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Clonidine modulates BAY K 8644-induced rat behavior and neurotransmitter changes in the brain

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Abstract

BAY K 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4[2-trifluoromethyl-phenyl]-pyridine-5-carboxylate), an activator of dihydropyridine-sensitive Ca^{2+} channels, injected in rats [2 mg/kg intraperitoneally (i.p.)], induces behavioral changes including ataxia, increased sensitivity to auditory stimulation, stiff tail, arched back, limb tonus and clonus, and rolling over. Neurochemical changes in the brain 45 min after application of 2 mg/kg were characterized by a significant decrease of noradrenaline in the amygdala (−27.8%, $P < 0.02$) and piriform cortex (−16.3%, $P < 0.02$). No significant changes of catecholamines were found in the hippocampal subregions CA1, CA3 and dentate gyrus or in the septum as compared to controls. The dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the amygdala were elevated by 60% ($P < 0.02$) and 66.7% ($P < 0.02$), respectively. In the septum, a 52.6% ($P < 0.02$) increase of HVA was observed. Analysis of amino acids revealed a marked increase of γ-aminobutyric acid (GABA) content (+50.4%, $P < 0.001$) in the septum. Pretreatment of the rats with the α_2-adrenoceptor agonist, clonidine (0.1 mg/kg i.p.), 30 min before BAY K 8644 (2 mg/kg i.p.) injection completely abolished the behavioral and neurochemical changes. The data suggest that the Ca^{2+}-dependent neurotransmitter release provoked by BAY K 8644 can be modulated by stimulation of presynaptic α_2-adrenoceptors. The effect of clonidine on the GABAergic system may represent an important mechanism involved in the prevention of BAY K 8644-induced behavior.

Keywords: Clonidine; BAY K 8644; GABA (γ-aminobutyric acid); Ca^{2+} channel, L-type; Limbic system; Septum, Epilepsy

1. Introduction

Influx of Ca^{2+} into the cell is an important process in the control of cell function and plays a prominent role as a trigger for the release of neurotransmitters (Rubin, 1970). Voltage-sensitive Ca^{2+} channels have been shown in neuronal tissue and classified into 3 types L, N and T (long-lasting, neuronal and transient), respectively, on the basis of their electrophysiological and pharmacological properties (Thayer et al., 1986; Tsien et al., 1988; Miller, 1987). Studies with dihydropyridine, a compound interacting preferentially with the L-type channel, revealed that dihydropyridine binding sites are present in the brain (Marangos et al., 1982; Cortés et al., 1984; Supavilai and Karobath, 1984; Glossmann et al., 1985). Within the brain, L-type and N-type Ca^{2+} channels have a heterogeneous spatial distribution. L-type Ca^{2+} channels are mainly localized at the cell soma (Cortés et al., 1983; Sanna et al., 1986; Miller, 1987). On the other hand, evidence is available that N-type Ca^{2+} channels are localized predominantly in the dendrites and synaptic terminals (Westenbroek et al., 1992; Wang et al., 1999) and mediate neurotransmitter release from nerve terminals (Miller, 1987). BAY K 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4[2-trifluoromethyl-phenyl]-pyridine-5-carboxylate), an activator of dihydropyridine-sensitive Ca^{2+} channels, which enhances the en-

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try of Ca^{2+} into the cell (Schramm et al., 1983; Woodward and Leslie, 1986), augments the K^+ or electrical stimulation-evoked release of several neurotransmitters, including noradrenaline, acetylcholine and serotonin (5-HT) from rat brain slices (Middlemiss, 1985; Middlemiss and Spedding, 1985; Tsuda et al., 1991; Sabrià et al., 1995). BAY K 8644 induces the release of endogenous dopamine from striatal synaptosomes (Woodward and Leslie, 1986) and, in in vivo studies, from the striatum (Watanabe et al., 1998). BAY K 8644 modulates K^+-evoked amino acid release from cerebellar tissue (Philibert and Dutton, 1989).

In vivo studies showed that intraperitoneal (i.p.) application of BAY K 8644 (2–10 mg/kg) to rats or mice induces dose dependently a number of behavioral changes consisting of increased sensitivity to auditory stimulation (cage tapping), ataxia, stiff tail, arched back, limb tonus, clonus and rolling over (Bolger et al., 1985; Petersen, 1986; Bourson et al., 1989). In mice, BAY K 8644 (5 mg/kg s.c.) did not significantly affect the threshold for electroconvulsion, but distinctly diminished the protection offered by conventional anti-epileptic drugs (Gasior et al., 1995) and dose dependently impaired rotarod performance (Cohen et al., 1997). Additionally, analgesic potency of BAY K 8644 was found in rats (Bourson et al., 1989). In vivo, BAY K 8644 (4 mg/kg) induced a significant increase in homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindolacetic acid (5-HIAA) in the cortex and striatum of the rat (Bourson et al., 1989; Colado et al., 1993). The various effects of BAY K 8644, however, are not easily antagonized by Ca^{2+} channel blockers. Only nifedipine and nimodipine antagonize the BAY K 8644-induced symptoms and/or reverse the increases in DOPAC, HVA and 5-HIAA, whereas flunarizine, diltiazem or verapamil are not effective (Petersen, 1986; Bourson et al., 1989; Colado et al., 1993). Diltiazem even triggers the BAY K 8644-induced behavioral symptoms (Petersen, 1986).

The aim of the present study was to further characterize the behavioral symptoms following i.p. injection of BAY K 8644 in the rat and to find possible correlates among neurotransmitter changes in various limbic areas of the brain. We focused on catecholamines, serotonin (5-HT) and putative neurotransmitter amino acids. It was also intended to search for pharmacological approaches other than Ca^{2+} channel blockers interfering with the behavioral and neurochemical changes associated with BAY K 8644. It is well established that the release of several neurotransmitters, including glutamate, is modulated by systems involving α_2-adrenoceptors (for review, see Starke et al., 1989; Kamisaki et al., 1992). Therefore, it is conceivable that activation of presynaptic α_2-adrenoceptors interferes with the release of neurotransmitters triggered by excess influx of Ca^{2+}. For this purpose we tested the influence of the α_2-adrenoceptor agonist, clonidine, on BAY K 8644-induced behavior and on neurotransmitter changes in the rat brain.

2. Materials and methods

2.1. Animals and materials

Male Sprague–Dawley rats (Forschungsinstitut für Versuchstierzucht, Himberg, Austria, 280–320 g body weight) were used. The animals were housed in groups of four to five per cage in a room with controlled temperature, humidity and controlled light/dark cycle (12 h light/12 h dark) and were given free access to laboratory chow and tap water.

BAY K 8644 (Bayer, Leverkusen, Germany) was dissolved in ''placebo'' solvent (consisting of 22% glycerol, 37% water and 41% polyethylene glycol 400). Only fresh solution was used that had been protected from light. Clonidine (Boehringer, Ingelheim, Germany) was dissolved in saline. All other chemicals were purchased from Sigma (Deisenhofen, Germany).

2.2. Experimental procedures

Experiments were started in the morning, between 9 and 10 a.m. The animals received i.p. injections of different doses of BAY K 8644 (0.1, 1, 2, 4 and 10 mg/kg). Corresponding control rats were injected with ''placebo'' solvent. There were five or six animals per group. Behavior was observed continuously for up to 2 h after injection. In particular, changes in motor activity were recorded. Because of findings with mice (Bolger et al., 1985) we also roughly tested the sensitivity to auditory stimulation by cage tapping and hand clapping. Subsequently, two separate groups of rats, receiving either 2 mg/kg BAY K 8644 or ''placebo'' solvent, were killed by decapitation 45 min after injection and their brains were analyzed neurochemically.

In the next set of experiments, rats were pretreated with clonidine (0.1 mg/kg i.p.) or saline 30 min before the administration of BAY K 8644 at a dose of 2 mg/kg (i.p.). Corresponding controls were rats pretreated with saline/''placebo'' solvent and clonidine/''placebo'' solvent. Behavioral changes were evaluated after the second injection for 45 min. At this point the rats were killed by decapitation. Behavior was observed in a blinded manner. For the neurochemical analyses the brains were quickly removed, put on ice and immediately dissected on a cold plate, based on König and Klippel (1970). The amygdala/piriform cortex area or amygdala and piriform cortex was taken separately from levels A 2.9 to 4.9 with the sulcus rhinalis as landmark for the piriform cortex. Septum (lateral and medial) was taken from levels A 7.0 to 8.6. The subregions of the hippocampus, CA1, CA3 and dentate gyrus were dissected by the method described by Berger et al. (1986). Dissected areas were immediately frozen, weighed and stored for up to 3–7 days at −70°C.

Table 1
Effect of clonidine (0.1 mg/kg i.p. 30 min before BAY K 8644 application) on symptoms induced by BAY K 8644 (2 mg/kg i.p.)

Symptoms	BAY K 8644 2 mg/kg i.p. ($n=7$)	Clonidine/BAY K 8644 0.1 mg/kg i.p. /2 mg/kg i.p. ($n=7$)
Ataxia	7/7	0/7[a]
Arched back	7/7	0/7[a]
Limb tonus and clonus	7/7	0/7[a]
Increased sensitivity to auditory stimuli	7/7	0/7[a]
Biting and scratching	7/7	0/7[a]
Stiff tail	7/7	0/7[a]
Reduced motor activity	7/7	7/7

Statistical significance of results was calculated using α^2-test; n = number of rats.
[a] $P < 0.0002$; $\alpha^2 = 14$, $df = 1$.

2.3. Biochemical analyses

Frozen areas were homogenized at 4°C by ultrasonication in 50 vols. of 0.1 N perchloric acid containing 0.4 mM $NaHSO_3$. Homogenization was followed by centrifugation at $25\,000 \times g$ for 10 min at 4°C. Aliquots of the supernatants were processed separately for determination of noradrenaline and dopamine according to Felice et al. (1978) with minor modifications (Sperk et al., 1983), 5-HT, DOPAC, HVA and 5-HIAA as described previously (Sperk, 1982) using high performance liquid chromatography (HPLC) with electrochemical detection. The levels of the putative neurotransmitter amino acids were measured by HPLC after post-column derivatization with the fluorogenic reagent, *o*-phthalaldehyde, according to the method described by Schmid et al. (1980). The following minor modifications were introduced: the column was packed with Aminex A-9 (sodium form) and maintained at 76°C; the two-step gradient of buffers consisted of 0.2 M sodium formate/H_3PO_4 (pH 3.3 to 3.4) and 0.2 M sodium acetate/H_3PO_4, pH 5.6; the buffers as well as the *o*-phthalaldehyde reagent were pumped at a flow rate of 0.8 ml/min.

2.4. Data analyses

All data are given as means ± S.E.M. For statistical analyses student's *t*-test and one-way analysis of variance (ANOVA) test with Fisher's least significant difference (LSD) test were used. Statistical significance of the effect of clonidine on BAY K 8644-induced behavior was calculated with the α^2-test.

3. Results

3.1. Behavioral changes

At a dose of 0.1 mg/kg i.p. BAY K 8644 no behavioral changes were observed. 1 mg/kg BAY K 8644 resulted in mild behavioral changes in 70% of the rats. 2 mg/kg BAY K 8644 induced in 100% of the animals within 10–15 min characteristic behavioral alterations consisting of ataxia, increased sensitivity to auditory stimulation, tense tail, arched back, stretching and twisting of fore- and/or hindlimbs, scratching, limb tonus and clonus and backwards motility sometimes resulting in backwards rolling. The symptoms induced by BAY K 8644 2 mg/kg lasted up to 1 h after injection then became weaker and had disappeared at 2 h. At higher doses (4 and 10 mg/kg) severe primary clonic and secondary tonic seizures activity occurred, and a number of animals died in a ''status epilepticus'' within the observation period.

Clonidine at a dose of 0.1 mg/kg, applied 30 min before 2 mg/kg BAY K 8644 injection, abolished the behavioral changes induced by the Ca^{2+} channel agonist (see Table 1). After clonidine/BAY K 8644 treatment the rats appeared sedated. Sedation was also observed after clonidine alone; however, this effect vanished within 1 h

Table 2
Effect of BAY K 8644 (2 mg/kg i.p.) on monoaminergic parameters in different brain regions at 45 min after injection

Region	Treatment	n	Noradrenaline	Dopamine	5-HT
Amygdala/piriform cortex	Control	5	0.47 ± 0.02	0.26 ± 0.02	0.65 ± 0.04
	BAY K 8644	6	0.33 ± 0.02[a]	0.16 ± 0.02[a]	0.69 ± 0.05
CA1	Control	5	0.49 ± 0.04	0.010 ± 0.003	0.17 ± 0.01
	BAY K 8644	6	0.48 ± 0.04	0.020 ± 0.004	0.16 ± 0.02
CA3	Control	5	0.35 ± 0.03	0.030 ± 0.002	0.46 ± 0.04
	BAY K 8644	6	0.40 ± 0.04	0.030 ± 0.003	0.38 ± 0.03
Dentate gyrus	Control	5	0.57 ± 0.04	0.020 ± 0.005	0.19 ± 0.01
	BAY K 8644	6	0.59 ± 0.04	0.020 ± 0.004	0.20 ± 0.02
Septum	Control	5	1.04 ± 0.10	1.76 ± 0.37	0.42 ± 0.02
	BAY K 8644	6	0.91 ± 0.04	1.97 ± 0.25	0.49 ± 0.05

Data are presented as ng/mg tissue (means ± S.E.M.); n = number of animals. Significance vs. control using unpaired student's *t*-test.
[a] $P < 0.01$.

Table 3
Effect of clonidine (0.1 mg/kg i.p. 30 min before BAY K 8644 application) on BAY K 8644 (2 mg/kg i.p. at 45 min after injection)-induced changes in catecholamine and metabolite levels in amygdala and piriform cortex

Treatment	*n*	Noradrenaline	Dopamine	DOPAC	HVA
Amygdala					
Control	5	0.54 ± 0.02	0.29 ± 0.01	0.10 ± 0.01	0.06 ± 0.01
BAY K 8644	6	0.39 ± 0.02[a,b,c]	0.21 ± 0.01[a]	0.16 ± 0.3[a,b,c]	0.10 ± 0.01[a,b,c]
Clonidine	5	0.50 ± 0.01[d]	0.23 ± 0.02	0.05 ± 0.1[a,d]	0.06 ± 0.01[d]
Clonidine + BAY K 8644	6	0.54 ± 0.03[d]	0.28 ± 0.06	0.07 ± 0.1[a,d]	0.07 ± 0.003[d]
Piriform cortex					
Control	5	0.43 ± 0.02	0.21 ± 0.01	0.08 ± 0.01	0.09 ± 0.02
BAY K 8644	6	0.36 ± 0.01[a,b,c]	0.18 ± 0.01	0.10 ± 0.02[b]	0.11 ± 0.01[b,c]
Clonidine	5	0.51 ± 0.02[a,c,d]	0.17 ± 0.01	0.06 ± 0.01[d]	0.05 ± 0.01[a,d]
Clonidine + BAY K 8644	6	0.44 ± 0.03[b,d]	0.16 ± 0.01	0.09 ± 0.01	0.05 ± 0.01[a,d]

Data are expressed as ng/mg tissue, means ± S.E.M.; *n* = number of rats. For statistical analysis one-way ANOVA with Fisher's LSD test was used.
[a] vs. control, significance $P < 0.02$.
[b] vs. clonidine, significance $P < 0.02$.
[c] vs. clonidine + BAY K 8644, significance $P < 0.02$.
[d] vs. BAY K 8644, significance $P < 0.02$.

after clonidine injection. Thus, at the time of decapitation (75 min after clonidine injection) sedation was no longer visible.

3.2. Neurochemical changes

3.2.1. Monoamines, metabolites and amino acids after BAY K 8644

The effect of BAY K 8644 (2 mg/kg i.p.) on noradrenaline, dopamine and 5-HT levels in various brain regions 45 min after drug injection is presented in Table 2. BAY K 8644 caused a significant reduction of noradrenaline (−30%, $P < 0.01$) and dopamine (−38%, $P < 0.01$) in amygdala/piriform cortex complex. No changes in 5-HT levels were noted. No effect of BAY K 8644 on noradrenaline, dopamine and 5-HT concentrations was seen in the hippocampal subregions CA1, CA3 and dentate gyrus and in the septum (see Table 2) except for a moderate increase of dopamine in CA1 (+50%, n.s.) and septum (+12%, n.s.) and a decrease of 5-HT in CA3 (−17%, $P = 0.05$).

The concentrations of the amino acids, taurine, aspartate and glutamate and γ-aminobutyric acid (GABA) were analyzed in the brain regions at 45 min after BAY K 8644 injection. Of all regions tested, only the septum had significantly increased GABA levels (+50%, $P < 0.001$) after BAY K 8644 administration (data not shown).

3.2.2. Effect of clonidine on BAY K 8644-induced neurochemical changes

In the next set of experiments, we analyzed the effect of clonidine pretreatment on BAY K 8644-induced neurochemical changes. Since the monoamine changes induced by BAY K 8644 were most pronounced in the amygdala/piriform cortex, we further dissected this area into amygdala and piriform cortex and observed biochemical differences between the two regions. BAY K 8644 induced a significant reduction of noradrenaline (−27%, $P < 0.001$) and dopamine (−27%, $P < 0.001$) in the amygdala, but in the piriform cortex only a mild reduction of noradrenaline (−16.3%, $P < 0.02$) and dopamine (−14.3%, n.s.) was observed (Table 3). DOPAC and HVA levels were elevated to a greater extent in amygdala (+60% and +66.7%, respectively, $P < 0.02$) than in piriform cortex (+25% and +22%, n.s., respectively). No significant changes of DOPAC and HVA were seen in the hippocampal subregions (data not shown). In the septum, the level of HVA was significantly increased [from 0.19 ± 0.01 ($n = 5$) to 0.29 ± 0.02 ($n = 6$); $P < 0.02$)].

Pretreatment with clonidine (0.1 mg/kg i.p.) completely suppressed all neurochemical alterations in amygdala and piriform cortex induced after BAY K 8644 application (see Table 3). The increase of HVA levels in septum after BAY K 8644 injection also was prevented by clonidine pretreatment. Clonidine completely abolished the BAY K 8644-induced elevation of GABA levels in septum (see Table 4). Furthermore, the decrease of GABA content in response to clonidine was not altered by co-administra-

Table 4
Effect of clonidine (0.1 mg/kg i.p. 30 min before BAY K 8644) on BAY K 8644 (2 mg/kg i.p.)-induced changes in GABA levels in septum 45 min after injection

Treatment	*n*	GABA (ng/mg tissue)
Control	5	266.0 ± 36.4
BAY K 8644	6	400.0 ± 12.3[a,b,c]
Clonidine	5	174.0 ± 9.9[a,d]
Clonidine + BAY K 8644	6	181.2 ± 10.3[a,d]

For statistical analysis, one-way ANOVA with Fisher's LSD was used; *n* = number of rats.
[a] vs. control, significance $P < 0.0001$.
[b] vs. clonidine, significance $P < 0.0001$.
[c] vs. clonidine + BAY K 8644, significance $P < 0.0001$.
[d] vs. BAY K 8644, significance $P < 0.0001$.

tion of BAY K 8644 (−31.9%, $P < 0.001$ vs. control). A significant reduction of the level of GABA by clonidine (−34.6%, $P < 0.001$), in the absence of BAY K 8644, occurred only in the septum, and in none of the other brain regions investigated (data not shown).

4. Discussion

This study was designed to further evaluate the action of the Ca^{2+} channel agonist, BAY K 8644, on rat behavior and on neurotransmitter markers in the brain. The similarity of BAY K 8644-induced symptoms and of kainic acid-produced seizure activity in rats (Baran et al., 1985) suggests the involvement of noradrenergic activity in the limbic system. In the kainic acid epileptic model a reduction of noradrenaline and dopamine was found in the amygdala/piriform cortex, hippocampus (Sperk et al., 1983; Baran et al., 1985, 1989) and septum (Baran, 1994). BAY K 8644 (2 mg/kg)-induced behaviors were accompanied by a decrease of noradrenaline and dopamine levels in the amygdala and a slight decrease of noradrenaline in piriform cortex without changes in the hippocampus. The non-significant changes of catecholamine levels in the septum were accompanied by a significant increase of HVA. The differences in effects of the two neurotoxins are likely due to the differences in the regional binding/action sites of kainic acid and of BAY K 8644 in the brain (Berger et al., 1986; Marangos et al., 1982).

It is of interest that the behavioral symptoms and neurochemical changes induced by BAY K 8644 were reversed by pretreatment with clonidine (0.1 mg/kg). This effect of clonidine indicates a link between the specific changes of noradrenergic function in amygdala/piriform cortex and the characteristic behavioral symptoms. Clonidine at a dose of 0.1–0.2 mg/kg effectively protects against kindling, kainic acid- and pentylenetetrazol-induced seizures (Papanicolaou et al., 1982; Lazarova and Samanin, 1983; Baran et al., 1985, 1989; Yoshioka et al., 2000), and this anticonvulsant effect has been proposed to be due to a direct action of clonidine on central α_2-adrenoceptors. The anticonvulsant potential of clonidine in pentylenetetrazol-induced seizures was, however, not confirmed by Fletcher and Forster (1988). On the contrary, blockade of noradrenergic activity by an α_2-antagonist, yohimbine (5 mg/kg), triggered kainic acid (10 mg/kg)-induced symptoms (Baran et al., 1985). A proconvulsant action of selective α_2-adrenoceptor antagonists has also been demonstrated in pentylenetetrazol- or bicuculline-induced seizures (Fletcher and Forster, 1988). The therapeutic efficacy of clonidine (0.1 mg/kg) has also been shown in noradrenaline-deficient rats, indicating the involvement of postsynaptic α_2-adrenoceptors. This supports a predominant involvement of the noradrenergic system in seizure regulation (Zis and Fibiger, 1975; Baran et al., 1989). Furthermore, clonidine (0.1 mg/kg) was more potent to reduce kainic acid-induced behaviors in noradrenaline-deficient rats or in rats with unaltered catecholamine function than was the anticonvulsant drug, diazepam (5 mg/kg) (Baran et al., 1985, 1989, 1994). In the present study, we restricted the dose of clonidine to 0.1 mg/kg. At higher doses of clonidine, the anticonvulsive action is lost or a proconvulsant action has even been observed and hypothermic side effects may occur (Gellman at al., 1987; Papanicolaou et al., 1982; Zis and Fibiger, 1975; Fletcher and Forster, 1988). Lower doses of clonidine are less effective in epileptic models (Gellman et al., 1987; Lazarova and Samanin, 1983; Papanicolaou et al., 1982) or are even proconvulsive (Wu et al., 1987). Although, clonidine causes sedation at 0.1 mg/kg dose, we found that the sedative effect of clonidine in the anticonvulsive dose range was markedly lower than that of diazepam 5 mg/kg (Baran et al., 1985, 1989).

The increase in GABA levels in septum after BAY K 8644 (2 mg/kg) may reflect a decreased release, thereby contributing to the initiation of the behavioral changes. In contrast, clonidine increases the release of GABA, as shown by in vivo microdialysis in the nucleus accumbens (Murai et al., 1998). Since, after co-administration of clonidine (0.1 mg/kg) and BAY K 8644 (2 mg/kg), the GABA levels remained decreased to the same extent as after clonidine alone, it is likely that the effect of clonidine on the GABAergic system predominates and, thus, may represent an important mechanism also responsible for the prevention of BAY K 8644-induced behavior. Activation of the GABA system has also been suggested to underlie the anticonvulsant effect of clonidine in the case of pentylenetetrazol-induced seizures in mice (Amabeoku et al., 1994). In this respect, it is noteworthy that the anticonvulsant, diazepam, at the dose of 30 mg/kg protected against BAY K 8644 (5 mg/kg)-induced spasticity (Petersen, 1986).

It cannot be excluded that the behavioral effects of BAY K 8644 were at least partly mediated by an increased presynaptic release of glutamate as has been demonstrated in various brain regions (Bonci et al., 1998). An inhibitory effect of clonidine on the release of glutamate has been demonstrated in synaptosomes of various brain regions, including cortex and hippocampus (Kamisaki et al., 1992). Thus, the antagonistic effect of clonidine on the central actions of BAY K 8644 may additionally involve activation of α_2-adrenoceptors located presynaptically on glutamatergic nerve terminals. The mechanism of the presynaptic α_2-adrenergic modulation of neurotransmitter release has been postulated to involve either inhibition of electrosecretory coupling or reduction of depolarization-evoked Ca^{2+} entry (for review see Starke et al., 1989). The latter possibility is supported by some reports showing that clonidine can reduce the Ca^{2+} influx both in preparations of rat preganglionic sympathetic nerves (Elliott et al., 1989) and in embryonic chick sympathetic neurons (Böhm and Huck, 1991). Our observation of an antagonistic effect

of clonidine on the behavioral and neurochemical actions of BAY K 8644 supports the notion that the inhibitory control of neurotransmitter release by presynaptic α_2-adrenoceptors is primarily due to the inhibition of depolarization-evoked Ca^{2+} entry. However, it cannot be excluded that the inhibitory effect of clonidine on neurotransmitter release possibly involves the modulation of N-type Ca^{2+} channels (Lipscombe et al., 1989; Xiang et al., 1990; Dolezal et al., 1996). In the case of presynaptic muscarinic receptors inhibiting the release of acetylcholine, close coupling with the N-type Ca^{2+} channels has been demonstrated (Dolezal and Tucek, 1999). If this mechanism also applies to α_2-adrenoceptors, blockade of L-type Ca^{2+} channel-induced neurotransmitter release by clonidine would underline the predominance of N-type Ca^{2+} channels in the presynaptic control of exocytotic release of neurotransmitters. A prominent role of N-type Ca^{2+} channels in the modulation of noradrenaline release has been described, e.g. in hippocampal nerve terminals, whereas, in the cortex there seems to be a similar contribution of both N-type and L-type Ca^{2+} channels (Sabrià et al., 1995). It has been suggested that L-type Ca^{2+} channels in the central nervous system are not normally active and have limited importance under normal conditions, but might play a role in some pathophysiological conditions such as ischemia or seizures (Bourson et al., 1989). Recently, Barger (1999) has shown that, in primary cultures of hippocampal neurons, BAY K 8644 exhibits a considerable neurotoxicity, which was at least partially reversed by K^+-mediated depolarization. Thus, the pharmacological activation of L-type Ca^{2+} channels may have more damaging effects than physiological activation by depolarization (Barger, 1999). This assumption is further supported by the fact that low doses of BAY K 8644 (0.1 and 1 mg/kg) triggered not only epileptic events but also increased neurotoxic damage in the amygdala/piriform cortex in the kainic acid-epileptic model (Baran, personal observation).

In conclusion, the present data suggest that the Ca^{2+}-dependent neurotransmitter release provoked by BAY K 8644 can be modulated by clonidine via stimulation of presynaptic α_2-adrenoceptors. The inhibition of the BAY K 8644-induced epileptic symptoms as well as the previously shown protective effect against kainic acid-, quinolinic acid- or pentylenetetrazole-induced seizures point to a possible antiepileptic potential of this α_2-adrenoceptor agonist, at least in various subtypes of epilepsy.

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