Protective Effect of Vinpocetine against Brain Damage Caused by Ischemia

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Received November 9, 1990 Accepted April 9, 1991

ABSTRACT—The effects of vinpocetine against hippocampal neuronal damage and on local cerebral blood flow (LCBF) were examined in a rat model of forebrain ischemia (10-min occlusion of the carotid arteries and hypotension). Histological evaluation of neuronal loss in the hippocampus was performed 7 days after ischemia. LCBF was measured before ischemia as well as after 2 min and 1 hr of recirculation. Vinpocetine (10 mg/kg) administered pre- or post-ischemically reduced the hippocampal neuronal necrosis, while pre-ischemic administration of 2 or 20 mg/kg vinpocetine was ineffective. Since vinpocetine increased the LCBF after 1 hr of recirculation, it cannot be excluded that blood flow improvements contribute to its neuroprotective activity. On the other hand, there is no clear evidence that an elevation of post-ischemic hypoperfusion could protect neurons against ischemic damage. It is, therefore, suggested that vinpocetine acts directly on brain cells.

Transient cerebral ischemia causes selective neuronal necrosis in certain vulnerable regions of the brain (1, 2). The pyramidal neurons in the CA1 subfield of the hippocampus are selectively vulnerable to ischemia (1, 2). Neurotoxic release of excitatory amino acids (3–5) and the resulting disturbance of intracellular calcium homeostasis (6–8) are thought to be critical factors that cause neuronal death. Thus, it was demonstrable that N-methyl-D-aspartate-receptor-antagonists (NMDA-antagonists) or calcium-antagonists were able to protect hippocampal neurons against ischemic damage (9–12). These neuroprotectants have been suggested to act directly on the cerebral parenchyma (12–14). Vinpocetine, a derivative of vincamine, also revealed protective activities in several models of hypoxia, anoxia and cerebral ischemia (15), but, to our knowledge, no information is available about the mechanism by which this drug is acting. The findings that vinpocetine causes a dilation of isolated cerebral arteries (16) and improves the global cerebral blood flow (17–19) suggest that cerebral circulatory effects may contribute to its cerebroprotective activity. To test this, the present study evaluates the pre- and post-ischemic LCBF of vinpocetine-treated rats. In addition, 2 histological series were set up to find out: 1) whether a dose-dependent effect of pre-ischemically administered vinpocetine on the inhibition of hippocampal damage is demonstrable, and 2) whether vinpocetine also has neuroprotective action after post-ischemic administration.

MATERIALS AND METHODS

Animals

Male Wistar rats (Ivanovas, Kisslegg, F.R.G.) weighing 250–300 g were used. They were maintained under controlled lighting and
environmental conditions (12 hr dark/light cycle, 23 ± 1°C, 55 ± 5% rel. humidity) and were fed a standard diet (Altromin, Lage, F.R.G.) and tap water ad libitum. Preceding the ischemic procedure, the rats were fasted overnight.

Materials

Vinpocetine was obtained from Thiemann Arzneimittel GmbH (Waltrop, F.R.G.). [14C]-Iodoantipyrine (IAP; spec. act., 60 mCi/mmol) was purchased from NEN (Dreieich, F.R.G.). All other chemicals were of reagent grade.

Induction of ischemia

Surgical procedure was performed according to Smith et al. (20). After anesthetizing with 3.5% halothane, the rats were connected to a Starling type respirator which delivered 0.8% halothane and 30% O₂ in N₂O. Muscle paralysis was achieved and maintained with suxamethonium chloride (Asta Pharma AG, Frankfurt, F.R.G.). The tail artery was cannulated for control of blood pressure and blood sampling. A catheter was advanced into the inferior vena cava, and heparin was injected (200 IU/kg). Halothane was then discontinued. Thirty minutes later, 5 mg/kg trimethaphan camphor sulfonate (Hoffmann-La Roche, Grenzach-Wyhlen, F.R.G.) was injected rapidly via the inferior vena cava, and cerebral ischemia was induced by clamping both carotid arteries and reduction of the blood pressure to 40 mmHg by exsanguination. After 10 min of cerebral ischemia, blood pressure was restored by removing the carotid clamps and reinfusion of the shed blood. A solution of NaHCO₃ (0.6 mmol/l) was injected to prevent a systemic acidosis. Finally, the rats were ventilated until they regained consciousness, disconnected from the pump and extubated. Body temperature was kept at 37°C with a heating lamp throughout the surgical procedures. After extubation, the rats stayed in cages at an environmental temperature of 30°C. These conditions result in normothermia of the brain during ischemia and postischemia (M. Seif el Nasr et al., unpublished results).

Local cerebral blood flow

LCBF was determined by using the [14C]-iodoantipyrine technique described by Sakura-da et al. (21). Before determination of LCBF in conscious non-ischemic rats, the femoral vessels were cannulated, and the rats were immobilized on a preparation desk. In this group, the experimental procedure started after 2 hr of recovery. In brief, 150 μCi/kg IAP was infused over 1 min using a ramp function. During infusion, 10 timed arterial blood samples were collected and analyzed for [14C]-iodoantipyrine content by liquid scintillation counting. The rats were decapitated shortly before the end of infusion, the brains were dissected out, immediately frozen in isopentane (−50°C), and sectioned in 20-μm slices which were exposed to Osray M3 film (Agfa-Gevaert, Leverkusen, F.R.G.) for 10 days. The optical densities of the autoradiograms were measured with a computer supported image analyzer (IBAS 2000, Kontron, Echingen, F.R.G.).

Histological assessment of ischemic neuronal damage

Seven days after ischemia, the rats were anesthetized with 1% halothane in a 2 : 1 mixture of nitrous oxide and oxygen. Brains were perfusion-fixed transcardially via the ascending aorta with a fixative containing 4% formaldehyde in a phosphate buffer of pH 7.35 after rinsing the brains with approximately 30 ml physiological saline. After a postfixation period of about 24 hr in the perfusion medium, the brains were dehydrated, embedded in Paraplast (Monoject Scientific, Kildare, Ireland), sectioned in 5-μm slices, which were stained with a mixture of 1% celestine blue and 1% acid fuchs in, and finally coverslipped. After this staining procedure, damaged neurons can be differentiated from intact neurons as densely stained violet cells (22). Intact and damaged neurons were counted in the entire given hippocampal CA1 subfield of frontal planes that were taken 6-mm rostral of
The tentorial incision. The percentage of neuronal damage per rat was calculated as the mean of the data obtained from the left and right hippocampus of one slice.

**Drug administration**

Vinpocetine, dissolved in acidic saline (pH = 5.5), was administered intraperitoneally 15 min before induction of cerebral ischemia as well as 15 min before determination of LCBF in non-ischemic rats. Single experiments of the series which would be compared statistically were carried out in a randomized manner. Post-ischemic application of vinpocetine (10 mg/kg, i.p.) was performed immediately after removing the carotid clamps and reinfusion of the shed blood. Controls received vehicle only.

**RESULTS**

Physiological parameters measured were within the range of normal values and were not altered after treatment with 2 or 10 mg/kg vinpocetine (Table 1). In contrast, a dosage of 20 mg/kg vinpocetine caused a decrease in the mean arterial blood pressure (Table 1).

In the hippocampal CA1 subfield of the rats, there were 1251 ± 98 pyramidal cells (mean ± S.D., n = 4) 7 days after sham-operation. Seven days after ischemia, 77% of the neurons were damaged in the CA1 subfield of the control rats (Figs. 1A and 2). Treatment of the rats with 2 mg/kg vinpocetine did not result in a significant reduction of hippocampal neuronal necrosis, while administration of 10 mg/kg vinpocetine reduced the ischemic neuronal damage in the CA1 subfield to 37% (Figs. 1B and 2). Interestingly, this neuroprotective effect was not demonstrable after treatment with 20 mg/kg vinpocetine (Fig. 2).

In a second series of experiments, we examined the effect of post-ischemic vinpocetine-application on the histologic outcome of the rat brain 7 days after ischemia. Here, vinpocetine (10 mg/kg) reduced the neuronal damage in the hippocampal CA1 subfield to 55% compared to 75% in the controls (Fig. 3).

LCBF of rats treated with vinpocetine (10 mg/kg i.p., 15 min prior to ischemia) was measured before (Fig. 4A) as well as 2 min (Fig. 4B) and 1 hr after ischemia (Fig. 4C). The LCBF in rats was not influenced by vinpocetine when measured before ischemia (Fig. 4A). Two minutes after ischemia, LCBF was only increased in some cortical brain areas of vinpocetine-treated rats (Fig. 4B). At 1 hr of recirculation, vinpocetine caused a significant increase of LCBF in nearly all brain structures.

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<th>Table 1. Physiological variables</th>
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Measurement of physiological variables in control and vinpocetine-treated rats was performed after 30 min of adaptation to the artificial ventilation. The values are given as means ± S.D. of 9 experiments. *P < 0.05 (Mann-Whitney test).
Fig. 1. Histological demonstration of neuronal damage in the CA1 subfield of untreated rats (A) and vinpocetine-pretreated (10 mg/kg) rats (B) 7 days after ischemia.
DISCUSSION

The histological examinations of the rat hippocampus presented in this study (Figs. 1–3) confirmed previous findings that a 10-min forebrain ischemia injured to a great extent the pyramidal neurons of the hippocampal CA1 subfield (1, 2). Pre- or post-ischemic application of 10 mg/kg vinpocetine prevented this post-ischemic neuronal damage, while pre-ischemic application of 2 or 20 mg/kg vinpocetine did not reduce the neuronal necrosis in the CA1 subfield. The lack of neuroprotection by 20 mg/kg vinpocetine was accompanied by pre- and post-ischemic reduction of the mean arterial blood pressure. This result provides some evidence that the protective effect of a drug against ischemic damage of brain tissue can be reduced by a post-ischemic hypotensive effect. However, it is unlikely that the slight reduction of the mean arterial blood pressure to 100 mmHg after treatment with 20 mg/kg vinpocetine (Table 1) causes an extracerebral steal phenomenon which results in a poorer histologic outcome 7 days later. This is supported by the findings of Mendelow et al. (23) who demonstrated that the LCBF was unchanged in rats when the mean arterial blood pressure was reduced to 60 mmHg. It is rather suggested that the hypotensive effect of vinpocetine impairs the physical conditions of the rat which counteracts its ameliorative activity.

Previous investigations have demonstrated an increase of global cerebral blood flow after intravenous administration of vinpocetine (17–19). Therefore, blood flow improvements by vinpocetine could be considered to be of value for the cell survival. In the present study, the pre-ischemic LCBF was not enhanced by vinpocetine 15 min after i.p. administration. Such contradictory results could be attributed to different doses and modes of drug-application. LCBF measurements performed 2 min after ischemia indicate that vinpocetine ameliorates cerebral blood flow in some cortical areas analyzed (Fig. 4C).
Fig. 4. Local cerebral blood flow of rats before (A) and at 2 min (B) and 1 hr (C) after ischemia. Vinpocetine was administered 15 min prior to induction of ischemia. Values are means ± S.D. of 10 experiments. Significantly different from the controls: **P < 0.01, *P < 0.05 (Mann-Whitney test). [] control, ☑ vinpocetine (10 mg/kg, i.p.).
only, whereas the hippocampal LCBF was not changed.

The low values given for LCBF of saline-treated rats 1 hr post-ischemia reflect the period of delayed hypoperfusion (24). Pre-ischemic treatment of rats with vinpocetine caused an increase in LCBF of all brain areas analyzed at this post-ischemic time point. This may lead to the assumption that an effect on brain vasculature contributes to the protection of hippocampal neurons by vinpocetine. However, the influence of hypoperfusion on the development of post-ischemic neuronal necrosis is not yet clear. It could be argued that hypoperfusion of post-ischemic brain tissue results in a decreased oxygen and glucose supply and in a reduced drainage of metabolites, promoting processes which induce neuronal necrosis. Thus it may be possible that the improvement of cerebral blood flow in this period of reperfusion can ameliorate the histological consequences of cerebral ischemia. On the other hand, it was reported that some calcium- and NMDA-receptor-antagonists exert a cerebroprotective effect on hippocampal neurons without changing the post-ischemic blood flow (12–14). Taking these data into consideration, it is concluded that the amelioration of hypoperfusion by vinpocetine alone cannot explain its neuroprotective effect. Although a direct effect on brain parenchyma seems to be likely, the mechanism of action of vinpocetine is not clear. Lamar et al. (15) demonstrated that vinpocetine has no calcium-antagonistic activity. In addition, neither antagonistic actions at the NMDA-receptor site nor barbiturate-like effects are described for vinpocetine. However, running experiments with cultured neurons in our laboratory demonstrate that vinpocetine increases the neuroprotective effect of adenosine (R. Rischke and J. Kriegstein, to be published).

To sum up our results, vinpocetine was demonstrated to protect hippocampal neurons against ischemic damage by pre- or post-ischemic intraperitoneal application. This effect was limited to the tested dose of 10 mg/kg. As already suggested for other neuroprotective drugs, a direct action on brain parenchyma is thought to be responsible for the reduction of hippocampal neuronal necrosis in vinpocetine-treated rats. However, it cannot be completely excluded that improvement of post-ischemic hypoperfusion caused by vinpocetine could contribute to its neuroprotective effect.

Acknowledgments
This research was supported by a scholarship (R.R.) of the Philipps-Universitat Marburg. The authors are grateful to Mrs. R. Seidel and Miss S. Engel for their skillful technical assistance.

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