Dihydropyridine Ligand Binding Decreases Earlier in Adolescent Than in Infant Swine After Global Cerebral Ischemia

Paul J. Hoehner, MD; Jeffrey R. Kirsch, MD; Mark A. Helfaer, MD; Travis F. Ganunis; Maureen T. Murphy, MD; Richard J. Traystman, PhD

Background and Purpose Voltage-dependent calcium channels (VDCCs) are thought to play a major role in the alteration of calcium homeostasis during ischemia. Tissue functional state as well as responsiveness to therapy with calcium channel blockers may be a function of regional changes in the density of VDCCs. This study determined whether VDCCs are altered by global ischemia in infant and adolescent swine.

Methods We employed the radioligand \(^3\)HPN200-110 to quantify the binding characteristics of VDCCs in cerebral cortex, caudate, and hippocampus by equilibrium binding analysis. Adolescent and infant pigs underwent 3, 5, 10, and 20 minutes of global cerebral ischemia without reperfusion by ligation of the brachiocephalic and left subclavian arteries combined with hypotension to a mean arterial blood pressure of 50 mm Hg. Brain cortex, hippocampus, and caudate samples were taken during ischemia and frozen immediately in liquid nitrogen, and crude synaptosomal membranes were isolated by differential centrifugation/filtration. \(^3\)HPN200-110 equilibrium binding assays were performed in the presence or absence of 1.0 \(\mu\)mol/L unlabeled nitrendipine to determine total and nonspecific binding.

Results Infant cortex maximal binding (\(B_{\text{max}}\)) increased to 176% of control after 5 minutes of global cerebral ischemia and remained significantly increased (172% of control) after 10 minutes before falling to near control levels by 20 minutes. Adolescent cortex \(B_{\text{max}}\) increased to 157% of control levels after 5 minutes but did not remain elevated, falling to 131% of control by 10 minutes and near control by 20 minutes. Infant caudate and hippocampus binding were significantly elevated after 10 (124% and 149% of control, respectively) and 20 (115% and 120% of control, respectively) minutes of ischemia. Adolescent caudate and hippocampus binding was either not significantly different from control levels (hippocampus at 10 minutes) or less than control after 10 and 20 minutes of global cerebral ischemia. The decrease in binding following the initial upregulation, which appeared earlier in the adolescent than the infant pigs, may indicate decreased tolerance to ischemia in the adolescent.

Conclusions The binding of \(^3\)HPN200-110 in brain is altered during 20 minutes of global cerebral ischemia, and these changes are region- and age-dependent. (Stroke. 1994;25: 2060-2066.)

Key Words • calcium channel blockers • calcium channels • cerebral ischemia, global • infants • nitrendipine

Intracellular (\(\text{Ca}^{2+}\)) accumulation has been implicated in contributing to neuronal infarction following ischemia.\(^1\) Calcium flux through agonist-operated calcium channels and voltage-dependent calcium channels (VDCCs) results from cellular energy failure caused by ischemia. Both agonist-operated calcium channels and VDCCs mediate enhanced neuronal calcium permeability. Tissue functional state as well as responsiveness to therapy with calcium channel blockers may result from regional changes in the density of these channels. Recent studies suggest ischemia may directly alter the pharmacokinetic properties of calcium channels. Specifically, \(L\)-type channels labeled by the dihydropyridine (DHP) ligand \(^3\)HPN200-110 (Isradipine) appear to be upregulated by short-term ischemia. Short-term, mild ischemia in the rat, produced by 1 hour of right carotid artery ligation, results in a 60% increase in binding.

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control values after global ischemia than mature pigs after 10 minutes of global ischemia produced by aortic cross-clamping10 and after 30 minutes of ischemia produced by elevation of intracranial pressure.11 The mechanisms responsible for the more rapid return of these variables to control levels remain uncertain.

The present study was performed to examine the temporal relation in DHP ligand binding to L-type VDCCs after short-term global cerebral ischemia in adolescent and infant pigs. We hypothesized that ligand binding to the L-type VDCC would be increased after short-term global cerebral ischemia in both infant and adolescent pigs and that subsequent decreased binding, reflecting sensitivity to ischemia and impending cell death, would occur sooner in the older pigs.

Materials and Methods

Surgical Preparation

Two groups of mixed-breed pigs were used in this study: 25 1- to 2-week-old (2 to 4 kg) and 21 6- to 8-month-old (60 to 80 kg) animals. Age ranges were supplied by the breeder (Thomas D. Morris). The pigs were anesthetized with pentobarbital sodium (65 mg·kg\(^{-1}\)·IP), and anesthesia was maintained with pentobarbital infusions (5 mg·kg\(^{-1}\)·h\(^{-1}\)·IV) before the onset of ischemia. All pigs were paralyzed with pancuronium bromide (1.0 mg·kg\(^{-1}\)·IM) to facilitate surgical preparation. Each animal was mechanically ventilated via a tracheostomy so that minute ventilation could be adjusted to maintain arterial P\(_{\text{CO}_2}\) between 25 and 40 mm Hg throughout the experiment. Supplemental oxygen was administered to maintain arterial P\(_{\text{O}_2}\) above 80 mm Hg. Rectal temperature was maintained at 38±1°C with the use of a heat lamp and a heating pad. A catheter was placed in a femoral vein and advanced into the inferior vena cava for administration of fluids and drugs. Mean arterial blood pressure (MABP) was monitored through a catheter inserted into the descending aorta via a femoral artery and simultaneously through a catheter placed in the right axillary artery via the brachial artery. Through a left thoracotomy, ligatures were loosely placed around the brachiocephalic and left subclavian arteries for later use in combination with controlled hypotension to produce global cerebral ischemia. After the ligatures were secured, the pig was turned prone and its head was stabilized so that the external auditory meatus was above the level of the heart (−10 cm in pigs, −5 cm in piglets). The brain was exposed by craniotomy, and intraparenchymal brain temperature was measured and maintained at 38±1°C.

MABP was measured continuously with a Statham P-23 pressure transducer and recorded on a Gould-Brush recorder. Arterial P\(_{\text{O}_2}\) (Pao\(_2\)), P\(_{\text{CO}_2}\) (Paco\(_2\)), and pH were measured with a self-calibrating Radiometer electrode system (ABL3). Arterial and cerebral venous oxygen content, saturation, and hemoglobin concentration were determined with a Hemoximeter (Radiometer, Model OSM3). The blood gas analyzer and CO-oximeter were calibrated routinely throughout each experiment.

Experimental Protocol

After control values were obtained for all variables, cerebral ischemia was induced by tightening the ligatures that were previously placed around the brachiocephalic and left subclavian arteries. This tightening, combined with hemorrhagic hypotension, resulted in an MABP of 50 mm Hg. In all groups, right axillary artery pressure was abolished during ligation. We have demonstrated that this technique reduces CBF to less than 5 mL·min\(^{-1}\)·100 gm\(^{-1}\) in piglets. At the end of the ischemic (or control) period, before the pigs were killed, brain cortex, hippocampus, and caudate samples were obtained and frozen immediately in liquid nitrogen. Ten adolescent and 11 infant pigs served as sham-operated controls (no ischemia). Four adolescent and 6 infant pigs underwent 10 minutes of cerebral ischemia, and 4 adolescent and 5 infant pigs underwent 20 minutes of cerebral ischemia. Additionally, 3 adolescent and 3 infant pigs underwent 10 minutes of cerebral ischemia, and brain cortex samples were taken by biopsy at 3 and 5 minutes of ischemia followed by brain cortex, hippocampus, and caudate samples at 10 minutes.

Brain Crude Synaptosomal Membrane Isolation

Brain crude synaptosomal membranes were prepared from the brain cortex, hippocampus, and caudate tissue by a modification of the method of Magnoni et al and described in detail elsewhere.3 Samples were stored in 1-mL aliquots at −80°C. Protein concentrations were determined by the Coomassie binding method by using bovine serum albumin as the standard.13

Equilibrium Binding Assays

Binding assays were performed by incubating 50 to 100 μg crude synaptosomal membrane protein in 5-cc glass vials at 25°C with 0.0525, 0.1050, 0.1837, 0.2630, 0.5250, and 1.0500 mmol/L \(^{3} \text{HPN200-110}\) (82 Ci/mmol, New England Nuclear) in 50 mmol/L Tris HCl (pH 7.5; total volume, 1.0 mL) in the presence or absence of 1.0 μmol/L unlabeled nitrendipine (NTP) as displacer to determine total and nonspecific binding. Samples were incubated for 60 minutes in an oscillating water bath at a constant temperature of 25°C rather than at 37°C to slow membrane degradation. Samples were shielded from light to prevent the degradation of \(^{3} \text{HPN200-110}\) and NTP. The reaction was terminated by rapidly filtering the samples under vacuum onto Whatman GF/C glass fiber filters by using a Brandel cell harvester (Brandel Research and Development Labs, Inc). The filters had been pretreated for 30 minutes with 0.5% polyethyleneamine and 10 μmol/L NTP in 50 mmol/L Tris HCl buffer (pH 7.5). The filters were washed three times with ice-cold buffer and allowed to air dry for at least 3 hours. They were then placed in scintillation vials with 5 mL 3a70 complete scintillation cocktail (Research Products International Corp) and counted in a Beckman LS8200 scintillation counter. Counting efficiency was at least 55%.

Two studies were performed in quadruplicate for each membrane sample and each \(^{3} \text{HPN200-110}\) concentration. Nonspecific binding was subtracted from total binding to obtain specific binding.

Data Analysis

Equilibrium binding data were analyzed by using an ENZFIT software package (Rabin J. Letherbarrow, Elsevier Science Publishers), which yields an explicitly weighted, nonlinear least-squares fit of the data to the following equation:

\[
\frac{\text{[PN200-110] bound}}{\text{[PN200-110] free}} = \frac{B_{\text{max}}}{K_{d} + \text{[PN200-110] free}}
\]

where \(B_{\text{max}}\) is the total number of binding sites, \(K_{d}\) is the dissociation constant, and \([\text{PN200-110}]_{\text{free}}\) is the concentration of \(^{3} \text{HPN200-110}\) in the reaction mixture. Data were linearized by a Scatchard transformation of the above equation:

\[
\frac{\text{[PN200-110] bound}}{\text{[PN200-110] free}} = \frac{1}{K_{d}} \frac{B_{\text{max}}}{K_{d}} + \frac{B_{\text{max}}}{K_{d}}
\]

Statistical analysis of the binding studies was performed by using ANOVA and paired \(t\) tests for comparing individual experiments. Physiological variables were compared between groups by using one-way ANOVA. Values were considered significantly different at \(P < .05\). All data are reported as mean±SEM.
Table 1. Arterial Blood Gas Values, Brain Temperature, and MABP at Baseline and After Ischemia in Infant and Adolescent Pigs

<table>
<thead>
<tr>
<th></th>
<th>pHa</th>
<th>Paco₂, mm Hg</th>
<th>Pao₂, mm Hg</th>
<th>Brain Temp, °C</th>
<th>MABP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
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<tr>
<td>Adolescent (n=10)</td>
<td>7.45±0.01</td>
<td>38.7±0.4</td>
<td>178±23</td>
<td>37.9±0.2</td>
<td>87±3</td>
</tr>
<tr>
<td>Infant (n=11)</td>
<td>7.41±0.02</td>
<td>35.5±1.3</td>
<td>156±19</td>
<td>38.2±0.2</td>
<td>83±4</td>
</tr>
<tr>
<td><strong>10 Minutes' ischemia</strong></td>
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<tr>
<td>Adolescent (n=7)</td>
<td>7.42±0.02</td>
<td>40.1±1.0</td>
<td>166±14</td>
<td>37.3±0.3</td>
<td>94±8</td>
</tr>
<tr>
<td>Infant (n=9)</td>
<td>7.39±0.01</td>
<td>39.4±1.0</td>
<td>132±15</td>
<td>37.6±0.2</td>
<td>79±4</td>
</tr>
<tr>
<td><strong>20 Minutes' ischemia</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Adolescent (n=4)</td>
<td>7.44±0.01</td>
<td>41.5±1.9</td>
<td>206±8</td>
<td>38.6±0.3</td>
<td>86±3</td>
</tr>
<tr>
<td>Infant (n=6)</td>
<td>7.40±0.02</td>
<td>40.3±1.5</td>
<td>173±22</td>
<td>38.0±0.3</td>
<td>83±2</td>
</tr>
</tbody>
</table>

MABP indicates mean arterial blood pressure; pHa, pH of arterial blood; and Brain Temp, parenchymal brain temperature. Data are mean±SEM; there were no significant differences.

Results

Table 1 shows MABP, brain temperature, and arterial blood gas values immediately prior to and during cerebral ischemia. There were no significant differences between any of the experimental groups.

³HPN200-110 binds in a reversible and saturable manner to the brain crude synaptosomal membrane preparation. Scatchard analysis of ³HPN200-110 revealed a linear plot supporting the assumption of a single binding site for the ligand (Fig 1). Control B_mₐₓ and K_d in the adolescent cortex were 369.0±6.6 fmol/mg protein and 0.13±0.01 nmol/L, respectively, and in the infant cortex were 399.1±5.8 fmol/mg protein and 0.19±0.01 nmol/L, respectively. Table 2 shows the ³HPN200-110 B_mₐₓ and K_d obtained in the cortex, hippocampus, and caudate nucleus for each experimental group. In adolescent pig cortex B_mₐₓ was significantly elevated above control values after 3 minutes of global cerebral ischemia and reached a peak of 580.2±21.9 fmol/mg protein at 5 minutes of ischemia (157% of control, P<.05 versus control). Maximal binding fell to 481.6±16.4 fmol/mg protein after 10 minutes of ischemia and was not significantly different from control values by 20 minutes. In infant pig cortex B_mₐₓ likewise was significantly elevated above control values after 3 minutes and reached a peak at 5 minutes of ischemia of 710.6±110.8 fmol/mg protein (178% of control, P<.05 versus control). Maximal binding remained elevated at 10 minutes of ischemia (687.9±24.6 fmol/mg protein, 172% of control). This was significantly higher than adolescent cortex binding at 10 minutes of ischemia when values were normalized for control (preischemic) values (Fig 2). B_mₐₓ then declined to near-control values by 20 minutes although still remaining significantly higher than control in infants but not adolescents (Table 2). The time course of these changes in B_mₐₓ as percent of preischemic baseline values is shown in Fig 2. K_d was significantly higher at 3 minutes of ischemia in infant cortex (0.33±0.05 nmol/L, P<.05 versus control). Otherwise, K_d did not significantly change in adolescent or infant cortex at any other time point.

![Graph showing ³HPN200-110 specific binding as a function of ³HPN200-110 concentration for control (○) and 5 minutes' ischemia (●) in adolescent cerebral cortex. Inset: Scatchard transformation of the data showing bound ligand plotted against the ratio of bound and free ligand. Each point represents the mean of all sample values.](http://stroke.ahajournals.org/)

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TABLE 2. \(^{3}H\)PN200-110 Maximal Binding and Binding Affinity at Baseline and During Ischemia in Infant and Adolescent Pigs

<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>Hippocampus</th>
<th>Caudate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B(_{max}), fmol/mg</td>
<td>K(_d), nmol/L</td>
<td>B(_{max}), fmol/mg</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescent</td>
<td>369.0±6.6(\dagger)</td>
<td>0.13±0.01(\dagger)</td>
<td>255.2±5.7(\dagger)</td>
</tr>
<tr>
<td>Infant</td>
<td>399.1±5.8(\dagger)</td>
<td>0.19±0.01</td>
<td>309.5±8.0(\dagger)</td>
</tr>
<tr>
<td>3 Minutes' ischemia</td>
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<tr>
<td>Adolescent</td>
<td>472.8±37.2(\dagger)</td>
<td>0.08±0.01(\dagger)</td>
<td></td>
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<tr>
<td>Infant</td>
<td>593.3±50.7(\dagger)</td>
<td>0.33±0.05(*)(\dagger)</td>
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<tr>
<td>5 Minutes' ischemia</td>
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<tr>
<td>Adolescent</td>
<td>580.2±21.9(\dagger)</td>
<td>0.14±0.03(\dagger)</td>
<td></td>
</tr>
<tr>
<td>Infant</td>
<td>710.6±110.8(\dagger)</td>
<td>0.33±0.09</td>
<td></td>
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<tr>
<td>10 Minutes' ischemia</td>
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<tr>
<td>Adolescent</td>
<td>481.6±16.4(\dagger)</td>
<td>0.11±0.01(\dagger)</td>
<td>282.7±17.5(\dagger)</td>
</tr>
<tr>
<td>Infant</td>
<td>687.9±24.6(*)</td>
<td>0.16±0.01</td>
<td>382.3±7.1(*)(\dagger)</td>
</tr>
<tr>
<td>20 Minutes' ischemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescent</td>
<td>394.1±14.9(\dagger)</td>
<td>0.17±0.01(\dagger)</td>
<td>212.3±8.3(\dagger)</td>
</tr>
<tr>
<td>Infant</td>
<td>456.7±14.6(*)</td>
<td>0.14±0.01</td>
<td>356.2±11.3(*)(\dagger)</td>
</tr>
</tbody>
</table>

B\(_{max}\) indicates maximal specific binding; K\(_d\), binding affinity. Data are mean±SEM.

\(*)P<.05 vs control; \(\dagger\)P<.05 adolescent vs infant; \(\ddagger\)P<.05 vs 5 minutes' ischemia.

In the adolescent hippocampus and caudate, binding was unchanged after 10 minutes of ischemia and was significantly lower than control values after 20 minutes of ischemia. K\(_d\) did not change significantly in hippocampus but fell to 0.08±0.01 nmol/L at 10 minutes of ischemia in the caudate. In the infant hippocampus and caudate, binding increased from control values of 309.5±8.0 and 317.7±13.2 fmol/mg protein, respectively, to 382.3±7.1 and 474.2±10.7 fmol/mg protein, respectively, at 10 minutes of ischemia. This remained statistically elevated at 20 minutes of ischemia (Table 2). K\(_d\) did not change in infant hippocampus, but was significantly lower in caudate after 10 minutes of ischemia (0.12±0.01 nmol/L versus control of 0.24±0.03 nmol/L, P<.05).

Discussion

This study shows that DHP ligand binding to L-type VDCCs in cerebral cortex increases early in ischemia (peaking at 5 minutes of ischemia) and then decreases to near-baseline values in both infant and adolescent pigs. The decrease in binding occurred by 10 minutes of ischemia in adolescent cortex but was not evident until 20 minutes of ischemia in infant cortex. Caudate and hippocampus binding was also higher than baseline in infants, but in adolescents was less than baseline by 20 minutes of ischemia.

Infant animals tolerate ischemia better than adults. In the adolescent hippocampus and caudate, binding was unchanged after 10 minutes of ischemia and was significantly lower than control values after 20 minutes of ischemia. K\(_d\) did not change significantly in hippocampus but fell to 0.08±0.01 nmol/L at 10 minutes of ischemia in the caudate. In the infant hippocampus and caudate, binding increased from control values of 309.5±8.0 and 317.7±13.2 fmol/mg protein, respectively, to 382.3±7.1 and 474.2±10.7 fmol/mg protein, respectively, at 10 minutes of ischemia. This remained statistically elevated at 20 minutes of ischemia (Table 2). K\(_d\) did not change in infant hippocampus, but was significantly lower in caudate after 10 minutes of ischemia (0.12±0.01 nmol/L versus control of 0.24±0.03 nmol/L, P<.05).

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Infant animals tolerate ischemia better than adults.\(^5\)\(^-\)\(^9\) We have demonstrated age-related differences in recovery of CBF, CMRO\(_2\), and electrical function during reperfusion after short-term (10 minutes)\(^10\) and prolonged complete (30 minutes)\(^11\) ischemia. Although there is no direct evidence, many factors have been implicated in the evolution of ischemic brain injury in...
immature brain that may explain the age-dependent effects of ischemia. Studies have speculated that lower CMRO$_2$ in younger animals, production of fewer oxygen-derived free radicals during reperfusion, age-dependent effects of excitatory neurotransmitter mediated injury, and differences in prostanoid and calcium fluxes may play a role. This study was designed to investigate what role, if any, regulation of VDCCs play in this age-dependent difference.

DHP VDCC ligand binding increases after ischemia in cerebral cortex with different time courses in adolescent and infant pigs. These results extend those studies in rat by Magnoni et al as well as our previous observations of increased DHP binding following short-term (10 minutes) global cerebral ischemia produced by ventricular fibrillation and apnea in dog. In dog PN200-110 $B_{max}$ increases to over 250% of control. Binding was assessed during varying periods of reperfusion and declined to baseline over 24 hours. We hypothesized that this increased DHP binding represents increased available L-type VDCCs in the cell membrane that contribute to increased Ca$^{2+}$ influx into cells during ischemia/early reperfusion, triggering neurochemical processes thought responsible for delayed, programmed neuronal death. Our current investigation did not study ligand binding during reperfusion. Rather, increasing the duration of ischemia decreased ligand binding. Peak binding in cortex appeared at 5 minutes of ischemia in adolescent pigs and between 5 and 10 minutes in piglets before decreasing. Hippocampus and caudate were not analyzed at shorter periods of ischemia because of the difficulty associated with obtaining multiple biopsy samples in the same animal for these deep structures. However, we hypothesize that the same peak and rapid decline in binding would have been observed as well. By 10 minutes of ischemia, binding in the hippocampus and caudate in adolescents may have already declined to or below control values. Binding appears to have remained elevated longer in infants. It is unlikely that these differences between adolescent and infant DHP binding represent age-related changes in the VDCCs since Golik et al have shown that aging does not change L-type VDCC binding parameters in mouse brain.

Differences between adolescent and infant pigs may be related to a difference between groups in the degree or extent of ischemia during temporary ligation of the brachiocephalic and left subclavian arteries. We believe that this is unlikely because both groups demonstrated similar ablation in right axillary blood pressure during vascular ligation, and there was no arterial bleeding while obtaining the brain biopsy samples in either group. In addition, this technique of producing cerebral ischemia appears to be valid across both species and age. For example, similar to infant pigs, in adult cat we have demonstrated a reduction of CBF to less than 5 mL·min$^{-1}$·100 g$^{-1}$ during combined temporary ligation of the brachiocephalic and left subclavian arteries in the presence of hypotension.

Several possible mechanisms for increased DHP ligand binding may be considered, although the precise mechanism is not known. Increased de novo channel synthesis may have produced increased ligand binding, but in view of the short time periods involved (3 to 5 minutes), the prevailing cellular energy failure, and the lack of evidence for protein synthesis during ischemia, this mechanism is unlikely. Increased ligand binding may also be due to increased channel numbers resulting from fusion of subcellular membrane stores of channels with the plasma membrane. Also, the uncovering of additional binding sites in existing receptors resulting from changes in membrane fluidity could result in increased DHP binding.

L-type VDCC channels are composed of four subunits: alpha 1, alpha 2, delta, beta, and gamma. Cloning studies have shown that the alpha 1 subunit alone acts as a functional, organic calcium channel blocker-sensitive calcium channel; the other subunits modify the DHP binding and gating properties. Coexpression experiments in LCa.$\alpha_{11}$ cells show a 100% increase in the $B_{max}$ of PN200-110 (no change in $K_d$) for the alpha 1-beta combination compared with alpha 1 alone. Other various subunit combinations show both significant increases and decreases in $B_{max}$ compared with alpha 1 alone. This may present an additional mechanism for the ischemia-induced increase in $B_{max}$. Ischemia-triggered calcium influx may activate endogenous lipases and proteases, which then act to degrade the plasma membrane and integral membrane proteins. This may result in dissociation of L-type channel subunits or anomalous subunit recombination with an accompanying alteration in the number of DHP binding sites. As membrane and protein degradation continues, the DHP binding would be expected to decrease as binding sites are catabolized.

There is no current evidence that increased DHP binding is related to increased VDCC activity. Several studies provide indirect evidence that increases in DHP binding represent functional channels in certain models. Ohnishi et al have demonstrated increases in DHP binding associated with enhanced channel function. We have also shown that changes in PN200-110 binding in ischemic dog striatum correlate with the magnitude of Gly-$N$-methyl-$d$-aspartate–stimulated dopamine release, a process dependent on L-type VDCC activity that can be blocked by NTP, an L-type VDCC antagonist.

Hakim and Hogan have shown a correlation between severity of ischemia and nimodipine binding by in vivo autoradiography in a middle cerebral artery and ipsilateral common carotid artery model of focal cerebral ischemia in rat. Activation of L-type VDCC binding followed by loss of binding was an indication of impending infarction. In this in vivo study, DHP binding was dependent on the state of the channel, i.e., high-affinity binding to the open or inactivated state. This in turn is related to membrane polarization. Hence, activation of L-type VDCC binding in their study is more a function of membrane depolarization (resulting in activation of L-type VDCCs, resulting in high-affinity ligand binding) than absolute increases in DHP binding sites. The VDCCs in isolated membrane vesicles as used in the current study are in the inactivated state, with high-affinity binding characteristics for DHPs, and reflect total binding sites. The decrease in DHP binding in the study of Hakim and Hogan is thought to be secondary to loss of channels from cell necrosis, which would also have been reflected in our binding studies. Hence, increased VDCC activation coupled with increased VDCC numbers would both act to increase available DHP binding sites and, presumably, Ca$^{2+}$ during ischemia.
emia and reperfusion. One may hypothesize that, since the increased infant DHP VDCC binding was more prolonged than adolescent, Ca\(^{2+}\) would be greater in the infant and result in greater cell damage. However, loss of cellular integrity, necrosis, and subsequent loss of DHP binding to the VDCCs would occur sooner in regions more vulnerable to ischemia and Ca\(^{2+}\) entry. If one assumes that the infant pig cerebral cortex is more resistant to ischemia than the adolescent pig, then this study supports our original hypothesis. Other mechanisms, including internal calcium handling and sensitivity to calcium-initiated processes, may be more important to differential cell vulnerability than the actual magnitude of calcium overload or calcium entry via L-type VDCCs. No direct evidence yet exists, however, supporting changes in VDCC activity contributing to neuronal injury as opposed to merely being a marker for impending cell death. Further research is needed in this area.

In conclusion, this study demonstrates increased DHP VDCC binding after short-term complete global ischemia in infant and adolescent pig cerebral cortex. Binding peaks sooner and thereafter decreases sooner in adolescent than in infant animals. Similar patterns of VDCC activity may have been present in the hippocampus and caudate if smaller time increments were measured. Although the early increase in binding may represent increased functional channels that contribute to Ca\(^{2+}\) early in ischemia, the earlier secondary fall in binding in the adolescent suggests that the differences may relate to earlier neuronal cell death in the older animals and thus are the result rather than the cause of the greater resistance in the infant animal. We hypothesize that differences in VDCC activity over time may provide a marker for the age dependence of ischemic tolerance between adolescents and infants and thereby identify vulnerable brain regions and define therapeutic windows of opportunity in models of cerebral ischemia.

Acknowledgments

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References

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