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Effect of Bay K 8644 on the synthesis and metabolism of dopamine and 5-hydroxytryptamine in various brain areas of the rat

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Abstract—The effects of Bay K 8644 (1, 2 and 4 mg kg^{-1}, i.p.) on the synthesis and metabolism of dopamine and 5-hydroxytryptamine (5-HT) in rat brain after *m*-hydroxybenzylhydrazine administration were studied. Bay K 8644 (2 and 4 mg kg^{-1}, i.p.) caused an increase in the synthesis of both dopamine in the striatum and 5-HT in the midbrain and striatum, measured as the accumulation of 3,4-dihydroxyphenylalanine (dopa) and 5-hydroxytryptophan, respectively. Moreover, Bay K 8644 at the dose of 4 mg kg^{-1} increased the turnover of dopamine in the striatum and of 5-HT in midbrain and striatum. These neurochemical changes were antagonized by the calcium channel antagonist nimodipine (10 mg kg^{-1}, i.p.). It is concluded that dihydropyridine receptors may mediate the brain region-specific changes in the dopaminergic and 5-HT-ergic neurotransmission which occur following activation of neuronal calcium channels.

The dihydropyridine derivate Bay K 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethyl-phenyl)-pyridine-5-carboxylate) is a calcium-channel activator that has been reported to penetrate readily the blood-brain barrier and interact competitively with high-affinity dihydropyridine-binding sites in the voltage-sensitive calcium channels in brain tissues (Janis et al 1984). These binding sites, localized in the cell soma, have been characterized as L-type calcium channels and appear to be involved in the regulation of neuronal functions (Miller 1987). Bay K 8644 activates L-type calcium channels in neuronal tissue thereby enhancing depolarization-induced neurotransmitter release. In this regard, it has been shown that Bay K 8644 is able to enhance potassium-stimulated calcium-dependent release of dopamine, 5-hydroxytryptamine (5-HT), noradrenaline, acetylcholine, γ-aminobutyric acid and glutamate from synaptosomes or brain slice preparations (Middlemiss 1985; Woodward & Leslie 1986). These effects are blocked by nifedipine or nimodipine, indicating that dihydropyridine receptors play an important role in the neurochemical actions of Bay K 8644 (Middlemiss 1985; White & Bradford 1986; Woodward & Leslie 1986).

On the other hand, intracerebroventricular and intraperitoneal administration of Bay K 8644 to rodents exerts marked behavioural changes including hyper-reactivity, Straub tail, arched back, hypersensitivity to auditory stimulation, convulsions, self-mutilation and deficits in motor function such as decreased motor activity and ataxia (Bolger et al 1985; Bourson et al 1989; O'Neil et al 1990). These effects may reflect a functional consequence of actions upon dopamine and 5-HT release via an activation of calcium channels in the central nervous system (CNS).

The present study was designed to obtain further insight into the actions of Bay K 8644 on dopamine and 5-HT synthesis in several brain areas rich in dopamine and 5-HT terminals and its antagonism by the dihydropyridine calcium antagonist nimodipine. Dopamine and 5-HT synthesis was measured as accumulation of 3,4-dihydroxyphenylalanine (dopa) and 5-hydroxytryptophan (5-HTP) respectively, after central L-amino acid-aromatic decarboxylase inhibition by *m*-hydroxybenzylhydrazine (NSD 1015).

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Materials and methods

Animals. Male Wistar rats, 175–200 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.

Drug treatments. Bay K 8644 (Química Farmacéutica Bayer SA, Spain) was suspended in 1% Tween 80 immediately before use and administered at doses of 1, 2 and 4 mg kg^{-1} 60 min before the animals were killed. NSD 1015 (Sigma Chemical Co., Poole, Dorset, UK) at a dose of 100 mg kg^{-1} was dissolved in saline and administered intraperitoneally 30 min before they were killed. Control animals were injected with vehicle and NSD 1015 following the same experimental schedule.

To study the ability of nimodipine to antagonize the effect of Bay K 8644, 10 mg kg^{-1} nimodipine (Química Farmacéutica Bayer, SA, Spain) dissolved in 1% Tween 80 was administered 30 min before NSD 1015 either together with Bay K 8644 or alone.

All drugs were injected in a volume of 5 mL kg^{-1}.

Analysis of monoamines. The animals were killed by decapitation 60 min after Bay K 8644 administration. The brain was removed quickly and the midbrain, medulla oblongata, hippocampus, striatum and cortex rapidly dissected out 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 20000 *g* for 20 min at 4°C. Samples of the supernatant were taken for analysis of dopa, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HTP, 5-HT and 5-hydroxy-3-indoleacetic acid (5-HIAA) by HPLC with electrochemical detection.

The mobile phase for 5-HTP, dopamine, DOPAC and HVA analysis consisted of 0·1 M Na_2HPO_4, 0·1 M citric acid (pH = 4·5), 1 mM octanesulphonic acid and 10% v/v methanol. The mobile phase for 5-HT and 5-HIAA was the same as above but without octanesulphonic acid. The mobile phase for dopa consisted of 0·1 M Na_2HPO_4, 0·1 M citric acid (pH = 3), 2 mM octanesulphonic acid and 8% v/v methanol. The flow rate was 1 mL min^{-1} and the working electrode potential was set at 0·8 V for 5-HTP, dopamine, DOPAC and HVA and 0·7 V for dopa, 5-HT and 5-HIAA.

The HPLC system consisted of a pump (Waters 510) linked to an automatic sample injector (Waters 712 WISP), a stainless steel reversed-phase column (Resolve C, 5 μm, 3·9 mm × 15 cm) with a precolumn (Resolve C_{18}) and an amperometric detector (Waters M640). The current produced was monitored using an integrator (Waters M745).

Statistics. Statistical evaluation of the data was carried out by one-way analysis of variance followed by Newman-Keuls test. The probability level of $P < 0.05$ was considered to be statistically significant.

Results

Intraperitoneal NSD 1015 (100 mg kg^{-1}) induced a marked accumulation of dopa and 5-HTP in the rat brain. In saline-

Table 1. Effect of Bay K 8644 (1, 2 and 4 mg kg^{-1}) on 5-HT metabolism in various brain areas of the rat after NSD 1015 administration.

	Vehicle	Bay K 8644 (mg kg^{-1}) 1	2	4
Midbrain				
5-HTP	322±27	326±26	421±30*	386±18*
5-HT	1147±45	1153±31	1296±78	1342±54
5-HIAA	217±14	227±20	219±8	258±10*
Medulla oblongata				
5-HTP	198±19	203±12	242±10	177±16
5-HT	921±40	982±36	969±20	876±55
5-HIAA	171±14	189±16	176±9	183±12
Hippocampus				
5-HTP	129±12	131±14	160±14	119±12
5-HT	758±66	881±44	832±68	877±58
5-HIAA	159±10	174±11	170±6	181±10
Striatum				
5-HTP	86±6	92±12	137±8**	105±6*
5-HT	666±32	654±42	644±24	677±25
5-HIAA	199±13	217±14	248±7*	279±8*
Cortex				
5-HTP	41±5	39±3	46±2	39±3
5-HT	209±29	185±3	192±6	214±8
5-HIAA	33±2	34±2	34±1	40±4

NSD 1015 (100 mg kg^{-1}) was given 30 min after Bay K 8644 administration, the rats being killed 30 min later. Values are expressed as ng (g wet tissue)$^{-1}$ ± s.e.m. (n = 8). * $P < 0.05$, ** $P < 0.01$ (Newman-Keuls test). 5-HTP: 5-hydroxytryptophan; 5-HIAA: 5-hydroxy-3-indoleacetic acid.

treated rats, reflecting the physiological situation, these amines could not be detected. The administration of NSD 1015 did not modify the levels of dopamine or 5-HT whereas the metabolites of these monoamines decreased in all areas studied compared with saline-treated rats. The changes in concentration of 5-HIAA (ng g^{-1}) were as follows: midbrain (372±22–217±14, $P < 0.01$), medulla oblongata (300±18–171±14, $P < 0.01$), hippocampus (238±11–159±10, $P < 0.01$), striatum (269±9–199±13, $P < 0.01$) and cortex (53±2–33±2, $P < 0.01$). In striatum DOPAC and HVA changed from 683±45–115±10 ($P < 0.01$) and 522±37–351±19 ($P < 0.01$), respectively. The effects of Bay K 8644 on dopamine and 5-HT metabolism in rat brain were restricted to two areas: midbrain and striatum. Bay K 8644 at 2 and 4 mg kg^{-1} administered to NSD 1015-treated rats induced an increase in the accumulation of 5-HTP in midbrain and striatum (Table 1) and of dopa in striatum (Table 2). Levels of dopamine, 5-HT and their metabolites were not modified by 2 mg kg^{-1} Bay K 8644 when compared with rats receiving NSD 1015 alone (Tables 1, 2). However, Bay K 8644, at the dose of 4 mg kg^{-1}, produced an increase in the levels of 5-HIAA in midbrain and striatum (Table 1) and in the levels of DOPAC and HVA in striatum (Table 2).

Table 2. Effect of Bay K 8644 (1, 2 and 4 mg kg^{-1}) on dopamine metabolism in rat striatum after NSD 1015 administration.

	Vehicle	Bay K 8644 (mg kg^{-1}) 1	2	4
Dopa	1106±71	1388±167	1578±151*	1460±64*
Dopamine	8129±213	8138±316	7877±189	8199±352
DOPAC	115±10	158±21	128±15	200±15**
HVA	351±19	370±34	344±37	660±36**

NSD 1015 (100 mg kg^{-1}) was given 30 min after Bay K 8644 administration, the rats being killed 30 min later. Values are expressed as ng (g wet tissue)$^{-1}$ ± s.e.m. (n = 8). * $P < 0.05$, ** $P < 0.01$ (Newman-Keuls test). Dopa: 3,4-dihydroxyphenylalanine; DOPAC: 3,4-dihydroxyphenylacetic acid; HVA: homovanillic acid.

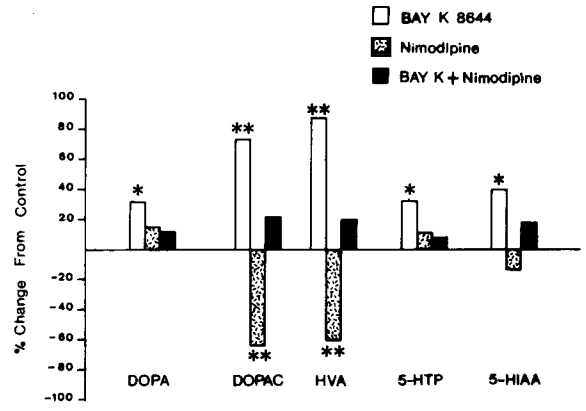

FIG. 1. The effects of Bay K 8644 (4 mg kg^{-1}) in combination with nimodipine (10 mg kg^{-1}) on the NSD 1015-induced accumulation of dopa and 5-HTP and on the levels of DOPAC, HVA, 5-HIAA in rat striatum. Nimodipine was administered 30 min before NSD 1015 either with Bay K 8644 or alone. Bars represent mean ± s.e.m. of eight animals. * $P < 0.05$, ** $P < 0.01$ vs vehicle-injected rats.

As shown in Fig. 1, when Bay K 8644 (4 mg kg^{-1}) was injected in combination with nimodipine (10 mg kg^{-1}) to NSD 1015-treated rats, the effects of Bay K 8644 on the synthesis and metabolism of dopamine and 5-HT were markedly reduced.

Discussion

The results reported in this paper indicate that Bay K 8644 administered intraperitoneally increases the synthesis of both dopamine in the striatum and 5-HT in the midbrain and striatum of the rat. These changes are antagonized by the calcium-channel antagonist nimodipine, indicating that Bay K 8644 potentiates the NSD 1015-induced accumulation of dopa and 5-HTP through an activation of dihydropyridine-sensitive calcium channels in the rat brain. Our results are in accordance with those described previously showing that, in the mouse, Bay K 8644 caused a significant increase in the synthesis of dopamine in striatum and in the limbic region (Pileblad & Carlsson 1987).

Bay K 8644, by interfering with the calcium transport, could mediate increases in the rate of tyrosine and tryptophan hydroxylation. Several lines of evidence suggest a role for calcium in the regulation of tyrosine hydroxylase. Thus, in depolarized dopaminergic terminals, the activation of this enzyme is directly linked to a calcium-dependent phosphorylation process (El-Mestikawy et al 1983).

With respect to the 5-HT-ergic system, the increase in 5-HT synthesis reported in this paper was restricted to midbrain and striatum; similar increases in the accumulation of 5-HTP following NSD 1015 administration were not detected in the medulla oblongata, hippocampus or cortex. These observations suggest that the actions of Bay K 8644 on 5-HT synthesis are region-specific and may depend (in the striatum) on the synaptic interaction between 5-HT-ergic and dopaminergic neurons.

Bay K 8644 not only potentiated the NSD 1015-induced accumulation of 5-HTP and dopa but also increased the levels of DOPAC and HVA in striatum and of 5-HIAA in midbrain and striatum. NSD 1015 is an inhibitor of both L-amino-acid-aromatic decarboxylase and monoamine oxidase (Carlsson et al 1972). Therefore, our results appear to indicate that high doses of Bay K 8644 are able to increase the metabolism of dopamine and 5-HT, these effects being antagonized by nimodipine. In this sense, results reported by other laboratories and our own (Bolger et al 1988; Bourson et al 1989; Colado et al 1991) have shown that Bay K 8644 increases the turnover of dopamine and 5-HT in several brain areas of the rat. Bay K 8644, in a dose

range similar to those tested in this study, exerts marked behavioural changes in rodents; these behavioural effects are antagonized by nifedipine and may reflect a functional consequence of the region-specific changes in dopaminergic and 5-HT-ergic neurotransmission via an interaction with dihydropyridine-binding sites within the CNS (Janis et al 1984; Bolger et al 1985).

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References

Bolger, G. T., Weissman, B. A., Skolnick, P. (1985) The behavioral effects of the calcium agonist Bay K 8644 in the mouse: antagonism by the calcium antagonist nifedipine. Naunyn Schmiedebergs Arch. Pharmacol. 328: 373–377

Bolger, G. T., Lesieur, P., Basile, A. S., Skolnick, P. (1988) Modulation of neurotransmitter metabolism by dihydropyridine calcium channel ligands in mouse brain. Brain Res. 438: 101–107

Bourson, A., Moser, P. C., Gower, A. J., Mir, A. K. (1989) Central and peripheral effects of the dihydropyridine calcium channel activator Bay K 8644 in the rat. Eur. J. Pharmacol. 160: 339–347

Carlsson, A., Davis, J. N., Kehr, W., Lindquist, M., Atack, C. V. (1972) Simultaneous measurement of tyrosine and tryptophan hydroxylase activities in brain in vivo using an inhibitor of aromatic amino acid decarboxylase. Naunyn Schmiedebergs Arch. Pharmacol. 275: 153–168

Colado, M. I., Alfaro, M. J., Lopez, F., Del Val, V., Martin, M. I. (1991) The effect of dihydropyridine calcium channel agents on 5-HT metabolism in the CNS of rat. J. Pharm. Pharmacol. 43: 662–664

El Mestikawy, S., Glowinski, J., Hamon, M. (1983) Tyrosine hydroxylase activation in depolarized dopaminergic terminals-involvement of Ca^{2+}-dependent phosphorylation. Nature 302: 830–832

Janis, R. A., Rampe, D., Sarmiento, J. G., Triggle, D. J. (1984) Specific binding of a calcium channel activator [^{3}H] Bay K 8644 to membranes from cardiac muscle and brain. Biochem. Biophys. Res. Commun. 121: 317–323

Middlemiss, D. N. (1985) The calcium channel activator, Bay K 8644, enhances K^+-evoked efflux of acetylcholine and noradrenaline from rat brain slices. Naunyn Schmiedebergs Arch. Pharmacol. 331: 114–116

Miller, R. J. (1987) Multiple calcium channels and neuronal functions. Science 235: 46–52

O'Neil, S. K., McKay, D. W., Campbell, N., Triggle, C. R., Crowley, M., Bolger, G. T. (1990) Comparative behavioral, neurochemical and pharmacological activities of dihydropyridine calcium channel activating drugs. J. Pharmacol. Exp. Ther. 253: 905–912

Pileblad, E., Carlsson, A. (1987) The Ca^{2+}-antagonist nimodipine decreases and the Ca^{2+}-agonist Bay K 8644 increases catecholamine synthesis in mouse brain. Neuropharmacology 26: 101–105

White, E. J., Bradford, H. F. (1986) Enhancement of depolarization-induced synaptosomal calcium uptake and neurotransmitter release by Bay K 8644. Biochem. Pharmacol. 35: 2193–2197

Woodward, J. J., Leslie, S. W. (1986) Bay K 8644 stimulation of calcium entry and endogenous dopamine release in rat striatal synaptosomes antagonized by nimodipine. Brain Res. 370: 397–400

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Dissimilarity in the mechanisms of action of KRN2391, nicorandil and cromakalim in canine renal artery

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Abstract—In the present study, we examined the mode of action of KRN2391 (*N*-cyano-*N'*-(2-nitroxyethyl)-3-pyridinecarboximidamide monomethanesulphonate) in isolated canine renal artery compared with those of nicorandil and cromakalim. KRN2391 (10^{-8}–3×10^{-5} M), nicorandil (10^{-7}–3×10^{-4} M) and cromakalim (10^{-8}–3×10^{-5} M) relaxed renal arteries contracted by 25 mM KCl in a concentration-dependent manner. KRN2391-induced relaxation was inhibited by methylene blue (10^{-5} M) and glibenclamide (10^{-6} M). Nicorandil-induced relaxation was inhibited by methylene blue, but not by glibenclamide. The concentration-relaxation curve for cromakalim displayed a rightward parallel shift in the presence of glibenclamide. In the control observation, KRN2391 and nicorandil also produced full relaxation, but cromakalim did not. The present results suggest that KRN2391 acts as both a nitrate and a potassium channel opener, and nicorandil acts only as a nitrate and only in canine renal artery.

KRN2391, *N*-cyano-*N'*-(*N*-(2-nitroxyethyl)-3-pyridinecarboximidamide monomethanesulphonate, is a novel agent possessing vasodilating action (Kashiwabara et al 1991). As shown in Fig. 1, KRN2391 has a nitrate moiety in its structure. KRN2391-induced relaxation was inhibited by both a guanylate cyclase inhibitor and a K^+-channel blocker in rat isolated aorta (Kashiwabara et al 1991). However, in canine large coronary artery, this relaxation was inhibited by a guanylate cyclase inhibitor, but not by a K^+-channel blocker (Fukata et al 1991). In canine cranial mesenteric artery, both a guanylate cyclase inhibitor and a K^+-channel blocker antagonized the relaxation induced by KRN2391 (Fukata et al 1991). Kingsbury et al (1991) also reported that KRN2391 predominantly behaved as a K^+-channel opener in resistive coronary arterioles of dogs. Though KRN2391 has a dual mechanism of action as a nitrate and as a K^+-channel opener, its mechanism of action is thought to depend on the segment of vascular bed and the type of blood vessel. Therefore, it is of interest whether KRN2391 shows its action as a K^+-channel opener or as a nitrate in renal artery,

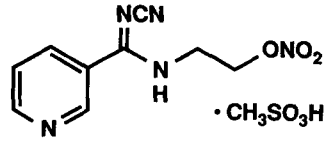

FIG. 1. Chemical structure of KRN2391.

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