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Calcium Antagonist Activity of Vinpocetine and Vincamine in Several Models of Cerebral Ischaemia

Jean-Claude Lamar, Hervé Poignet, Michèle Beaughard, and Georges Dureng

Department of Pharmacology, Riom Laboratoires-Cerm, 63203 Riom Cedex, France

ABSTRACT

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The potency and selectivity (i.e., the central vs. peripheral vascular smooth muscle activity) of the calcium antagonist (CA) effects of vinpocetine and vincamine have been compared with those of the standard CAs: flunarizine, verapamil, diltiazem, and nimodipine in rabbit basilar and splenic artery preparations. The cerebral antiischemic activity of these substances also was evaluated in five well-documented in vivo models, i.e., hypobaric and normobaric hypoxia, global cerebral ischemia to $MgCl_2$, cytotoxic anoxia with KCN, and cerebral edema induced by triethyl tin. Both vinpocetine and vincamine possess only weak CA activity, the potency order being: nimodipine > diltiazem > flunarizine = verapamil > vinpocetine > vincamine, with vinpocetine and flunarizine, in contrast to other compounds, showing a clear, 6- to 13-fold selectivity for cerebral vascular smooth muscle. In the in vivo models, vinpocetine and flunarizine, together with vincamine, proved most active and had a larger spectrum of activity than the other CAs. These results suggest that the cerebrally selective CA effects of vinpocetine are at most only partly responsible for the effects of this compound in the in vivo models of cerebral ischemia.

Key words: vinpocetine, vincamine, calcium antagonist activity, models of cerebral ischaemia

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Address reprint requests to Hervé Poignet, Riom Laboratoires-Cerm, 63203 Riom Cedex, France.

INTRODUCTION

Vinpocetine is used in several countries for the treatment of cognitive and behavioural symptoms associated with vascular and degenerative disorders of the central nervous system [Otomoto et al., 1985]. The mode of action of vinpocetine still remains to be elucidated, although several mechanisms have been postulated, including effects on cellular translocations of calcium [Anderson et al., 1986]. Given that much evidence implicates calcium ion overload in the mediation of neuronal death following cerebral ischemia and reperfusion [Siesjo, 1981; Raiche, 1983; White et al., 1983; Meldrum et al., 1985], a CA effect of vinpocetine may contribute to the action of this drug in cognitive disorders.

The purpose of the present study was to evaluate the CA activity and the selectivity of vinpocetine and also the standard drug vincamine for cerebral vs. peripheral vascular smooth muscle and to evaluate their effects in five well-documented pharmacologic models of cerebral ischemia that have different etiologies. Flunarizine, nimodipine, diltiazem, and verapamil were used as reference CAs.

MATERIALS AND METHODS

Calcium-Antagonist Activity

Simultaneous measurements (i.e., in the same organ bath) of circular muscle contraction of rabbit basilar and splenic arteries were carried out. The arteries were set up in an organ bath containing a depolarizing solution (zero Ca^{++}, 40 mM KCl Krebs' solution) at 37°C and aerated with carbogen. Cumulative concentration-response curves with $CaCl_2$ were established. After two consecutive, identical control curves, the test substance was added to the bath; 15 min later, another $CaCl_2$ concentration-response curve was elicited in the presence of the test drug, and CA activity was quantified in terms of the calculated pA_2 value.

Normobaric Hypoxia

Male mice were pretreated intraperitioneally (i.p.) with test compound or vehicle 30 min prior to exposure to an atmosphere of 96% N_2 + 4% O_2. The time until death, as indicated by the last respiratory gasp, was measured for each animal.

Hypobaric Hypoxia

Male mice were pretreated i.p. with test compound or vehicle 30 min before establishing a constant barometric depression of 600 mmHg. The depression was created by a vacuum pump within 30 ± 2s. The time until death, as indicated by the last respiratory gasp, was measured for each animal.

Global Cerebral Ischaemia

Global cerebral ischaemia was induced by an intravenous (i.v.) injection of $MgCl_2$ (0.1 ml saturated solution), which caused immediate cardiac arrest followed by gasping (an indicator of severe hypoxia). The interval between cardiac arrest and the final gasp was used as the evaluation parameter. Control and drug-treated animals were given the vehicle or test compound i.p. 30 min before the $MgCl_2$ injection.

Cytotoxic Anoxia

Cytotoxic anoxia was produced in 180–200 g male Sprague-Dawley rats by rapid i.v. injection of 4 mg/kg of KCN. KCN was lethal within 1 min in 93% of untreated animals ($n = 237$). The number of animals surviving 2 hr after KCN injection was recorded in control animals and also in those receiving test drug orally 1 hr before challenge with KCN.

TABLE 1. Calcium Antagonist Activity and Tissue Specificity*

Drugs	pA_2		Ratio: basilar vs. splenic
	Basilar artery	Splenic artery	
Vinpocetine	5.85 ± 0.22(4)	5.10 ± 0.16(5)	6
Vincamine	5.43 ± 0.23(2)	5.31 ± 0.14(3)	1
Flunarizine	7.10 ± 0.15(9)	6.00 ± 0.25(9)	13
Nimodipine	11.00 ± 0.56(4)	10.47 ± 0.33(3)	3
Diltiazem	7.31 ± 0.07(3)	6.85 ± 0.58(2)	3
Verapamil	6.99 ± 0.40(3)	6.90 ± 0.55(3)	1

*( ) Number of experiments.

Cerebral Edema

Male Wistar rats weighing about 260 g were treated orally with 5 mg/kg/day of triethyl tin (TET) for five days. Placebo or test compound was administered orally twice daily during five consecutive days. On the fifth day, the animals were decapitated, the brains removed, and both wet and dry weights of the brain were recorded. Body weights were noted for all animals on each day of the study. On Days 1 and 5, neurological function was evaluated and results expressed as an index using a simple scoring system. Brain edema also was measured as the difference between the wet weight and dry weight, expressed in terms of 100 g brain tissue.

RESULTS

Calcium-Antagonist Activity (Table 1)

All six drugs had CA properties, but of varying potency and selectivity. Nimodipine was the most active, with calculated pA_2 values of 11.0 ± 0.56 and 10.47 ± 0.33 for basilar and splenic arteries, respectively. Vincamine was the least active compound in the basilar artery preparation (pA_2 = 5.43 ± 0.23), and it possessed similar activity in the splenic artery (pA_2 = 5.31 ± 0.14). Other compounds had intermediate activity that varied from vinpocetine with a basilar artery pA_2 value of 5.85 ± 0.22 to diltiazem with a value of 7.31 ± 0.07. Interestingly, only flunarizine and vinpocetine possessed a selective effect on the cerebral artery with basilar: splenic artery ratios of 13 and 6, respectively (Table 1).

Normobaric Hypoxia (Fig. 1)

In the normobaric hypoxia test, vincamine (10–20 mg/kg i.p.) was the most active compound, increasing the survival time (ST) index of the animals by 127% at the dose of 20 mg/kg i.p. Vinpocetine (10–50 mg/kg i.p.) was also active, increasing the ST index by 59% at the highest dose tested. The other reference CAs—flunarizine, nimodipine, diltiazem, and verapamil—all were inactive in this test.

Hypobaric Hypoxia (Fig. 2)

Vincamine (15, 25, and 50 mg/kg i.p.) was extremely active in this test, prolonging the ST index by 54, 135, and 201%, respectively. Vinpocetine also had significant activity in this test at 100 mg/kg i.p., increasing the ST index by nearly 75%. The reference CA drugs flunarizine and nimodipine were without significant activity in this model.

Global Cerebral Ischaemia (Fig. 3)

Vincamine at doses of 25, 50, and 100 mg/kg i.p. dose-dependently increased the ST index by 16, 21, and 41%, respectively in mice given a lethal intravenous injection of $MgCl_2$. At the same dose levels, however, vinpocetine was inactive. The reference CAs flunarizine

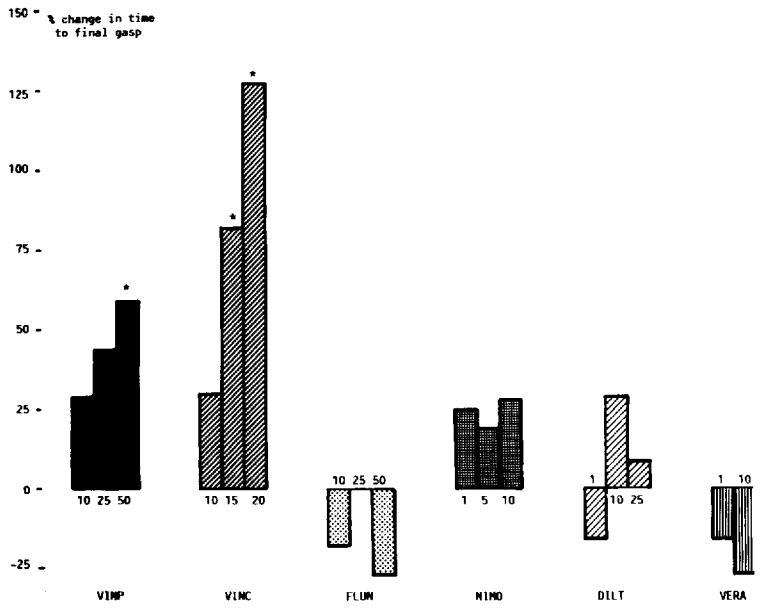

Fig. 1. Effects of vinpocetine (VINP), vincamine (VINC), flunarizine (FLUN), nimodipine (NIMO), diltiazem (DILT), and verapamil (VERA) on the survival time of mice in normobaric hypoxia induced by 96% N_2, 4% O_2. *$P < 0.05$ (analysis of variance: Newman-Keuls test).

(25–100 mg/kg i.p.), nimodipine (1–10 mg/kg i.p.), diltiazem (1–25 mg/kg i.p.), and verapamil (1–25 mg/kg i.p.) possessed moderate but significant activity in this test at some dose levels.

Cytotoxic Anoxia (Fig. 4)

In this model, vinpocetine (10–100 mg/kg p.o.) increased the survival rate of rats given an i.v. injection of KCN. Vinpocetine possessed significant activity at the lowest dose tested, but increasing the dose did not lead to a proportional increase in the survival rate (Fig. 4). Although vincamine (25–100 mg/kg p.o.) was less potent than vinpocetine in this test, a dose-related effect was observed. Flunarizine at 50 and 100 mg/kg p.o. was also active, but as with vinpocetine there was no clear dose-response relationship. Nimodipine was inactive in this test.

Cerebral Edema Induced by TET (Fig. 5)

Vincamine (50 mg/kg p.o., twice daily), vinpocetine (100 mg/kg p.o. twice daily), flunarizine (50 and 100 mg/kg p.o. twice daily), but not nimodipine (50 mg/kg p.o. twice daily), were active in this test. Vincamine (50 mg/kg p.o. bid) was approximately twice as active as vinpocetine in reducing the cerebral edema (−63%), the neurological deficit (−56%), and the body weight loss (−25%) induced by chronic triethyl tin (TET) treatment. Flunarizine (100 mg/kg p.o. twice daily), although reducing the cerebral edema to an extent similar to that of vincamine and vinpocetine, was less active than the latter drugs on the neurological deficit and had no significant effect on the body weight loss induced by TET.

DISCUSSION

The pathophysiological disorders related to cerebral ischaemia lead to neuronal cell death. Among the most important factors generating this neuronal necrosis is a cytosolic

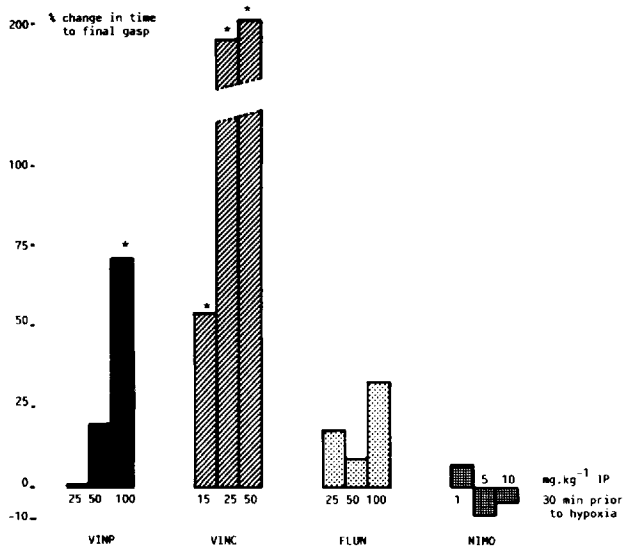

Fig. 2. Effects of vinpocetine, vincamine, flunarizine, and nimodipine on the survival time index of mice exposed to a hypobaric hypoxia. $^{*}P < 0.05$ (analysis of variance: Newman-Keuls test).

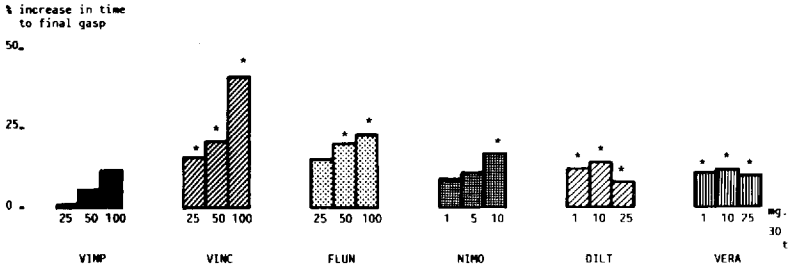

Fig. 3. Effects of vinpocetine, vincamine, flunarizine, diltiazem, and verapamil upon the survival time index to global cerebral ischaemia induced by cardiac arrest using intravenous $MgCl_2$. $^{*}P < 0.05$ (analysis of variance: Newman-Keuls test).

accumulation of calcium, the so-called calcium overload phenomenon [Farder, 1981]. With regard to how this excess calcium accumulates in cells, one possible route is via the calcium channels in the sarcolemma; it is interesting that marked differences now have been shown to exist between the calcium channels present in neurones and those found in vascular smooth muscle [Tsien et al., 1987]. There is a dense vascularization of brain tissue, and CAs also are known to reduce the hypoperfusion that occurs following cerebral ischaemia and restoration of flow. Thus, CAs theoretically may afford protection directly by preventing neuronal calcium accumulation and cell necrosis or indirectly by vasodilatation and preservation of cerebral blood flow.

The marked differences in the chemical structure of CA drugs make it likely that they possess distinct loci of action in or around the slow C^{++} channel and at other (e.g., intracellular) sites. Functional and ligand binding studies have led to recent proposals of CA

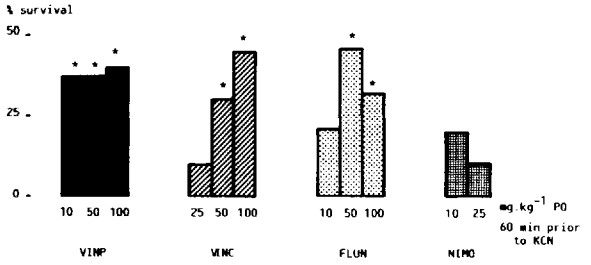

Fig. 4. Effects of vinpocetine, vincamine, flunarizine, and nimodipine on cytotoxic anoxia in the rat induced by KCN. $*P < 0.05$ (χ^2 test).

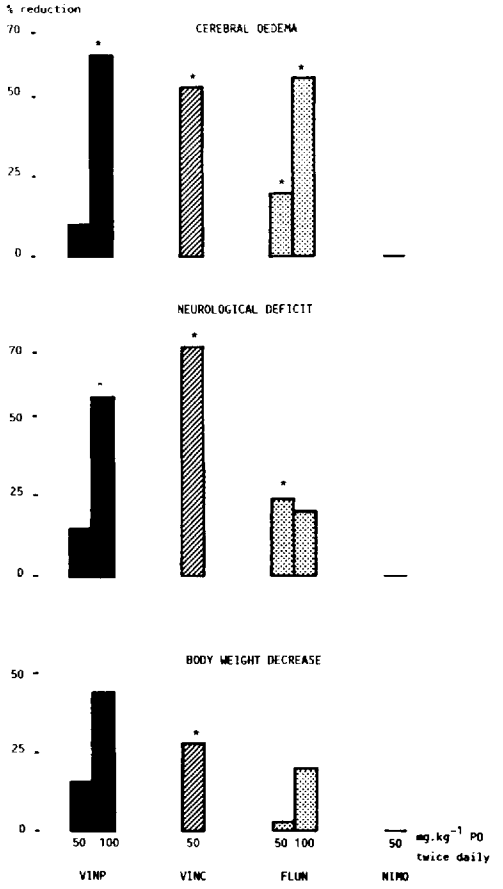

Fig. 5. Effects of vinpocetine, vincamine, and nimodipine in the cerebral edema, neurological deficit, and body-weight loss induced by chronic treatment of rats with triethyl tin. $*P < 0.05$ (analysis of variance: Newman-Keuls test).

subgroups: Group I = dihydropyridines, Group II = verapamil and diltiazem, and Group III = diphenylalkylamines (e.g., flunarizine) [Spedding, 1985]. At least three CA subgroups have been proposed, with the Group III compound flunarizine, in contrast to Group I and II CAs, being found to be particularly effective in animal models thought to be indicative of cerebral antihypoxic activity. In this respect, it has recently been termed a "calcium overload blocker" rather than a calcium channel blocker [Van Zwieten, 1985, 1986].

In experiments using vascular smooth muscle, vincamine and vinpocetine possessed relatively weak CA activity compared with the reference compounds, being some 10,000 times less potent than the most active compound, nimodipine. The other reference compounds—flunarizine, diltiazem, and verapamil—possessed intermediate activity in this test. Vinpocetine, like flunarizine, but in contrast to the other compounds, exhibited selectivity for the cerebral vs. the peripheral blood vessel, albeit vinpocetine's weak activity; a relatively good, broad spectrum of activity is noted for these two compounds in the in vivo cerebral ischaemia models. That this is not an entirely satisfactory explanation is evident from the anomalous results with vincamine, which, despite its relative lack of cerebral selectivity in the CA tests in vitro, possessed marked effects in all five in vivo models. The relationship between CA activity and activity in certain in vivo models of cerebral hypoxia, therefore, depends not only on cerebral selectivity, but also on the type of CA effect produced, with Group III-like activity (i.e., flunarizine-like) being particularly effective in this respect. Clearly, additional experiments with vinpocetine and vincamine are required to determine the significance of the weak CA activity observed in this study.

Vinpocetine, like vincamine, was generally active in the in vivo models, with one exception: the global cerebral ischaemia model induced by cardiac arrest. In this respect, these drugs, together with flunarizine, could be distinguished easily from nimodipine, diltiazem, and verapamil, whose main action at therapeutic dose levels is to block calcium entry into the cell. The protective effect of vincamine and vinpocetine against hypoxia in mice has been reported previously [Linee et al., 1977; Milanova et al., 1983; Anderson et al., 1986; Lamar et al., 1986; King, 1987]. Flunarizine is especially active in the cytotoxic anoxia test, against TET-induced edema, and in the global cerebral ischemia test; nimodipine, diltiazem, and verapamil were active only in the single model of global cerebral ischaemia.

In conclusion, these studies have shown that vinpocetine possesses protective effects in several animal models of hypoxia and ischemia. This action clearly is unlikely to be due to an effect upon calcium ion movements in vascular smooth muscle. Nevertheless, additional experiments are required to completely characterize the drug in this respect and to study its action at the level of cerebral neurones.

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