
原 著

Ca²⁺ Antagonist and Protection of Brain against Ischemia. Nicardipine Levels in Rat Brain Following Intraperitoneal Administration

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Summary

Levels of nicardipine, a dihydropyridine Ca²⁺ antagonist, in the brain of rats were determined following an intraperitoneal injection using a method of radioligand competitive-binding assay. [³H] nitrendipine was used as a ligand. Brain level of nicardipine 15 minutes after an intraperitoneal administration was 15.85 ng/g, or 31.8 nmol/kg, namely the brain tissue nicardipine levels effective to mediate the pharmacological effects on neuronal metabolism were achieved.

Among the proposed strategies for acute cerebral ischemia calcium antagonists have recently gained much interests [10, 17, 19]. Free calcium ion (Ca²⁺) has been proposed to be a trigger of the process of ischemic neuronal death [18]. Besides their vascular effects, therefore, Ca²⁺ antagonists are expected to show also the beneficial neuronal metabolic effects [1, 13, 19].

Using an experimental model of global cerebral ischemia in rats, we have found that a dihydropyridine Ca²⁺ antagonist nicardipine effectively attenuates the liberation of free fatty acids (FFA's) in the ischemic brain [14]. As FFA's of the brain have been known as one of the biochemical markers indicating ischemic damage of the brain cell membrane, our results seem to support a possible protective effect of nicardipine against cerebral ischemia. Prior to study further the beneficial neuronal metabolic effects of the drug, however, it seems essential to confirm that the drug is present in the brain tissue at a concentration sufficient to mediate its pharmacological effects. We have determined, therefore, brain nicardipine levels following parenteral administration, using a radioligand competitive-binding technique.

Key words: Cerebral ischemia, Ca²⁺ antagonist, Nicardipine; Brain drug level, Rat.

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Materials and Methods

Male Wistar rats weighing from 220 to 240 g were used. Animals were decapitated with a guillotine exactly 15 minutes after an intraperitoneal injection of 1 mg/kg of nicardipine (dissolved in the physiologic saline at a concentration of 1 mg/ml). The cerebral hemispheres were quickly removed, divided into two halves, and a unilateral hemisphere was used for the quantitation of brain nicardipine levels (5 rats). At the same time, the mixed blood was collected from 3 rats, incubated at 4°C for 3 hours and the serum was separated by centrifugation at $900 \times g$ for 30 minutes. Four rats that received an intraperitoneal injection of 1 ml/kg of the physiologic saline served as the control.

The cerebral hemispheres were weighed, homogenized in a Teflon homogenizer with 4 volumes of methanol-0.1 N HCl (9 : 1, v/v), and the homogenate was centrifuged for 15 minutes at $900 \times g$ and at 4°C. Two ml of the supernatant was desiccated under vacuum in a rotatory evaporator at 38°C, redissolved in the solution consisting of 40 μ l of methanol and 360 μ l of 0.1% ascorbic acid, centrifuged for 15 minutes at $900 \times g$ and 4°C, and the final supernatant (methanol extract) was used for a radioreceptor assay.

For preparation of brain membranes to be used in the binding assay, another group of Wistar rats were sacrificed by decapitation. The cerebral hemispheres were quickly removed without preparatory perfusion, weighed, cooled in the iced 0.85% NaCl solution, and homogenized in a Teflon homogenizer with 9 volumes of the iced assay buffer consisting of 50 mM Tris-HCl and 10 mM EDTA, pH 7.7. The sample of the homogenate was centrifuged at $900 \times g$ and 4°C for 10 minutes, and the supernatant was further centrifuged at $18000 \times g$ for 20 minutes. The precipitate was washed twice by Tris buffer (50 mM Tris-HCl, 0.1 mM EDTA, pH 7.7), and kept at -80°C until the use.

[3H]-nitrendipine (193.1 dpm/fmol, 1 μ Ci/ μ l) was purchased from New England Nuclear. For preparation of the standard solution, nicardipine-HCl was dissolved in the diluent consisting of 10% methanol and 0.1% ascorbic acid at a concentration of 0.2 mM, and then diluted to the concentrations of 2, 6, 20 and 60 nM.

The binding assay was performed by the method of Gould et al [8]. Two ml of the brain membrane preparation (25.4 mg/ml protein) was dissolved in 7 ml of the calcium-containing buffer (50 mM Tris-HCl, 0.1 mM EDTA and 1.25 mM CaCl_2 , pH 7.7). Three Ci/ μ l of [3H]-nitrendipine was diluted with 4.5 ml of the calcium-containing buffer. One hundred μ l of the prepared brain membrane solution and 50 μ l of [3H]-nitrendipine solution were added to 800 μ l of the calcium-containing buffer. Fifteen μ l in volume of either the diluent for total binding, the standard solution of nicardipine, the methanol extract for assay, or the five-fold diluted serum, was further added to the mixture. After the incubation in the dark at 25°C for 60 minutes, the mixture was rapidly filtered under vacuum with a Whatman GF/B filter. The filter was then washed four times with 4 ml of the iced Tris buffer (50 mM Tris-HCl, 0.1 mM EDTA, pH 7.7), and the radioactivity was measured by use of a liquid scintillation counter (Packard TRI-CARB 4640).

Total binding was obtained from the counts of the diluent, and non-specific binding was obtained from those under the presence of 0.2 mM nicardipine. Specific binding was obtained as the difference between the binding of each sample and of non-specific binding. The standard curve was obtained from the data from the known concentrations of nicardipine-HCl in the diluent by the linear regression of logit-log method by DeLean et al [5]. At least two samples were examined for each rat, and the measurements were duplicated for each sample.

Results

As has been described previously [2, 3, 4, 6, 15, 16], [3H]-nitrendipine binding to brain cell membrane was monophasic and saturable, with the apparent affinity constant (KD) of 0.37 nM and the maximal number of binding sites (Bmax) of 144.6 fmol/g (wet weight) in this experiment. A plot of [3H]-nitrendipine bound correlated with concentration of unlabeled nicardipine yielded the standard curve, with the derived formula of:

$$F(x) = 6082 - 5150 / (1 + 0.2436 / X).$$

The concentrations of nicardipine in the brain and serum were measured using this standard curve. Fifteen minutes after an intraperitoneal injection of nicardipine, measured levels of the drug was 15.85 ± 1.51 ng/g in the brain and 34.12 ± 4.63 ng/ml in the serum. Brain to serum ratio was thus $43.12 \pm 3.77(\%)$. In the control group injected with the physiologic saline, the levels of the drug was 0 ng/ml in the serum, and 0.35 ± 0.13 ng/g in the brain which apparently was within the limit of an experimental error.

Discussion

Previously known dihydropyridine Ca²⁺ antagonists bind directly the voltage-dependent calcium channels and compete with considerable potency for these sites. The binding of dihydropyridines has been widely studied and high-affinity receptors have been found in the brain of rats and gerbils as well as in the human frontal lobe cortex [3, 4, 7, 9, 16].

Radioligand competitive-binding assays have been widely used to measure the blood levels of various Ca²⁺ antagonists. We have used this technique to measure the drug levels in the brain and the serum of rats following an intraperitoneal administration of unlabeled nicardipine. The rat brain had not been perfused in this study. In the separate experiment using the perfused cat brain, however, similar brain levels of nicardipine (10 to 18 ng/g) were obtained, and we believe that the contamination with the drug present in the cerebral blood pool is negligible in the rat study. Dissociation constant (Ki) of nicardipine against [3H]-nitrendipine is approximately 0.9 nM [20, 21], and the Bmax of nimodipine in the gerbil brain is 12 nmol/kg (12 pmol/g) [11]. Accordingly, the brain level of nicardipine achieved 15 minutes after a 1 mg/kg dose in this study (15.9 ng/g, or 31.8 nmol/kg) is three times higher than the Bmax and seems to be sufficient to mediate the pharmacologic effects.

Using gas chromatography, Higuchi et al [12] reported that the serum level of nicardipine in the rat reached ca. 50 ng/ml 10 minutes after an intravenous injection of 0.1 mg/kg of the drug. In the present study, the level of nicardipine was 34.14 ng/ml 15 minutes after a

1.0 mg/kg dose, but the drug was given intraperitoneally. The calculated brain to serum ratio of the drug amounts to 43%, but this may be an underestimate as nicardipine, which easily binds with the serum protein, might have been partly lost in the precipitate during the process of methanol extraction.

In conclusion, we have demonstrated that nicardipine accumulates quickly in the brain following an intraperitoneal injection in rats, and that the brain levels of the drug capable of mediating pharmacologic effects on cerebral metabolism can be achieved at the physiological dosage with minimal risk of side effects such as systemic hypotension. The present result seems to substantiate the possible beneficial effect of Ca^{2+} antagonist nicardipine on neuronal metabolism in the ischemic brain, besides its vascular effects.

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和文抄録

カルシウム拮抗剤と脳虚血に対する保護作用 —ラットにおけるニカルジピン腹腔内 投与後の脳への移行について—

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木戸岡 実, 松田 昌之, 半田 譲二

最近2・3のカルシウム拮抗剤が脳血管拡張作用のほかに虚血脳に対し直接保護作用をもつことが知られてきた。ニカルジピンもその例に洩れないが、この薬剤の脳内移行は確認されていなかった。

本実験ではラットに血圧低下などを来さない1mg/

kg量のニカルジピンを腹腔内投与し、15分後に脳を摘出し、radioligand competitive binding assayを用いて、脳内に生理作用を呈するに十分な量(34.12±4.63 ng/ml)が移行していることを証明した。