Research report

Acute protective effect of nimodipine and dimethyl sulfoxide against hypoxic and ischemic damage in brain slices

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Abstract

Nimodipine and dimethyl sulfoxide (DMSO) were tested (alone and in combination) regarding their ability to increase hypoxic tolerance of brain slices under ‘hypoxic’ (deprivation of oxygen) or ‘ischemic’ (hypoxia+withdrawal of glucose) conditions. Direct current (DC) and evoked potentials were recorded in the CA1 region of hippocampal slices of adult guinea pigs. After induction of hypoxia or ischemia, the latency of anoxic terminal negativity (ATN) of the DC potential was determined during superfusion with artificial cerebrospinal fluid alone (aCSF), and during superfusion with aCSF containing DMSO [0.1\% (14.1 mmol/l) and 0.4\% (56.3 mmol/l)] with the addition of nimodipine (40 μmol/l). Latencies of ATN with first hypoxia were 6.7±3.7 min in the control group, 9.3±4.2 min in the 0.4\% DMSO group and 12.3±5.5 min \((P=0.007)\) in the nimodipine/0.4\% DMSO group. Latencies of ATN with first ischemia were 2.9±2 min in the control group, 4.1±1.6 min in the 0.1\% DMSO group, 7.1±3.9 min in the 0.4\% DMSO group \((P=0.006)\), 5.3±1.5 min in the nimodipine/0.1\% DMSO group and 7.6±3 min \((P<0.001)\) in the nimodipine/0.4\% DMSO group. DMSO (0.4\%), either alone or in combination with nimodipine, increase the latency of the ATN after acute onset of hypoxia and ischemia. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Disorders of the nervous system

Topic: Ischemia

Keywords: Ischemia; Brain slice; Neuroprotection; Nimodipine; Dimethyl sulfoxide

1. Introduction

Subarachnoid hemorrhage often causes cerebral artery spasms from day 3 after the event onwards. These spasms are due to an influx of Ca\textsuperscript{2+} into the smooth muscle cells causing vessel contraction and, finally, a critically reduced cerebral perfusion [12,14,27,33,35,50]. Dihydropyridine calcium antagonists, such as nimodipine, inhibit transmembrane calcium influx into vascular smooth muscle cells and thus influence cerebral artery spasm [13,54]. Furthermore, calcium antagonists can directly interfere with neuronal excitability, which is influenced by transmembrane calcium movements, particularly through L-type calcium channels [15,45]. Several investigations showed protective effects of nimodipine both in vitro and in vivo, as well as in clinical trials, although, in the latter, controversial findings were also published [48,49]. Often these studies were focused on cerebral artery spasm or effects of nimodipine on the regional cerebral blood flow [8,16,17,20,25,26,34,37]. In most of these investigations, an interfering and possibly neuroprotective effect of the solvents used as vehicle was not taken into account.

The aim of these experiments was to analyze the acute effect of nimodipine on the bioelectric activity of isolated hippocampal brain slices during hypoxia and ischemia. Nimodipine and dimethyl sulfoxide (DMSO), the latter being widely applied as a solvent for a variety of drugs, were tested in combination and alone [23].

Hypoxic conditions were induced by deprivation of
oxygen, whereas ischemic conditions were imitated by combined deprivation of oxygen and glucose.

Bioelectrical changes in brain tissue during hypoxia/ischemia are well described. Under critical hypoxic/ischemic conditions, a sudden negative shift of the direct current (DC) potential, so-called anoxic terminal negativity (ATN), is brought about by the anoxic depolarization of neurons. This sudden depolarization, which is accompanied both in vivo and in vitro by an excessive transmembranous influx of calcium and sodium ions, a release of glutamate, an increase in extracellular potassium concentrations (up to 60 mmol/l), and a reduction of the extracellular volume fraction, is the endpoint of energy depletion of neuronal tissue during persistent hypoxia/ischemia. These changes are at least partially reversible if hypoxia/ischemia is terminated upon reaching the peak of ATN [1,10,23,24,28,31,50].

2. Material and methods

Adult guinea pigs (n=54; 340–440 g) were anesthetized with the short acting barbiturate methohexital (250 mg/kg i.p.). The brain was rapidly removed and chilled in artificial cerebrospinal fluid (aCSF) at 4°C. The aCSF contained (in mmol/l): NaCl 124, KCl 4, NaH₂PO₄ 1.24, MgSO₄ 1.3, NaHCO₃ 26, glucose 10 and CaCl₂ 2). The hippocampus was dissected, transverse slices were cut (500 μm) using a McIlwain chopper (Mickle Lab Eng. Co., Gomshall, UK). The slices (n=92) were immediately placed in a submerged-type preincubation chamber containing aCSF. The solution was equilibrated with 95% O₂ and 5% CO₂ (pH 7.35–7.45). The temperature was held at 28°C.

After a preincubation period of 1–2 h, slices were transferred into the recording chamber [21] at the interface of aCSF and humidified gas (95% O₂ and 5% CO₂; 34°C, measured adjacent to the slice). A constant flow rate of 2 ml/min was maintained (chamber volume=1.4 ml). One slice was used for each experiment. Schaffer collaterals were stimulated with bipolar electrodes (2 ms/1 s) that were placed in the stratum radiatum between areas CA2 and CA3. Direct current and evoked potentials (EPs) were recorded using NaCl-filled glass micropipettes (0.5–1 MΩ) from CA1 (str. pyramidalis). In all experiments, the amplitudes of EP were stable for 30 min prior to initiation of hypoxia or ischemia. Slices with an EP amplitude <4 mV were excluded.

Hypoxic conditions (n=44) were established by replacing N₂ for O₂ in the fluid and gaseous phase of the chamber, whereas ischemic conditions (n=48) were established by additionally lowering the glucose concentration of the perfusate from 10 to 2 mmol/l. Hypoxic and ischemic episodes were induced up to three times and were terminated when the so-called ATN of the DC potential had reached its peak. Recovery periods after each hypoxic/ischemic episode were 30 min. Latency of ATN was determined from the beginning of the N₂ application to the point at which 10% of the ATN peak amplitude had been reached [43]. The amplitude of the DC potential was measured from baseline to peak. The amplitudes of the EP were measured from the interpolated baseline to peak. Thirty min after hypoxia/ischemia, the EP amplitude was compared with the initial amplitude at the beginning of the experiment. Statistical analysis was performed using the Student’s t-test and the Mann-Whitney-Rank-Sum test, with P<0.05 considered to be significant. All data are given as means and standard deviation (S.D.).

Parallel experiments were done in two recording chambers of similar construction to compare two slices from the same animal under both hypoxic and ischemic conditions. In some experiments, the pO₂ of the slice surface during the whole experiment was monitored online with an optical monitoring system (phosphorescence quenching method) [29].

Besides control experiments without the addition of drugs, different solutions containing nimodipine and DMSO were added to the aCSF. Solutions given under hypoxic conditions: 40 μmol/l nimodipine with 0.4% (56.3 mmol/l) DMSO, and 0.4% DMSO alone. Solutions given under ischemic conditions: 40 μmol/l nimodipine with 0.1% (14.1 mmol/l) DMSO, 40 μmol/l nimodipine with 0.4% DMSO, 0.1% DMSO and 0.4% DMSO alone. Experiments with nimodipine were performed in a darkened room. The concentration of nimodipine (40 μmol/l) was chosen in view of the well known protein-binding properties of the substance [30] and results of experiments demonstrating massive diffusion barriers and substance accumulation in the uppermost cut surface of the slices, which even preclude fast diffusion of small ions [2,46].

3. Results

3.1. Latencies and amplitudes of ATN under hypoxic conditions

With hypoxic conditions (deprivation of oxygen), ATN occurred in all slices (n=44). Latencies of ATN with the first episode of hypoxia in the nimodipine/0.4% DMSO group were significantly longer than in the control group (P=0.007). Latencies of ATN generally lasted longer in the nimodipine/0.4% DMSO group also with the second and third episodes of hypoxia, although the differences were not statistically significant.

With the first episode of hypoxia, amplitudes of ATN in the 0.4% DMSO group (P=0.034) and in the nimodipine/0.4% DMSO group (P=0.034) were significantly smaller than in the control group. No statistically significant differences of ATN amplitudes could be observed with the second and third episodes of hypoxia (Table 1, Figs. 1 and 2).
Table 1
Latencies and amplitudes of anoxic terminal negativity and amplitudes of evoked potentials after inducing three hypoxic or three ischemic episodes in hippocampal slices from guinea pig

<table>
<thead>
<tr>
<th>Latencies of ATN (min)</th>
<th>Control</th>
<th>0. 1% DMSO</th>
<th>0.4% DMSO</th>
<th>Nimo+0.1% DMSO</th>
<th>Nimo+0.4% DMSO</th>
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<tbody>
<tr>
<td>Hypoxia</td>
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<tr>
<td>1st</td>
<td>6.7±3.7 (n=21)</td>
<td>9.3±4.2 (n=11)</td>
<td>12.3±5.5 (n=12)</td>
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</tr>
<tr>
<td>2nd</td>
<td>3.6±2.1 (n=21)</td>
<td>3.8±1.1 (n=11)</td>
<td>4.8±2.0 (n=12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd</td>
<td>3.0±1.9 (n=21)</td>
<td>3.4±1.1 (n=11)</td>
<td>4.5±2.2 (n=12)</td>
<td></td>
<td></td>
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<tr>
<td>Ischemia</td>
<td></td>
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<tr>
<td>1st</td>
<td>2.9±2.0 (n=17)</td>
<td>4.1±1.6 (n=6)</td>
<td>7.1±2.9 (n=9)</td>
<td>5.3±2.0 (n=8)</td>
<td>7.6±3.0 (n=9)</td>
</tr>
<tr>
<td>2nd</td>
<td>1.3±1.0 (n=15)</td>
<td>2.1±0.7 (n=5)</td>
<td>3.2±1.6 (n=9)</td>
<td>2.9±1.0 (n=7)</td>
<td>4.2±1.8 (n=8)</td>
</tr>
<tr>
<td>3rd</td>
<td>1.1±1.0 (n=12)</td>
<td>1.8±0.6 (n=5)</td>
<td>2.5±1.6 (n=9)</td>
<td>2.5±1.1 (n=7)</td>
<td>2.9±0.9 (n=7)</td>
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</table>

<table>
<thead>
<tr>
<th>Amplitudes of ATN (mV)</th>
<th>Hypoxia</th>
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</thead>
<tbody>
<tr>
<td>1st</td>
<td>13.5±7.8 (n=21)</td>
<td>7.9±4.4 (n=11)</td>
<td>9.1±4.3 (n=12)</td>
<td></td>
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<tr>
<td>2nd</td>
<td>14.1±5.3 (n=21)</td>
<td>12.5±3.7 (n=11)</td>
<td>14.7±5.3 (n=12)</td>
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<tr>
<td>3rd</td>
<td>14.4±6.2 (n=21)</td>
<td>13.4±4.5 (n=11)</td>
<td>16.2±6.6 (n=12)</td>
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<td></td>
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<tr>
<td>Ischemia</td>
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<tr>
<td>1st</td>
<td>16.8±5.7 (n=17)</td>
<td>13.3±2.5 (n=6)</td>
<td>13.7±6.7 (n=9)</td>
<td>13.7±5.0 (n=8)</td>
<td>8.9±3.7 (n=9)</td>
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<td>2nd</td>
<td>17.3±7.1 (n=15)</td>
<td>15.1±2.5 (n=5)</td>
<td>17.0±8.1 (n=9)</td>
<td>15.8±6.8 (n=7)</td>
<td>7.9±4.9 (n=8)</td>
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<td>3rd</td>
<td>16.6±7.0 (n=12)</td>
<td>16.2±2.0 (n=5)</td>
<td>19.6±8.9 (n=9)</td>
<td>16.6±6.9 (n=7)</td>
<td>9.6±6.0 (n=7)</td>
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<thead>
<tr>
<th>Recovery of EP 30 min after hypoxia/ischemia (%)</th>
<th>Hypoxia</th>
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<tr>
<td>After 1st</td>
<td>94.7±52.3 (n=21)</td>
<td>108.0±18.1 (n=11)</td>
<td>99.2±49.1 (n=12)</td>
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<tr>
<td>After 2nd</td>
<td>88.6±60.0 (n=21)</td>
<td>85.9±41.7 (n=11)</td>
<td>85.2±56.7 (n=12)</td>
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<tr>
<td>Ischemia</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>After 1st</td>
<td>84.2±32.6 (n=15)</td>
<td>99.2±20.8 (n=6)</td>
<td>118.4±65.5 (n=9)</td>
<td>87.4±39.2 (n=8)</td>
<td>76.4±53.3 (n=9)</td>
</tr>
<tr>
<td>After 2nd</td>
<td>73.4±43.4 (n=12)</td>
<td>111.6±27.3 (n=5)</td>
<td>102.7±56.6 (n=9)</td>
<td>86.7±56.2 (n=7)</td>
<td>57.3±44.3 (n=8)</td>
</tr>
</tbody>
</table>

*Hypoxia was defined as oxygen deprivation only, ischemia was defined as oxygen deprivation and glucose reduction. DMSO, dimethyl sulfoxide; Nimo, nimodipine. All data are given as the mean±S.D.

3.2. Recovery of evoked potentials under hypoxic conditions

With the beginning of hypoxia, amplitudes of the EP decreased and disappeared before the occurrence of ATN. Recovery of evoked potentials was determined 30 min after termination of the first and second episodes of hypoxia. The initial amplitude before the first episode of hypoxia was taken as 100%. No statistically significant differences were seen among the groups (Table 1).

3.3. Latencies and amplitudes of ATN under ischemic conditions

With ischemic conditions (deprivation of oxygen and decrease in glucose), ATN occurred in all slices (n=48). Latencies of ATN with 1st ischemia lasted significantly longer in the DMSO 0.4% group (P=0.006) and in the nimodipine/DMSO 0.4% group (P<0.001) than in the control group. Prolongations of ATN latency after application of DMSO 0.4% and nimodipine/DMSO 0.4% were also evident with the following ischamias.

In the nimodipine/DMSO 0.4% group amplitude of ATN was significantly smaller (P=0.001) with the 1st ischemia than in the control group. Although there is no statistically significant difference of ATN amplitudes with the 2nd and 3rd hypoxia, amplitudes of the nimodipine/DMSO 0.4% group were smaller than in the other groups (Table 1).

3.4. Recovery of evoked potentials under ischemic conditions

No statistically significant differences could be observed concerning the recovery of the evoked potentials.

4. Discussion

Hippocampal slice preparations of either rats or guinea pigs are frequently used to evaluate cerebroprotective effects of drugs on neurons and glia. This technique has the advantage that experimental parameters such as pH, flow and temperature of the bath solution can be effectively manipulated and monitored, and that the neuro-glial compartment can be observed in isolation, without being dependent on regional cortical blood flow [23,24,31,32,43,52]. Under these circumstances, ex-
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**Fig. 1.** Time course of anoxic terminal negativity (ATN) of the DC-potential during repetitive hypoxia (deprivation of oxygen) and ‘ischemia’ (deprivation of oxygen and reduction of glucose) in hippocampal slices of guinea pigs. Fig1a: * indicates $P < 0.007$; Fig. 1b: * indicates $P < 0.006$, ** indicates $P < 0.001$. Mean±S.D., Student’s $t$-test, Mann-Whitney-Rank-Sum test.

Experimental conditions such as $pO_2$ during hypoxic episodes, which declines to $2±0.1$ kPa [29], are uniform throughout the experiments. At the same time, changes of the ionic microenvironment ($K^+$, $Ca^{2+}$, $Na^+$) occur in a manner that is nearly identical to that found in hypoxic conditions in vivo. Slice experiments in this respect thus closely imitate the situation in the whole animal [22,43], except for the temperature (34°C) which, as common experience has shown, in the slice experiments, cannot be substantially raised without compromising the viability of the preparations. Indeed, in experiments in vitro, lower temperatures (<32°C) have been demonstrated to be protective [9,19,42,47,51].

The parameter for a protective effect was defined as prolongation of the latency of ATN. The precipitating event of ATN includes an extensive accumulation of intracellular calcium, causing an extracellular increase in excitatory amino acids, a depletion of high energy phosphates, failure of membrane ion pumps with subsequent membrane depolarization, a decrease in intracellular pH and an increase in fatty acids, with the production of oxygen free radicals [6,22]. Comparing the results with hypoxia (oxygen deprivation) and ischemia (concomitant oxygen deprivation and reduction of glucose), the biochemical effects were more pronounced with ischemia, resulting in a smaller variability in the tissue reactions. However, both with the first episode of hypoxia and ischemia, a significant prolongation of the latency of ATN was seen with 40 μmol/l nimodipine+0.4% DMSO. With the first episode of ischemia, a smaller but also significant effect on the latency of ATN was seen with 0.4% DMSO alone, whereas the prolongation with the first episode of
hypoxia was not statistically significant. The increasing latency after application of nimodipine and/or DMSO became less clear with the second and third episodes of hypoxia/ischemia, which could be explained by a critical depletion of cellular ATP stored, due to the preceding hypoxia/ischemia. Nimodipine is only soluble with solvents that are expected to be protective [36], so no control experiments with nimodipine alone could be conducted.

The prolonged latency of ATN with the application of DMSO during hypoxia/ischemia may be explained by a direct stabilization of the cell membrane by DMSO interfering either with the lipid bilayer or with voltage-gated ion channels. Thus, DMSO (0.3–10%) has been reported to block cellular ion channels directly, for example, sodium and chloride channels in myocytes. Furthermore, DMSO might suppress neuronal synaptic activity and consequently reduce energy consumption [38–41,44]. Investigations of GABA-mediated and glutamate-induced currents in dorsal root ganglia revealed a depression of the currents by DMSO at concentrations higher than 0.1% [39]. Other authors observed increased ATP synthesis after DMSO application in rabbit skeletal muscle, although this effect was reached with higher concentrations of DMSO (i.e., 20%) [11]. Taken together, the literature data show that an increase in ATP level, combined with reduced synaptic activity and, therefore, reduced ATP consumption, would preserve the Na⁺/K⁺-pump function during hypoxic and ischemic conditions. Consequently, the membrane potential can be maintained longer and ATN should be expected to be delayed. Because energy depletion is higher with ischemia, the effect of DMSO is more distinct with ischemia than with hypoxia. The prolonging effect of DMSO on the latency of ATN was enhanced by an additional application of 40 μmol/l nimodipine. Experimental and clinical data indicate that nimodipine does dilate small resistance vessels in particular, suggesting an improvement in pial collateral circulation in hypoperfused areas [3–5]. This is due to the inhibition of transmembrane calcium influx into vascular smooth muscle cells and, thus, a reduction in glutamate release [15,18]. Due to a dilatation of small cerebral arteries, an increase in regional blood flow and a better reperfusion of the penumbra has been observed [7]. As there is no vessel system in our experimental setup, these cerebroprotective effects can be ruled out as a reason for the latency prolongation of ATN. As a consequence, an anti-ischemic effect of nimodipine, directly acting on neuronal tissue, seems to be likely [53]. The presence of specific and stereoselective binding sites in neuronal tissue, which regulate the intracellular entry of calcium, suggests that nimodipine may modify neuronal
function by a direct action on nerve cells [15,53]. By limiting, and thus decelerating, excessive neuronal calcium entry, the application of nimodipine increases the latency of ATN. In our experiments, this effect became obvious with a concentration of 40 μmol/l nimodipine. A small unpublished series on four hippocampal slices of guinea pig was performed with 80 μmol/l nimodipine dissolved in 0.4% DMSO under hypoxic conditions. This series of experiments showed no differences compared to the present data with 40 μmol/l nimodipine, which thus appears to be the saturating dose.

In conclusion, a vessel-independent neuroprotective effect of nimodipine and DMSO, and of DMSO alone, could be observed. The effect of DMSO on the latency and amplitude of ATN can be augmented by the administration of 40 μmol/l nimodipine.

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References


