Nimodipine levels in gerbil brain following parenteral drug administration

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Nimodipine binding to the particulate fraction of gerbil brain homogenate was characterized using tritiated (3H)-nimodipine as the radioactive ligand. Binding was monophasic and saturable, with the apparent affinity constant \( K_d \) = 0.4 nM and the maximum number of binding sites \( B_{max} \) = 12 nmol/kg wet wt. A competitive binding assay was validated for the measurement of nimodipine using gerbil brain as the source of receptors for the drug. Binding characteristics were sufficiently similar in specimens from different animals to allow the use of homogenates from individual animals as the source of both membrane-binding sites and competing ligand. Nimodipine could be detected in the brains of animals sacrificed soon after drug injection, and reached a peak level within 15 minutes. Brain drug level at a given time was a linear function of dose administered. One hour after a 1-mg/kg dose, the level of drug measured in brain was approximately 100 nmol/kg wet wt, more than 200 times the \( K_d \). Sufficient drug to mediate a maximal pharmacological effect accumulated in brain even after a dose of only 0.25 mg/kg. Thus, in this species, effective tissue nimodipine levels may be achieved at doses which minimize the risk of systemic hypotension.

KEY WORDS - nimodipine - calcium channel blocker - brain drug level - drug delivery - gerbil

CALCIUM channel blockers, in particular the dihydropyridine, nimodipine, have been investigated as possible therapeutic agents for the treatment of cerebrovascular disease of varied etiology. Attention has focused primarily on their ability to prevent vasoconstriction in order to explain an observed protection against neurological injury during ischemia. We have demonstrated effects of nimodipine on cerebral metabolism in the gerbil during cerebral ischemia and recirculation (DS Heffez, et al., in preparation). In order to attribute a protective effect of nimodipine to an effect on cerebral metabolism, it is essential to demonstrate that the drug is present in brain tissue at concentrations sufficient to mediate pharmacological effects. In the present study, binding of tritiated (3H)-nimodipine to gerbil brain membrane has been characterized. Brain nimodipine levels have been determined using a radioligand competitive-binding technique, in which a given animal provides both the unlabeled ligand and the source of membrane receptors. The time course and dose dependence of brain tissue nimodipine levels following intraperitoneal administration of the drug have been determined. The implications of the findings to the clinical use of nimodipine are discussed.

Materials and Methods

Three-month-old female gerbils (Meriones unguiculatus), weighing 50 to 80 gm each, were maintained in standard animal quarters, with water and Purina rat chow.

A 10-mg/ml stock solution of unlabeled nimodipine and polyethylene glycol-400 (PEG-400) (PEG-400) was made. An aliquot of this solution was diluted with normal saline to give a 0.5-mg/ml suspension immediately prior to intraperitoneal administration. Standard concentrations of unlabeled nimodipine were made from the stock solution by serial dilution with brain homogenate. Nifedipine was dissolved in PEG-400 to a concentration of \( 10^{-3} \) M and then diluted to \( 10^{-5} \) M with assay

* Nimodipine and PEG-400 obtained from Miles Laboratories, West Haven, Connecticut.
† Nifedipine was a generous gift from Dr. Randolph Patterson.
buffer. The \( ^3\text{H}-\text{nimodipine}\) was diluted appropriately with assay buffer for use in the binding experiments.

For preparation of brain membranes to be used in the binding assays, animals were sacrificed by cervical dislocation and briefly perfused with normal saline through a cardiac cannula to remove the cerebral blood. The cerebral hemispheres were removed, weighed, and homogenized by polytron$ in 80 volumes of iced assay buffer consisting of 50 mM Tris HCl, pHi 7.7, 4 mM CaCl\(_2\), and 0.1% ascorbic acid.\footnote{Polytron pcu-2-110 manufactured by Kinemetica, Lucerne, Switzerland.} An 800-\(\mu\)l sample of the brain homogenate was incubated with 100 \(\mu\)l of \(10^{-9}\) M \(^3\text{H}-\text{nimodipine}\) in a final volume brought to 1 ml with buffer or unlabeled nifedipine. Tubes were incubated in the dark for 1 hour at 24°C and the contents were filtered under vacuum through Whatman GF/B filters with three 1.5-ml washes of iced assay buffer. Filters were counted in 10 ml of aqueous counting scintillation medium,\footnote{Liquid scintillation manufactured by Amersham Corp., Arlington Heights, Illinois.} with an efficiency of 35% to 40%. Specific binding of \(^3\text{H}-\text{nimodipine}\) was obtained as the difference in counts bound in the presence or absence of unlabeled nifedipine \(10^{-6}\) M. All incubations were performed in duplicate. Precautions were taken at all times to avoid exposure of nimodipine or nifedipine to light.

Unknown concentrations of nimodipine in brain were determined by comparison with a standard curve generated using known concentrations of unlabeled nimodipine, or by a mathematical formula derived from the analogous competitive-binding of substrate to enzyme.\footnote{\(^3\text{H}-\text{nimodipine}\) was supplied by Dr. George S. Allen. \(^3\text{H}-\text{nimodipine}\) was supplied by Dr. George S. Allen. Polytron pcu-2-110 manufactured by Kinemetica, Lucerne, Switzerland.} The equation was used in the form:

\[
I = (1 + F/B)(1 - B/Bo)(B_{\text{max}}),
\]

where \(I\) is the unknown concentration of drug, \(F\) indicates counts/minute (cpm) of free \(^3\text{H}-\text{nimodipine}\), \(B\) indicates cpm of bound \(^3\text{H}-\text{nimodipine}\) in the presence of unlabeled drug, \(Bo\) indicates cpm of bound \(^3\text{H}-\text{nimodipine}\) in the absence of unlabeled drug, and \(B_{\text{max}}\) is the maximum number of binding sites in an aliquot of gerbil brain homogenate.

\section*{Results}

The monophasic saturable binding of nimodipine to gerbil brain membranes is depicted in the representative Scatchard plot shown in Fig. 1. The maximum number of binding sites, \(B_{\text{max}}\), determined in three animals was 11.9 ± 1.7 nmol/kg wet wt brain, and the apparent affinity constant, \(K_D\), was 0.42 ± 0.06 nM.

A plot of \(^3\text{H}-\text{nimodipine}\) bound correlated with concentration of unlabeled nimodipine is shown in Fig. 2. Experimental points are indicated along with a predicted curve derived from the formula outlined in the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{A representative Scatchard plot of \(^3\text{H}-\text{nimodipine}\) binding to the particulate fraction of gerbil brain homogenate. Gerbil brain was homogenized in 80 volumes of buffer, and 800-\(\mu\)l aliquots were incubated with 0.065, 0.175, 0.34, or 0.68 nM \(^3\text{H}-\text{nimodipine}\) in a final volume of 1 ml. Tubes were incubated, and the contents filtered as described in the Materials and Methods section. Specific binding was obtained as the difference in counts bound in the presence or absence of competing nifedipine \(10^{-6}\) M. Free nimodipine was obtained as the difference between total counts added and specific bound counts. Each point represents the mean of triplicate determinations in one animal. For this preparation, \(K_D = 0.44\) nM and \(B_{\text{max}} = 14\) nmol/kg wet wt; \(r = 0.979\).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Standard curve showing relative binding of \(^3\text{H}-\text{nimodipine}\) correlated with the concentration of unlabeled drug. Gerbil brain homogenates and specific binding of \(^3\text{H}-\text{nimodipine}\) were obtained as described in the Materials and Methods section. Dilutions of stock nimodipine were prepared in brain homogenate to give concentrations of 0.31, 0.62, 1.25, and 2.5 nM in the final incubation. Experimental points are plotted along with a predicted curve derived using the equation described in the text. Each point represents the mean of duplicate determinations.}
\end{figure}
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FIG. 3. Brain nimodipine level correlated with the dose of drug administered. Gerbils were sacrificed 1 hour after receiving an injection of PEG-400 or 0.25, 0.5, 1, or 2 mg/kg of intraperitoneal nimodipine. After perfusion, brains were homogenized, the homogenates were incubated and filtered, and brain nimodipine levels were calculated as described in the Materials and Methods section. Each point represents the mean and standard error for duplicate determinations in three animals.

FIG. 4. Time course of brain nimodipine levels following drug administration. Gerbils were sacrificed immediately or 15, 30, 60, or 240 minutes after a 1-mg/kg intraperitoneal (IP) dose of nimodipine. Control animals were injected with PEG-400 and sacrificed immediately. Brain nimodipine levels were determined as described in the Materials and Methods section. Each point represents the mean and standard error for duplicate determinations in three animals. Values obtained at 4 hours or immediately after injection were near the limits of detection using this method.

Discussion

All dihydropyridine calcium channel blockers bind directly to the voltage-dependent calcium channel. While there are differences among the various compounds with regard to the affinity for the calcium channel, all known dihydropyridines compete with considerable potency for these sites. This binding is monophasic, saturable, and specific. The binding of dihydropyridines has been extensively studied in various tissue preparations. High-affinity receptors are present in guinea pig and rat brain and in the human frontal cortex. Measured K_D ranges from 0.2 to 1.1 nM, and the B_max varies from 6 nmol/kg wet wt in the human to 60 nmol/kg wet wt in the guinea pig. Autoradiographic studies in the rat using ^3H-nitrendipine have demonstrated regional variations in the density of these receptors. Our data show that the characteristics of nimodipine binding to gerbil brain membranes are similar to those reported in other species, with K_D = 0.42 nM and B_max = 12 nmol/kg wet wt.

Radioligand competitive-binding assays have been used to measure the blood levels of various calcium channel blockers. These assays detect parent drug and all therapeutically active metabolites. We have demonstrated that the measurement of drug levels in brain tissue following in vivo administration of unlabeled nimodipine is also possible using similar techniques. In the current study, each gerbil brain served as the source of unlabeled nimodipine and of membrane-bound receptors. This introduces some biological variability in the value of K_D and B_max and therefore in the determined concentration of nimodipine. This variability could be eliminated by extracting the drug from the individual brains and using a single source of brain membranes for the actual binding assay. The agreement between the experimental and theoretical standard curves and the reproducibility of drug levels determined in different experiments indicates that such variability is not great.

Nimodipine is rapidly absorbed after an intraperitoneal injection in gerbils; the drug can be measured in the brains of animals sacrificed soon after a 1-mg/kg intraperitoneal dose. Peak brain levels of nimodipine are achieved within 15 minutes. Concentrations 200 times that required to half-saturate the high-affinity receptors are maintained for more than 1 hour. Four hours after administration, much lower drug levels are
measured; this time course is compatible with the dosage interval used in a recent clinical trial of nimodipine. Brain tissue levels of nimodipine vary directly with the intraperitoneal dose administered. Levels achieved 1 hour after a 0.25-mg/kg dose are almost 100 times the value of \( K_D \). It is unlikely that the levels determined represent the free concentration of drug available to high-affinity receptors. In vivo, some of the drug is likely to be compartmentalized and therefore cannot equilibrate with these receptors. The act of homogenization disrupts this distribution. The linear Scatchard plot, nevertheless, suggests that other tissue-binding sites must have much lower affinity for the drug, and would be expected to buffer the level of free nimodipine at a concentration well above the \( K_D \) for the high-affinity receptor. The level of nimodipine measured in the cerebrospinal fluid of patients receiving the drug to prevent vasospasm following subarachnoid hemorrhage was 1.8 nM; this concentration is insufficient for a maximal vascular effect, and yet a significant protection against severe neurological deficit was observed in the treated patients. In view of our present findings, much higher drug concentrations may have been achieved in the brain tissue. It is possible that nimodipine affects cerebral metabolism as well as cerebral blood flow. If so, our data suggest that a lower dose of drug than previously recommended may be adequate. Concentrations of nimodipine sufficient to mediate a maximal pharmacological effect on cerebral metabolism can easily be achieved at doses that minimize the risk of significant side effects.

In conclusion, we have demonstrated that nimodipine binding in gerbil brain is similar to that reported in other species. Nimodipine accumulates quickly in the brain following intraperitoneal injection in gerbils. Concentrations achieved are dose-dependent in a linear fashion, and may be sufficient to fully saturate the voltage-dependent calcium channels even at the lowest dose administered (0.25 mg/kg). Thus, brain levels of nimodipine capable of mediating pharmacological effects on cerebral metabolism can be achieved at doses that minimize the risk of side effects such as hypotension.

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References


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