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The effect of dihydropyridine calcium channel agents on 5-HT metabolism in the CNS of the rat

M. I. COLADO*, M. J. ALFARO, F. LÓPEZ, V. DEL VAL, M. I. MARTÍN, *Instituto de Farmacologia y Toxicologia, Departamento de Farmacologia, Facultad de Medicina, Universidad Complutense, 28040 Madrid, Spain*

Abstract—The effects of dihydropyridines on the levels of 5-hydroxytryptamine (5-HT) and 5-hydroxy-3-indole acetic acid (5-HIAA) in the spinal cord and various brain regions of the rat have been studied. Nimodipine, nitrendipine and nifedipine (10 mg kg^{-1}), nisoldipine (5 mg kg^{-1}), and BAY K8644 (0·2 and 2 mg kg^{-1}) were administered i.p. 1 h before killing. The administration of nifedipine and nitrendipine increased 5-HT turnover in all of the areas studied except for the spinal cord. Nisoldipine increased 5-HT turnover in midbrain, hippocampus and cortex, while the effect of nimodipine was restricted to midbrain. BAY K8644 at 2 mg kg^{-1} produced the same effects as nifedipine and nitrendipine; however, at low doses (0·2 mg kg^{-1}), this compound increased 5-HT turnover only in midbrain and medulla oblongata. These results indicate that both dihydropyridine calcium channel agonist and antagonists are able to activate the 5-HT-ergic system in the central nervous system of the rat in-vivo. Therefore, it seems likely that such effects could be due to indirect actions or to interactions of the compounds with receptors other than the voltage-sensitive calcium channels.

Experimental evidence has indicated that neurons may possess several types of voltage-sensitive calcium channels (VSCC) (Miller 1985; Nowycky et al 1985). The dihydropyridines interact specifically with the L-subtype calcium channel and using radiolabelled dihydropyridine compounds it has been possible to demonstrate high-affinity, saturable and steroselective dihydropyridine binding sites in both the peripheral and central nervous system (CNS) (Glossmann et al 1982). The role of the VSCC in the control of centrally mediated events, such as neurotransmitter release and its functional consequences is currently a subject of great interest.

The dihydropyridine calcium channel agonist, BAY K8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate), has been reported to facilitate the release of a number of neurotransmitters, i.e. dopamine, noradrenaline, acetylcholine and 5-hydroxytryptamine (5-HT), from a number of in-vitro preparations of rodent brain (Middlemiss 1985; Middlemiss & Spedding 1985; Woodward & Leslie 1986). It also caused marked behavioural changes in the mouse (Bolger et al 1985) and rat (Bourson et al 1986) which appear to be mediated via activation of calcium channels in the brain and may represent a functional consequence of effects upon neurotransmitter release. These changes included ataxia, Straub tail, sedation, arched back and convulsions.

In contrast to the marked effects of BAY K8644 on both behavioural and neurotransmitter release, dihydropyridine calcium channel antagonists are ineffective in blocking neurotransmitter release (Ogura & Takahaski 1984; Middlemiss & Spedding 1985; Annunziato et al 1986) or in affecting behaviour in rodents in physiological conditions (Bolger et al 1985). It has been suggested that these compounds affect neuronal calcium channels only when these are activated either by pharmacological manipulation (BAY K8644) or by the presence of a pathological factor such as ischaemic damage.

Thus, there is substantial evidence that dihydropyridines may modify neuronal function in-vitro; however, the effects of these compounds in-vivo have not been extensively studied.

Recently, it has been reported that the dihydropyridine calcium channel agonist, BAY K8644, and the dihydropyridine calcium channel antagonists are active in the despair-behaviour test (Mogilnicka et al 1987, 1988), used for screening antidepressants (Porsolt et al 1977). However, data on the effect of these drugs on 5-HT turnover in-vivo are scarce. For this reason and taking into account that 5-HT metabolism is known to be involved in the mechanism of action of antidepressants, we have studied the influence of various dihydropyridine calcium channel antagonists and agonists (BAY K8644) on 5-HT turnover in the CNS of the rat.

Materials and methods

Male Sprague-Dawley rats, 250–300 g, were housed in clear plastic cages in groups of six under a 12 h light/dark cycle, with free access to food and water.

Nifedipine (10 mg kg^{-1}), nimodipine (10 mg kg^{-1}), nitrendipine (10 mg kg^{-1}), nisoldipine (5 mg kg^{-1}) and BAY K8644 (0·2 and 2 mg kg^{-1}) were dissolved in ethanol and water (final ethanol concentration: 15%) immediately before use (Nencini & Woolverton 1988). The drugs were administered (i.p.) 1 h before the rats were killed by decapitation. Control animals were injected with the vehicle. All the rats were killed between 1000 and 1400 h.

Immediately after death, the spinal cord and brain were removed and the midbrain, medulla oblongata, hippocampus and cortex rapidly dissected at 4 C and stored at −80 C until analysis. The tissues were homogenized with 0·4 M $HClO_4$ containing 0·1% $Na_2S_2O_5$ and centrifuged at 20 000 *g* for 15 min at 4 C.

Samples of the supernatant were taken for analysis of 5-HT and 5-HIAA by HPLC with electrochemical detection. The ratio 5-HIAA/5-HT was calculated in each CNS area and taken as an index of 5-HT turnover. The mobile phase consisted of 0·1 M Na_2HPO_4, 0·1 M citric acid (pH 4) and 10% v/v methanol. Elution was performed at a flow rate of 1 mL min^{-1} and the working electrode potential was set at 0·7 V.

The HPLC system consisted of a pump (Waters 510), linked to a Rheodyne 7125 injector, a stainless steel reversed-phase column (Resolve C18; 5 μm; 3·9 mm × 15 cm), a pre-column (Resolve C18) and an amperometric detector (Waters M-460). The current produced was monitored using an integrator (Waters M-745).

Results

The treatment with dihydropyridine calcium channel antagonist was well tolerated by all the rats and did not induce any perceptible behavioural change. BAY K8644 at the high dose (2 mg kg^{-1}) produced a number of profound behavioural effects in rat characterized by sedation, convulsions, ataxia, Straub tail, arched back and increased sensitivity to auditory stimulation. At

Correspondence to: M. I. Colado, Instituto de Farmacologia y Toxicologia, Departamento de Farmacologia, Facultad de Medicina, Universidad Complutense, 28040 Madrid, Spain.

low concentration (0·2 mg kg^{-1}) all the behavioural effects were reduced.

Under our experimental conditions, the vehicle used to dissolve dihydropyridine calcium channel agonist and antagonists did not affect 5-HT or 5-HIAA levels in the central nervous system of the rat (data not shown).

Table 1 shows the results of the analysis of 5-HT and 5-HIAA in the CNS of the rat after treatment with the drugs.

Discussion

Our results indicate that both dihydropyridine calcium channel agonists and antagonists increase 5-HT-ergic neurotransmission in the rat CNS. BAY K8644 produced an increase in the concentration of 5-HIAA in a dose dependent way, but did not alter significantly the concentration of 5-HT. These findings suggest that BAY K8644 increased the turnover of 5-HT in-vivo.

Nitrendipine, nisoldipine, nimodipine and nifedipine, dihydropyridine calcium channel antagonists, also enhanced 5-HT turnover in most of the CNS areas, mainly by increasing the levels of 5-HIAA. In the cortex, however, the increase in the 5-HT turnover produced by these drugs was due to a decrease in the content of 5-HT. The fact that dihydropyridine calcium channel agonists and antagonists modify 5-HT-ergic neurotransmission in a similar way may be because there are only small structural differences between dihydropyridine agonists and antagonists and indeed, agonists can behave as antagonists under certain experimental conditions (Triggle & Rampe 1989). It is interesting to note that racemic BAY K8644 causes calcium agonistic and antagonistic actions. The (−)-enantiomer has the calcium agonistic properties of the racemic compound while its antipode exerts characteristic effects of calcium antagonistic drugs (Franckowiak et al 1985).

Other authors have also reported that dihydropyridines exert complex changes in 5-HT function. Recently, Green et al (1990) indicated that pretreatment of rats with felodipine, 15 min before tranylcypromine, results in animals displaying enhanced locomotor activity and the complete 5-HT behavioural syndrome. Pretreatment with BAY K8644 did not modify the effect produced by felodipine; moreover, the administration of BAY K8644 and felodipine produced a greater total response than with felodipine only. These results are consistent with a lack of involvement of calcium channels in the effect of dihydropyridine derivates on 5-HT function.

Similar results were obtained by Pileblad & Carlsson (1987), who reported a reduced accumulation of 5-hydroxytryptophan after administration of both nimodipine and BAY K8644 in mice. This may represent dihydropyridine-mediated increases of tryptophan hydroxylation.

These observations provide evidence to suggest that the effects induced by dihydropyridine derivates could be due to indirect actions or to interaction with various receptor types. In this context, it is worth noting that nifedipine potentiates the effects of adenosine or interacts with an adenosine receptor (Swanson & Green 1986), inhibits the inositol phospholipid turnover (Kendall & Nahorski 1985) or interacts with Ca^{2+}-ATPase (David-Dufilho et al 1984). Furthermore, all the compounds studied produce marked effects on cardiac function and blood pressure (Bourson et al 1989). Such peripheral effects could also mediate strong actions on the brain.

The doses used in the present work are similar to those that were able to modify the duration of immobility in the despair behaviour test (Mogilnicka et al 1987, 1988). Thus, it seems likely that such compounds, which have been proposed for treatment of a variety of CNS disorders (Raeburn & Gonzales 1988) may share some properties with antidepressants.

On the other hand, we have found that nifedipine and BAY K8644 are the most potent dihydropyridines in modifying the 5-HT-ergic function. This is consistent with data obtained by Bartrup & Stone (1990) in the rat hippocampal slice showing that both compounds are able to enhance the inhibitory action of adenosine, while other calcium channel antagonists such as nitrendipine and nimodipine are less effective.

We did not observe a biphasic effect of BAY K8644, unlike

Table 1. Effects of nifedipine, nimodipine, nitrendipine, nisoldipine and BAY K8644 on the levels of 5-HT and 5-HIAA in various regions of the rat central nervous system.

	Control	Nifedipine (10 mg kg^{-1})	Nimodipine (10 mg kg^{-1})	Nitrendipine (10 mg kg^{-1})	Nisoldipine (5 mg kg^{-1})	BAY K8644 (0·2 mg kg^{-1})	BAY K8644 (2 mg kg^{-1})
Midbrain							
5-HT	1253 ± 70·9	1346 ± 48·9	1208 ± 83·8	1272 ± 65·7	1357 ± 77·5	1203 ± 68·1	1399 ± 77·5
5-HIAA	536 ± 24·7	751 ± 51·8*	611 ± 41·6	647 ± 38·7*	692 ± 49·9*	664 ± 30·6*	791 ± 43·6*
5-HIAA/5-HT	0·43 ± 0·02	0·55 ± 0·02*	0·51 ± 0·03*	0·51 ± 0·02*	0·51 ± 0·01*	0·57 ± 0·03*	0·57 ± 0·02*
Medulla oblongata							
5-HT	845 ± 49·9	841 ± 52·6	999 ± 56·6*	956 ± 29·4	912 ± 38·2	857 ± 50·6	1027 ± 33·7*
5-HIAA	315 ± 17·1	395 ± 35·7	415 ± 30·2*	423 ± 21·0*	392 ± 21·5	372 ± 20·1	513 ± 25·2*
5-HIAA/5-HT	0·38 ± 0·02	0·47 ± 0·03*	0·42 ± 0·02	0·45 ± 0·02*	0·43 ± 0·01	0·44 ± 0·02*	0·50 ± 0·02*
Hippocampus							
5-HT	408 ± 31·7	358 ± 29·9	451 ± 36·5	391 ± 15·4	389 ± 24·9	422 ± 32·5	384 ± 31·0
5-HIAA	199 ± 15·5	257 ± 17·3*	265 ± 22·1*	267 ± 15·8*	254 ± 14·5*	251 ± 19·5*	283 ± 12·3*
5-HIAA/5-HT	0·50 ± 0·05	0·74 ± 0·06*	0·59 ± 0·03	0·69 ± 0·04*	0·66 ± 0·05*	0·60 ± 0·06	0·75 ± 0·04*
Cortex							
5-HT	517 ± 45·2	313 ± 27·9*	382 ± 42·1*	340 ± 28·8*	367 ± 35·2*	521 ± 45·5	439 ± 22·3
5-HIAA	205 ± 20·6	200 ± 18·0	165 ± 25·2	192 ± 14·9	185 ± 17·2	243 ± 24·3	285 ± 8·4*
5-HIAA/5-HT	0·39 ± 0·02	0·61 ± 0·03*	0·45 ± 0·08	0·57 ± 0·04*	0·51 ± 0·03*	0·46 ± 0·02	0·65 ± 0·03*
Spinal cord							
5-HT	987 ± 76·6	940 ± 65·2	1064 ± 69·5	1606 ± 58·2*	1278 ± 148·4*	947 ± 73·6	1008 ± 111·2
5-HIAA	167 ± 10·9	189 ± 12·8	197 ± 14·1	284 ± 23·9*	227 ± 25·4*	182 ± 12·0	178 ± 13·4
5-HIAA/5-HT	0·17 ± 0·01	0·20 ± 0·01	0·19 ± 0·01	0·18 ± 0·01	0·18 ± 0·01	0·20 ± 0·01	0·18 ± 0·01

Values are expressed as ng per g wet tissue ± s.e.m. (n = 8). Statistical significances were calculated by one-way analysis of variance followed by Duncan's *t*-test; * $P < 0·05$.

Hoffmeister & Tettenborn (1986) who reported that low doses attenuate, and high doses potentiate, fentanyl antinociception, indicating that the calcium agonistic effect of this compound, prominent in low concentrations, declines at higher concentrations. However, since the route of administration used by those authors was i.v. while we used the i.p. route, any comparison between the respective results should be treated with caution.

This study shows that several dihydropyridine calcium channel antagonists (nifedipine, nitrendipine, nimodipine and nisoldipine) and a dihydropyridine calcium channel agonist (BAY K8644) induce an increase in the brain 5-HT turnover in-vivo. These compounds affect 5-HT-ergic neurotransmission in a similar manner, suggesting that such effects could have been produced through a mechanism independent of VSCC interaction. Furthermore, in agreement with the results published by Gaggi & Gianni (1990), the effect produced by these drugs was evident not only in hippocampus and cortex, i.e. in the brain regions with the highest density of VSCC (Gould et al 1985), but also in midbrain and medulla oblongata, areas almost devoid of dihydropyridine binding sites.

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