylamine	1·6
Antischizophrenic-neuroleptics	
Diphenylbutylpiperidines	
Fluspirilene	2·7
Pimozide	2·3
Clopimozide	3·0
Penfluridol	5·0
Butyrophenone Haloperidol	0·5

[a] The potencies of nitrendipine in both preparations have been used as the unity value from which all others have been calculated.

suggest the possible heterogeneity of [^{3}H]nitrendipine binding sites in different tissues. Gould et al (1984) have reported on the different properties of [^{3}H]nitrendipine binding in various tissues (e.g. skeletal muscle) and suggested that it could be related to the varying tissue sensitivity to organic calcium channel antagonists.

Antischizophrenic drugs of the diphenylbutylpiperidine type are also potent inhibitors of [^{3}H]nitrendipine binding sites (Table 1). Fluspirilene is the most potent analogue on [^{3}H]nitrendipine binding in both tissues. Pimozide and clopimozide possess moderate affinities for these sites while penfluridol is weaker. As shown in Table 2, the diphenylbutylpiperidines are 2·5–5·0 times more active in the brain than in the heart. The findings also support the concept of tissue heterogeneity of [^{3}H]nitrendipine binding sites. Thus, drugs structurally related to the diphenylbutylpiperidine-diphenylbutylpiperazine type could possibly act preferentially on brain calcium channel antagonist binding sites. Moreover, the replacement of the piperidine ring by a piperazine structure is likely to decrease the activity of the compound on dopamine receptors (Gould et al 1983) and possibly generate a more selective brain calcium channel antagonist.

The possible significance of the activity of the diphenylbutylpiperidines on [^{3}H]nitrendipine binding is unknown. However, it has been shown that these drugs are more effective than other neuroleptics in reversing emotional withdrawal and poverty of speech, the negative schizophrenic symptoms (Singh 1973; Lapierre & Lavallée 1975; Lapierre 1978; Crow 1980; Haas & Beckmann 1982). It has already been suggested that these selective effects of the diphenylbutylpiperidines in the treatment of schizophrenia are related to their actions on calcium channel binding sites (Gould et al 1983). Thus, the design of a potent diphenylbutylpiperidine-diphenylbutylpiperazine analogue selective for the [^{3}H]nitrendipine binding site could be useful for a more effective treatment of the negative symptoms of schizophrenia.

The relative selectivity of the diphenylbutylpiperidines for brain [^{3}H]nitrendipine binding sites correlates well with the absence of cardiovascular side effects related to calcium antagonism of these drugs (Gould et al 1983). On the other hand, the butyrophenones such as haloperidol appear to be more active on [^{3}H]nitrendipine binding sites in cardiac than in brain membranes (Table 2). This action of haloperidol on calcium channel binding sites might be related to some of the known cardiovascular side effects of the butyrophenones (Baldessarini 1980).

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Dose-dependent effect of calcium and magnesium etidronate on salicylic acid absorption in the rat

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Disodium etidronate affected salicylic acid absorption from the rat small intestine, in-situ, when instilled into a jejunal segment for different exposure times before the salicylic acid absorption was measured. At low etidronate concentrations and short exposure times, the salicylic acid absorption rate was significantly increased compared with saline controls. At high etidronate concentrations and longer exposure times, the absorption rate was reduced. Etidronate precomplexed with calcium or magnesium ions at low concentrations still enhanced salicylic acid absorption but at high concentrations absorption of salicylic acid was close to saline controls. Intestinal mucosa exposed to high etidronate concentrations showed a progressive structural destruction but with the complexes, there was no visible alteration. It is proposed that a solubilized etidronate complex, formed either in-situ or administered as such, is responsible for enhancing salicylic acid absorption. This effect is hidden at high etidronate concentrations because of the deterioration of the mucosal surface and at high complex concentrations because these decrease the absorbing surface area and increase the viscosity of the lumen contents.

Disodium etidronate (etidronate, disodium 1-hydroxyethylidenediphosphonate) is used in reducing ectopic calcification and excessive bone resorption. It affects the absorption rate of salicylic acid from the rat small intestine, in-situ (Shrewsbury et al 1982). At low concentrations and with short exposures, etidronate causes an increase, while at higher concentrations and with longer exposure it causes a decrease in absorption. It also causes histological changes in the mucosa at all concentrations and exposures studied.

Etidronate binds calcium both in solution and on crystalline surfaces (Francis 1969; Grabenstetter & Cilley 1971) forming polynuclear complexes (Grabenstetter & Cilley 1971; Wiers 1971) so it has been proposed that calcium depletion from the intestinal membrane is responsible for the structural changes. A similar mechanism has been suggested for the increase in membrane permeability to various substances caused by edetic acid (Windsor & Cronheim 1961; Schanker & Johnson 1961; Tidball 1964; Cassidy & Tidball 1967; Poiger & Schlatter 1979).

We have examined the proposed mechanism by which etidronate causes histological changes in the intestinal mucosa.

Methods

Male,albino, Sprague-Dawley rats, 190–310 g, housed in wide mesh metal cages with free access to water, were fasted 14–16 h before surgery for which a modification

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