ELSEVIER

European Journal of Pharmacology 286 (1995) 99–103

ejp

Short communication

Effect of vinconate on the extracellular levels of dopamine and its metabolites in the rat striatum: microdialysis studies

Toshiaki Iino, Masashi Katsura, Kinya Kuriyama *

Department of Pharmacology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602, Japan

Received 1 June 1995; revised 21 August 1995; accepted 25 August 1995

Abstract

The effects of (±)-methyl-3-ethyl-2,3,3*a*,4-tetrahydro-1*H*-indolo[3,2,1-*de*][1,5]naphthyridine-6-carboxylate hydrochloride (vinconate), an indolonaphthyridine derivative, on the extracellular levels of dopamine and its metabolites in the rat striatum were examined using brain microdialysis. Single administration of vinconate (10, 100 mg/kg i.p.) increased the extracellular level of dopamine and its metabolites. This enhancing effect of vinconate was antagonized by scopolamine (10 μM), a muscarinic receptor antagonist, which was added to the perfusate from 30 min before vinconate treatment. These findings suggest that vinconate, even when systemically administered, enhances the endogenous release of dopamine in the striatum, probably via the stimulation of presynaptic muscarinic receptors.

Keywords: Vinconate; Dopamine release; Muscarinic receptor; Striatum; Microdialysis

1. Introduction

The effects of (±)-methyl-3-ethyl-2,3,3*a*,4-tetrahydro-1*H*-indolo[3,2,1-*de*][1,5]naphthyridine-6-carboxylate hydrochloride (vinconate), an indolonaphthyridine derivative, on the release of dopamine and its metabolites in the rat striatum were examined using brain microdialysis.

Previous studies indicated that vinconate protected from neuronal damage after cerebral ischemia in the brain of rodents (Araki and Kogre, 1989; Iino et al., 1992), and had an antiamnesic effect on learning impairments in rodents (Kinoshita et al., 1992a,b). These reports suggest that vinconate may be effective for the treatment of cerebral disorders such as cerebral ischemia and neuropsychiatric symptoms associated with senile dementia.

Neurotransmitter release is modulated by auto- and heteroreceptors in the nerve terminals. Striatal dopamine release has been reported to be regulated in an accentuated manner by muscarinic receptors (Marchi et al., 1992; Raiteri et al., 1984), although contradictory results have been reported (Carter et al., 1988; Westerink et al., 1994). Vinconate has been reported to stimulate the muscarinic acetylcholine receptor-mediated phosphatidylinositol turnover in cerebral cortical slices (Katsura et al., 1993). Moreover, vinconate has been reported to increase the KCl-evoked [^{3}H]dopamine release from rat striatal slices (Koda et al., 1989). These findings were obtained from in vitro experiments. Therefore, we have examined in this study the effect of vinconate on the release of dopamine and its metabolites in the rat striatum using microdialysis to clarify the in vivo effect. In addition, we examined the effect of scopolamine on the vinconate-induced changes in the release of dopamine and its metabolites to clarify whether the effect of vinconate was related to an action on presynaptic muscarinic receptors in the dopaminergic nerve terminals.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 200–300 g were purchased from Japan SLC (Hamamatsu, Japan). The animals had free access to laboratory chow (MF, Oriental Yeast Co., Chiba, Japan) and tap water.

* Corresponding author. Tel.: 81-75-251-5333; fax: 81-75-241-0824.

SSDI 0014-2999(95)00545-5

2.2. Drug treatments

Vinconate (Tokyo Tanabe Co., Tokyo, Japan) was dissolved and diluted in distilled water. The solution of vinconate (10 and 100 mg/kg) and the same volume of vehicle were administered intraperitoneally (i.p.). Scopolamine (10 μM) was added to the perfusate from 30 min before the treatment with vinconate or vehicle.

2.3. Brain microdialysis

The rats were anesthetized with 0.5% halothane in a mixture of 30% O_2 and 70% N_2O and placed in a stereotaxic frame (Narishige, Tokyo, Japan). A microdialysis guide cannula with dummy cannula was implanted into the striatum at the following coordinates relative to the bregma: anterior 0.2, lateral 3.0 and ventral 6.5 mm according to the stereotaxic atlas of Paxinos and Watson (1982). After 1–2 days allowed for surgical recovery, the rat was reanesthetized and a vertical-type microdialysis probe (2 mm, CMA-10, Carnegie Medicin, Stockholm, Sweden) was inserted. Rectal temperature was maintained at 37 ± 0.5°C with a temperature controller (CMA 150, Carnegie Medicin, Stockholm, Sweden). The microdialysis probe was perfused with Ringer's solution (NaCl 147, KCl 4 and $CaCl_2$ 2.3 mM) containing 10 μM pargyline at a flow rate of 2.0 μl/min, using a microinfusion pump (EP 50, Eicom, Kyoto, Japan). The probes had in vitro recoveries of 14–15% for dopamine and its metabolites, and we did not correct for probe recovery. Following the start of perfusion, dialysate samples were discarded over the first 180 min to allow recovery from the acute effects of the implantation procedure of the probe. Dialysate samples were then collected continuously for 30-min periods. Following a 90-min baseline period, the drugs were injected i.p. or added to the perfusate, and further dialysate samples were collected for 270 min. Dialysate samples (60 μl) were collected in a 100-μl sample loop and automatically injected into a high performance liquid chromatograph (HPLC) at 30-min intervals by using an automated on-line sample injection system (AS-10, Eicom, Kyoto, Japan). The position of the probe was verified by visual inspection of brain slices at the end of each experiment.

2.4. Biochemical analysis

Dialysate concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by HPLC with electrochemical detection. Dopamine and its metabolites were separated by reversed-phase liquid chromatography (Eicompak MA-5ODS, Eicom, Kyoto, Japan) equipped with a precolumn (AC-ODS, Eicom, Kyoto, Japan). The mobile phase, consisting of a mixture of 36.3 mM

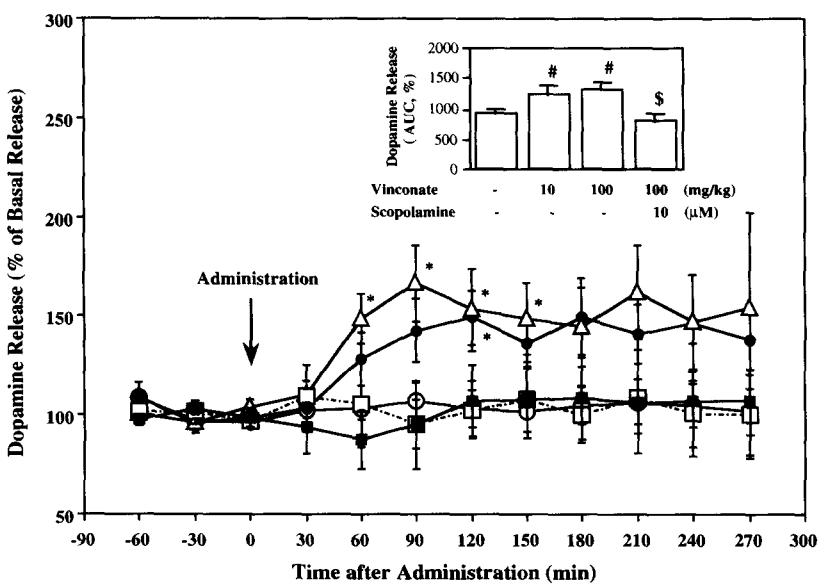

Fig. 1. Effects of vinconate and scopolamine, alone or in combination, on the basal release of dopamine in the rat striatum. The basal release of dopamine is expressed as a percentage of the basal level (the average value of three stable fractions obtained before vinconate or vehicle administration). The symbols are as follows: control group (○), vinconate-treated groups (10 (●) and 100 (△) mg/kg), scopolamine-treated group (10 μM (□)) and combinational group (vinconate (100 mg/kg) and scopolamine (10 μM), (■)). Vinconate or vehicle was administered i.p. (arrow). Scopolamine was added to the perfusate from 30 min before the administration of vinconate or vehicle. Insert: The same data (expressed as the area under the curve (AUC) values for 0–270 min after administration) are shown in the upper part of the figure. Each value represents the mean ± S.E. ($n = 5$–6). $^{*}P < 0.05$, versus the control values at the same time point (Bonferroni's test). $^{\#}P < 0.05$, versus the control value (without vinconate and scopolamine) (Ryan's test). $^{\$}P < 0.05$, versus the value for the vinconate (100 mg/kg)-treated group (Cochran-Cox's t-test).

sodium acetate, 51.7 mM citric acid, 0.013 mM Na_2EDTA, 0.74 mM sodium 1-octanesulfonate and 12% (v/v) methanol, adjusted to pH 3.9, was delivered at a rate of 1.0 ml/min. The glassy carbon working electrode was set at 750 mV against the Ag/AgCl reference electrode of the electrochemical detector (ECD-100, Eicom, Kyoto, Japan).

2.5. Reagents

The reagents used were halothane (Hoechst Japan, Tokyo, Japan); dopamine and DOPAC (Sigma Chemical Co., St. Louis, MO, USA); HVA, sodium 1-octanesulfonate, Na_2EDTA, methanol and scopolamine hydrobromide (Nacalai Tesque, Kyoto, Japan); sodium acetate and citric acid (Kanto Chemical Co., Tokyo, Japan). Other reagents used were of the highest grade commercially available.

2.6. Statistical analysis

The average of three stable fractions obtained before drug treatment was considered as the basal level and was defined as 100%. All values given are expressed as percentage of the basal levels. The area under the curve (AUC) (0–270 min after administration) was also calculated for each animal. All results are expressed as means ± S.E. The effects of vinconate and scopolamine at each time point were analyzed using an analysis of variance followed by Bonferroni's test. AUC values were analyzed using Ryan's multiple range test and Cochran-Cox's *t*-test.

3. Results

3.1. Basal extracellular levels

The average basal extracellular levels (as absolute values) of dopamine, DOPAC and HVA detected in all animals used in this study were 5.2 ± 0.3, 1180.3 ± 144.1 and 1721.4 ± 75.7 fmol/min, respectively. In control rats (treated with vehicle), the extracellular levels of dopamine and HVA remained stable for 360 min, whereas a moderate decrease was observed in that of DOPAC (data not shown).

3.2. Effect of vinconate on the extracellular levels of dopamine, DOPAC and HVA

Treatment with vinconate (10 mg/kg i.p.) caused a significant and transient increase of extracellular dopamine to a maximal increase of 149.4% (Fig. 1). The same treatment with vinconate also significantly increased the AUC (0–270 min) values, the total extracellular levels for 270 min after administration, of dopamine and DOPAC compared with those of control values (Fig. 1 and Table 1).

Vinconate, at 100 mg/kg, produced more pronounced increases of extracellular dopamine and HVA, to a maximal increase of 166.5% (Fig. 1) and 126.9% (Table 1), respectively. The AUC (0–270 min) values of dopamine, DOPAC and HVA were also significantly increased by the same treatment with vinconate (Fig. 1 and Table 1).

Table 1
Summary of effects of vinconate on striatal extracellular basal and peak levels and AUC (0–270 min) values for dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA)

	Treatment	Level (% of basal)		AUC (0–270 min)
		Basal	Peak	
Dopamine	Control	96.2 ± 2.0	106.8 ± 11.0	936.6 ± 75.1
	Vinconate (10 mg/kg i.p.)	98.6 ± 1.7	149.4 ± 13.6 [a]	1245.4 ± 129.1 [c]
	Vinconate (100 mg/kg i.p.)	104.4 ± 3.6	166.5 ± 19.2 [a]	1302.1 ± 121.1 [c]
	Scopolamine (10 μM)	97.4 ± 4.2	109.3 ± 15.9	854.4 ± 139.8
	Vinconate + scopolamine	95.8 ± 4.6	109.1 ± 21.8	786.7 ± 145.0 [e]
DOPAC	Control	101.5 ± 1.5	96.2 ± 4.9	484.1 ± 67.1
	Vinconate (10 mg/kg i.p.)	92.5 ± 2.5	80.3 ± 3.4	734.9 ± 48.8 [d]
	Vinconate (100 mg/kg i.p.)	99.1 ± 2.3	95.7 ± 9.9	764.9 ± 111.6 [d]
	Scopolamine (10 μM)	99.7 ± 9.3	105.8 ± 16.8	629.7 ± 97.0
	Vinconate + scopolamine	95.4 ± 3.2	102.6 ± 15.9	688.6 ± 87.8
HVA	Control	96.7 ± 1.6	98.8 ± 3.2	818.6 ± 47.5
	Vinconate (10 mg/kg i.p.)	98.6 ± 1.4	97.1 ± 4.5	859.7 ± 33.0
	Vinconate (100 mg/kg i.p.)	101.5 ± 1.6	126.9 ± 4.7 [b]	1057.0 ± 36.5 [d]
	Scopolamine (10 μM)	94.7 ± 1.5	91.0 ± 5.6	808.1 ± 120.2
	Vinconate + scopolamine	103.6 ± 2.1	95.3 ± 8.2	773.6 ± 81.0 [e]

Basal values represent the last preinjection values. Data are means ± S.E. values. [a] $P < 0.05$, [b] $P < 0.01$ versus the control values at the same time point (Bonferroni's test). [c] $P < 0.05$, [d] $P < 0.01$ versus each control value (Ryan's test). [e] $P < 0.05$ versus vinconate (100 mg/kg i.p.) alone (Cochran-Cox's *t*-test).

3.3. Effect of scopolamine on the vinconate-induced changes in the extracellular levels of dopamine, DOPAC and HVA

Scopolamine (10 μM) alone did not affect the extracellular levels of dopamine, DOPAC and HVA (Fig. 1 and Table 1). Pretreatment with scopolamine antagonized significantly the vinconate (100 mg/kg)-induced increases of extracellular dopamine and HVA, but not that of DOPAC (Table 1 and Fig. 1).

4. Discussion

This study was designed to assess whether vinconate could change the release of endogenous dopamine and of its main free acid metabolites, DOPAC and HVA, in the rat striatum when measured directly by in vivo microdialysis. Monoamines were detected in 30-min dialysates collected from the posterior striatum using a specifically designed HPLC-ECD assay that allowed the separation of dopamine and its metabolites in a signal run. It is likely that a major proportion of dopamine and its metabolites in the perfusates arises from dopaminergic nerve terminals rather than noradrenalinergic nerve terminals, in which dopamine is a precursor, since specific lesions of the rat forebrain noradrenaline systems did not affect regional brain tissue levels of either dopamine or its metabolites (Westerink and De Vries, 1985). The present findings have demonstrated that vinconate increases the release of endogenous dopamine in vivo. This is in agreement with the previous finding that vinconate increased the KCl-evoke [^{3}H]dopamine release from rat striatal slices (Koda et al., 1989). Pretreatment with scopolamine, a muscarinic receptor antagonist, blocked this vinconate-induced increase of endogenous dopamine release. This supported the previous finding that atropine antagonized the vinconate-induced increase of KCl-evoked [^{3}H]dopamine release from rat striatal slices. The present findings are consistent with previous in vitro findings in many respects. However, this is the first report of an in vivo study in which changes in endogenous dopamine release have been monitored simultaneously with measurements of its metabolites in the striatum following vinconate administration. In addition, the findings obtained with the dialysis technique and in vitro studies are consistent with the view that muscarinic heteroreceptors facilitate dopaminergic neurotransmission in the striatum (Marchi et al., 1992; Raiteri et al., 1984), although there have also been reported contradictory results, i.e. that muscarinic heteroreceptors are unable to modify striatal dopamine release (Carter et al., 1988; Westerink et al., 1994).

Vinconate facilitates phosphatidylinositide hydrolysis by mainly stimulating the muscarinic receptor and non-muscarinic mechanisms (Katsura et al., 1993). Phosphatidylinositide hydrolysis leads to production of diacylglycerol and inositol triphosphate. The former activates protein kinase C and the latter mobilizes intracellular Ca^{2+} from internal store sites (Berridge and Irvine, 1984; Nishizuka, 1984). Neurotransmitter release is likely to occur mainly by exocytosis, an event triggered by an increase in the free cytoplasmic Ca^{2+} concentration within the varicosity of the axon terminals (Miledi and Parker, 1981). Therefore, the facilitation of endogenous dopamine release by vinconate in the striatum may be due to the argumentation of the free cytoplasmic Ca^{2+}. Such studies on the molecular mechanisms involved in the facilitation of dopamine release by vinconate are underway in our laboratory.

In summary and conclusion, vinconate, an indolonaphthyridine derivative, even when systemically administered, facilitated the release of endogenous dopamine in the striatum, mainly via the stimulation of muscarinic receptor. This action seems to be an important characteristic of vinconate.

References

Araki, T. and K. Kogre, 1989, Prevention of delayed neuronal death in gerbil hippocampus by a novel vinca alkaloid derivative (vinconate), Mol. Chem. Neuropathol. 11, 33.

Berridge, M.J. and R.F. Irvine, 1984, Inositol triphosphate, a novel second messenger in cellular signal transduction, Nature 312, 315.

Carter, C.J., R. L'Heureux and B. Scatton, 1988, Differential control by *N*-methyl-D-aspartate and kainate of striatal dopamine release in vivo: a trans-striatal dialysis study, J. Neurochem. 51, 462.

Iino, T., M. Katsura and K. Kuriyama, 1992, Protective effect of vinconate on ischemia-induced neuronal damage in the rat hippocampus, Eur. J. Pharmacol. 224, 117.

Katsura, M., T. Iino and K. Kuriyama, 1993, (±)-Methyl-2,3,3*a*-4-tetrahydro-1*H*-indolo[3,2,1-*de*][1,5]naphthyridine-6-carboxylate monohydrochloride facilitates phosphatidylinositol hydrolysis: possible involvement of muscarinic and non-muscarinic mechanisms, J. Pharmacol. Exp. Ther. 267, 192.

Kinoshita, H., T. Kameyama, T. Hasegawa and T. Nabeshima, 1992a, Effects of vinconate on spatial learning impairments induced by medial septal lesion in rats, Life Sci. 51, 267.

Kinoshita, H., T. Kameyama, T. Hasegawa and T. Nabeshima, 1992b, Effects of vinconate, a novel vinca alkaloid, on spatial learning deficits induced by the basal forebrain lesion in rats, Pharmacol. Biochem. Behav. 42, 19.

Koda, H., T. Hashimoto and K. Kuriyama, 1989, Muscarinic receptor-mediated regulation of OM-853-enhanced dopamine release in striatum of rat, Eur. J. Pharmacol. 162, 501.

Marchi, M., G. Volpe, A. Augliera, A. Codignola, G. Lunardi and M. Raiteri, 1992, Cholinergic modulation of dopamine release in corpus striatum and substantia nigra of the rat, Neurochem. Int. 20, 271.

Miledi, R. and I. Parker, 1981, Calcium transients recorded with arsenazo III in the presynaptic terminal of the squid giant synapse, Proc. R. Soc. Lond. B. 212, 197.

Nishizuka, Y., 1984, The role of protein kinase C in cell surface signal transduction and tumor promotion, Nature 308, 693.

Paxinos, G. and C. Watson, 1982, The Rat Brain in Stereotaxic Coordinates (Academic Press, New York).

Raiteri, M., R. Leardi and M. Marchi, 1984, Heterogeneity of presynaptic muscarinic receptors regulating neurotransmitter release in the rat brain, J. Pharmacol. Exp. Ther. 228, 209.

Westerink, B.H.C. and J.B. De Vries, 1985, On the origin of dopamine and its metabolite in predominantly noradrenergic innervated brain areas, Brain Res. 330, 164.

Westerink, B.H.C., P. De Boer, M. Santiago and J.B. De Vries, 1994, Do nerve terminals and cell bodies of nigrostriatal dopaminergic neurons of the rat contain similar receptors?, Neurosci. Lett. 167, 109.