Effects of Dextromethorphan on Rat Brain During Ischemia and Reperfusion Assessed by Magnetic Resonance Spectroscopy

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Using proton and phosphorus magnetic resonance spectroscopy, we evaluated the metabolic effects of preischemic administration of the N-methyl-D-aspartate antagonist dextromethorphan (50 mg/kg i.p.) during global forebrain ischemia and subsequent reperfusion in rats. Dextromethorphan-treated animals (n=10) showed less lactate formation during ischemia than untreated animals (n = 11, p<0.001). During reperfusion, the lactate level in the treated group was reduced (p<0.05). Tissue pH declined less in the treated group during ischemia (p <0.01). There was no difference in the phosphocreatine/inorganic phosphate peak height ratio between groups. During ischemia, the N-acetylaspartate resonance peaks decreased in both groups. Histologic damage assessed in the hippocampal CA1 region 7 days after the ischemic insult was more severe in the untreated group (p <0.05). There was a significant correlation between end-ischemic tissue pH and hippocampal damage (r=-0.73, p<0.05). In the dextromethorphan-treated animals, 90% of the rats survived compared with 47% of the untreated animals (p <0.05). These results support findings in previous studies that dextromethorphan attenuates ischemic damage. (Stroke 1991;22:343-350)

There is considerable evidence that excitatory amino acids, in particular glutamate, play an important role in the pathogenesis of ischemic neuronal injury.1-4 During ischemia, neuronal depolarization following energy depletion induces the enhanced synaptic release of glutamate. The cellular re-uptake of glutamate, being adenosine triphosphate (ATP)-dependent, is diminished, leading to a further increase in the extracellular glutamate concentration, which results in the stimulation of glutamate receptors such as the kainate- and quisqualate-preferring subtypes. This in turn leads to a massive sodium (Na+) influx balanced by the uptake of chloride (Cl-) with osmotically obligated water.1-4 Membrane depolarization relieves the magnesium (Mg2+) blockade of the ion channel linked to the N-methyl-D-aspartate (NMDA)-preferring glutamate receptor. The unique property of this receptor-ionophore complex is that it is permeable not only to monovalent cations, but also to Ca2+. Activation of the NMDA receptor by glutamate then leads to a substantial Ca2+ influx.1-3 The intracellular Ca2+ accumulation is further enhanced by the membrane depolarization-induced activation of voltage-dependent calcium channels.1,2 The increase in the intracellular concentration of Na+ enhances the intracellular accumulation of Ca2+ by reversing the Na+/Ca2+ exchanger. These events cause toxic cell swelling that finally leads to membrane disruption and a subsequent nonspecific Ca2+ leakage into the cell.1 The intracellular Ca2+ accumulation is considered to be an important factor in the progressive metabolic deterioration of the cell through the activation of Ca2+-dependent enzymes that catalyze the breakdown of proteins, lipids, and nucleic acids, ultimately leading to death of the nerve cell.6,8-10

Recent reports show that dextromethorphan, a dextrorotatory morphinan and a common antitussive agent, noncompetitively blocks the NMDA receptor by acting at a site in the receptor-linked ion channel.6,12 Blockade of the NMDA receptor under ischemic conditions only partially diminishes the Na+ influx but greatly reduces the Ca2+ influx.1,4 Dext-
tromethorphan can also block voltage-gated calcium channels. By blocking the receptor-mediated and voltage-gated Ca\(^{2+}\) influx, dextromethorphan might limit the metabolic derangements that follow cerebral ischemia. Because of these properties, dextromethorphan is believed to have a cerebroprotective effect under hypoxic or ischemic conditions.

This study was undertaken to investigate the metabolic effects during ischemia and reperfusion of the preischemic administration of dextromethorphan. We used a rat model of global forebrain ischemia lasting for 30 minutes followed by reperfusion. Cerebral metabolic changes, in particular the formation of lactate (Lac), tissue pH, and high-energy phosphate levels, were assessed by proton (\(^1\)H) and phosphorus (\(^{31}\)P) nuclear magnetic resonance spectroscopy (MRS) during ischemia and early reperfusion. We compared these results with histologic findings in the hippocampal CA1 region 7 days after the ischemic insult.

**Materials and Methods**

Twenty-one male Wistar rats weighing 200–250 g were anesthetized with 0.25 mg/100 g i.p. diazepam and 0.05 ml/100 g i.m. of 10 mg fluanisone plus 0.315 mg fentanyl citrate/ml. A \(^1\)HP double-tuned surface coil covering both cerebral hemispheres was fixed on each rat's skull with acrylic resin after removal of the overlying tissue. After 4 days of recovery each ad libitum-fed rat was anesthetized with 7.5 mg/100 g i.p. thiobutabarbital, spontaneous respiration being maintained. The femoral artery and jugular vein on the right side were cannulated. Both common carotid arteries were exposed, avoiding distortion of the adjacent vagal nerves, and a remote-controlled occlusion device was placed. This device consisted of a piece of silicone tube with three incomplete cuts holding two nylon wires. The occluding wire was looped around the sternomastoid muscle and carotid artery on both sides. Continuous traction on the ends of this wire folds the tube around these structures, occluding both arteries. Removing the traction on the occluding wire and shortly pulling the opening wire stretches the tube again, opening the carotid arteries. A schematic presentation of the device is shown in Figure 1.

The anesthetized rat was placed in a 7.0-cm-diameter probe. A controlled heated airflow of 4 l/min kept the temperature in the chamber at 37°C, thus minimizing fluctuations in brain temperature. Dextromethorphan (Roche Products Ltd., Mijdrecht, The Netherlands, 50 mg/kg) was administered intraperitoneally in 10 rats 30 minutes before the induction of ischemia. The remaining rats received no treatment. The probe was inserted into a Bruker MSL 200 spectrometer with a vertical bore and a magnetic field strength of 4.7 T. The magnetic field homogeneity was optimized using the line width of the water signal. A homogeneity of 0.11 parts per million (ppm) was obtained in all experiments, leading to a constant line width. The excitation pulse was optimized by maximizing the water signal in the \(^1\)H spectra and by maximizing the phosphocreatine (PCr) signal in the \(^{31}\)P spectra.

One \(^1\)H spectrum and one \(^{31}\)P spectrum were made 20 minutes before the induction of ischemia and served as baseline spectra. The \(^1\)H spectral acquisition consisted of a 1-1-2-2 phase-cycled spin/echo sequence used for water suppression. A \(^1\)H spectrum was acquired in 4.5 minutes using 128 averages with a 2-second repetition time. The \(^{31}\)P spectra were acquired using a phase-cycled single-pulse collection sequence. A \(^{31}\)P spectrum was acquired in 4.5 minutes using 256 averages with a 1-second repetition time.

Global cerebral ischemia was induced by remote-controlled occlusion of both common carotid arteries by gentle continuous traction (100 g) on the occluding wire and subsequent lowering of the mean arterial blood pressure (MABP) to 50 mm Hg by the rapid withdrawal of venous blood. In each rat the \(^1\)H spectra were made at the intervals 0–5, 10–15, and 20–25 minutes of ischemia. The \(^{31}\)P spectra were made at the intervals 5–10, 15–20, and 25–30 minutes of ischemia. At 25 minutes of ischemia blood samples were taken for analysis of serum glucose concentrations.

After 30 minutes of ischemia, reperfusion was induced by the rapid infusion of the collected blood and subsequent opening of the occlusion device. The \(^1\)H spectra were assessed at 0–5, 10–15, 20–25, and 30–35 minutes of reperfusion. The \(^{31}\)P spectra were assessed at 5–10, 15–20, 25–30, and 35–40 minutes of reperfusion. At the end of the experiment the occlusion site was inspected to confirm the proper restoration of blood flow.
The 1H spectra were processed by the convolution-difference method using line broadenings of 5 and 150 Hz followed by Fourier transformation. The spectra were analyzed by measuring the peak heights of the choline (3.15 ppm), (phospho)creatine (Cr, 3.05 ppm), \( N \)-acetylaspartate (NAA, 2.02 ppm), and Lac (1.32 ppm) resonances. To compare individual experiments, ratios of the Lac and NAA peak heights were expressed as the Lac/Cr and NAA/Cr ratios, assuming no gross change in the total Cr pool.20

The 31P spectra were processed with line broadenings of 20 and 250 Hz. The spectra were analyzed by measuring the peak areas of inorganic phosphate (Pi, ±4.9 ppm), PCr (0 ppm), and the \( \gamma, \alpha, \) and \( \beta \)-ATP resonances (−2.8, −7.8, and −16.2 ppm, respectively). Peak areas were used instead of peak heights because of the lower signal-to-noise ratio compared with the 1H spectra. The chemical shift difference because of the lower signal-to-noise ratio compared with the 1H spectra. The chemical shift difference between Pi and PCr was measured to calculate the tissue pH as 6.77+log[(Pi−3.29)/(5.68−Pi)]21. The PCr peak area divided by the Pi peak area was used as an indicator of the cellular energy state.22

The 13 rats that survived for 7 days (eight treated and five untreated) were sacrificed using 25 mg pentobarbital i.p. The brains were perfused with heparin-saline, followed by a mixture of 4% formaldehyde, 6% purified water, 10% acetic acid, and 80% methanol. Three nonserial 5-μm hematoxylin and eosin-stained sections from a midcoronal plane 4 mm from the bregma were studied in each rat. The hippocampal CA1 sector was examined on each side in every section. The limits of this sector were clearly defined in rats with major ischemic damage, and comparable limits were applied in rats with minimal or no damage. In each section, the number of CA1 pyramidal neurons with anoxic change of the classical homogenizing type and the number of normal neurons were counted. To confirm statistically the trend of higher survival rates among the treated rats, a separate group of nine animals (two treated and seven untreated) underwent the same ischemia protocol without MRS measurements.

The MRS data acquired before, during, and after ischemia were analyzed separately to study the effects of dextromethorphan during these different stages of the experiment. Statistical analysis was performed using Student’s t test for measurements made at one time and repeated-measures analysis of variance for measurements made at multiple times. Histologic data (anoxic cell counts) of the treated and untreated groups were compared using Wilcoxon’s two-sample rank sum test. Survival percentages were compared using Fisher’s exact test. All probability values are given for two-tailed testing. Metabolic parameters as assessed by MRS were correlated with histologic findings. Results are given in the text as mean±SD.

Results

Preischemic MABP was 106±10 and 98±14 mm Hg, and postischemic MABP was 103±11 and 100±12 mm Hg in the untreated and treated groups, respectively, indicating no significant difference. There was no proper restoration of blood flow in five rats (two treated and three untreated). The MRS measurements made during reperfusion and the survival and histologic data in these rats were excluded from analysis. Serum glucose concentration measured at 25 minutes of ischemia was 10.1±3.5 and 10.6±4.9 mmol/l in the untreated and treated groups, respectively, indicating no significant difference.

A series of typical 1H spectra is shown in Figure 2, top. In every baseline spectra, at 1.32 ppm a small peak was present resulting from basal Lac and residual lipid. The 1H spectra measured in the untreated group during ischemia showed a rapid increase of the Lac signal at 1.32 ppm; the Lac elevation in the treated group was less pronounced. The 1H spectra measured during reperfusion showed a gradual decrease in Lac concentration in both groups. Values of the Lac/Cr ratios during ischemia and reperfusion are shown in Figure 3, top left. Statistical analysis revealed a significant difference between groups in Lac/Cr ratios during ischemia (\( p<0.001 \)) and reperfusion (\( p<0.05 \)) (Table 1).

A series of typical 31P spectra is shown in Figure 2, bottom. Tissue pH decreased in both groups after the induction of ischemia. During ischemia there was a significantly smaller reduction in tissue pH in the treated group (\( p<0.01 \)) than in the untreated group. During reperfusion tissue pH in both groups increased, resulting in a significantly higher pH in the treated group for only the first measurement (\( p<0.05 \)) (Table 1). Tissue pH values are shown in Figure 3, top right.

In the 31P spectra of both groups, the resonance peak of PCr decreased rapidly after the induction of ischemia, followed by a more gradual decrease of the ATP resonances. The peak of Pi increased rapidly. When the circulation of the brain was restored, the \( \beta \)-ATP resonance showed a rapid but incomplete restoration toward normal values (data not shown). The increase in the PCT/Pi ratio was more gradual and also incomplete. Values of the PCT/Pi ratios during ischemia and reperfusion in both groups are shown in Figure 3, bottom left. Statistical analysis revealed a significant difference between groups for only the first measurement during ischemia (\( p<0.05 \)).

The 1H spectra in both groups showed a significant (\( p<0.001 \)) decrease in the NAA/Cr ratio at 5 and 15 minutes of ischemia that failed to recover during reperfusion. Values of the NAA/Cr ratios of both groups are shown in Figure 3, bottom right. Statistical analysis did not reveal any significant difference between groups during ischemia and reperfusion. However, the preischemic values of the two groups differed significantly (\( p<0.05 \)).

The histologic findings are presented in Figures 4 and 5. Anoxic damage in the hippocampus was more severe in the untreated rats. The proportionate anoxic cell count in the treated group was 0.21±0.12 (\( n=8 \)); in the untreated group this value was
There was a linear correlation between end-ischemic tissue pH and morphologic damage in the total population of treated and untreated rats (r = -0.73, n = 13; p < 0.05). The Lac-pH correlation decreased during early reperfusion (r = -0.54, n = 15; p < 0.05). Ninety percent of the treated rats survived for 7 days, compared with 47% of untreated rats. This difference in survival was significant (p < 0.05).

Discussion

This study confirms the usefulness of MRS in sequentially studying cerebral metabolism and evaluating potential therapeutic modalities for cerebral ischemia. Using a double-tuned coil, the time course of Lac formation, tissue pH, and high-energy phosphate metabolite levels can be studied noninvasively during ischemia and subsequent reperfusion.

In the treated group lactic acidosis was significantly reduced compared with the untreated group, indicating that even during severe metabolic stress dextromethorphan had a mitigating effect on Lac production. Lac formation and the concomitant development of intracellular acidosis is highly dependent on serum glucose levels. However, the glucose concentrations were similar in the two groups.

Previous studies have demonstrated that activation of NMDA receptors leads to increased metabolic...
FIGURE 3. Bar graphs of mean±SD (top left) lactate/creatine peak height ratio showing significant difference during ischemia (p<0.001) and reperfusion (p<0.05) between dextromethorphan-treated (filled bars) and untreated (shaded bars) rats. Top right: Tissue pH showing significant difference during ischemia (p<0.01). Bottom left: Phosphocreatine/inorganic phosphate (PCr/Pi) peak area ratio. Bottom right: N-Acetylaspartate/creatine (nAA/creatine) peak height ratio. pre, baseline value; i, minutes of ischemia; r, minutes of reperfusion.

activity, reflected in an elevated rate of Lac formation. A possible explanation for the less pronounced lactic acidosis after dextromethorphan administration found in our study is the partial blockade of both NMDA receptor-mediated and voltage-gated Ca2+ influx. The cellular energy demanded for the extrusion of intracellular Ca2+ is reduced by blockade of these two routes of Ca2+ influx into the cells. An intracellular Ca2+ overload adversely affects mitochondrial function by uncoupling oxidative phosphorylation. By blocking the Ca2+ influx, NMDA antagonists might preserve mitochondrial oxidation and allow oxidative metabolism to continue in cells not totally deprived of oxygen, leading to a diminished production of Lac.

During ischemia tissue pH correlated strongly with Lac concentration. However, during reperfusion the acidosis showed a tendency toward rapid recovery, despite elevated Lac levels. This phenomenon has also been observed by others and is presumably caused by a continued Na+/H+ exchange with a delayed consumption of Lac.

It is claimed that excessive tissue lactic acidosis attributes to morphologic alterations. This is consistent with the strong correlation we found between tissue pH during ischemia and histologic damage at 7 days. The correlation between pH and histologic damage suggests that the pH measured by 31P nuclear magnetic resonance spectroscopy in the clinical setting could have value in predicting the final morphologic damage.

Our finding that histologic damage diminishes after dextromethorphan administration is in accor-
dance with the results of other studies showing that this agent protects against morphologic changes under hypoxic or ischemic conditions in cultured neuronal cells and in vivo. In interpreting our histologic data, we must point out that only the "surviving" rats were compared. Since almost half of the untreated animals died, presumably with severe anoxic damage, the true difference between groups would probably be even more pronounced than that observed.

A consistent feature in the $^1$H spectra is a significant decrease of the NAA concentration during ischemia in both groups. This drop has been observed in other studies. Its importance, however, is not completely clear. NAA is found almost exclusively in neurons of the central nervous system. The de-
TABLE 1. Lactate/Creatine Ratio and Intracellular pH During Ischemia and Reperfusion in Rats

<table>
<thead>
<tr>
<th>Time</th>
<th>Lactate/creatine ratio</th>
<th>Intracellular pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dextromethorphan-treated</td>
<td>Untreated controls</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.29±0.06</td>
<td>0.30±0.06</td>
</tr>
<tr>
<td>Ischemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>0.94±0.22</td>
<td>1.39±0.22</td>
</tr>
<tr>
<td>15 min</td>
<td>1.59±0.16</td>
<td>2.07±0.33</td>
</tr>
<tr>
<td>25 min</td>
<td>1.91±0.19</td>
<td>2.42±0.58</td>
</tr>
<tr>
<td>Reperfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>2.07±0.26</td>
<td>2.44±0.48</td>
</tr>
<tr>
<td>15 min</td>
<td>1.91±0.29</td>
<td>2.43±0.46</td>
</tr>
<tr>
<td>25 min</td>
<td>1.45±0.37</td>
<td>2.05±0.46</td>
</tr>
<tr>
<td>35 min</td>
<td>0.98±0.40</td>
<td>1.44±0.56</td>
</tr>
<tr>
<td>Baseline</td>
<td>7.13±0.05</td>
<td>7.14±0.05</td>
</tr>
<tr>
<td>Ischemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>6.66±0.20</td>
<td>6.48±0.09</td>
</tr>
<tr>
<td>20 min</td>
<td>6.61±0.15</td>
<td>6.46±0.11</td>
</tr>
<tr>
<td>30 min</td>
<td>6.54±0.13</td>
<td>6.29±0.17</td>
</tr>
<tr>
<td>Reperfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>6.75±0.22</td>
<td>6.43±0.34</td>
</tr>
<tr>
<td>20 min</td>
<td>6.96±0.22</td>
<td>6.86±0.21</td>
</tr>
<tr>
<td>30 min</td>
<td>6.94±0.34</td>
<td>7.07±0.14</td>
</tr>
<tr>
<td>40 min</td>
<td>7.11±0.13</td>
<td>7.03±0.14</td>
</tr>
</tbody>
</table>

NS, not significant.

crease in the NAA resonance signal during ischemia might be due to a leakage of this substance out of severely damaged neurons. An increased excretion in urine samples was demonstrated in patients with cerebral palsy. Other investigators have suggested an active degradative metabolic mechanism for NAA in injured neurons. The unexpected difference in baseline measurements of the NAA/Cr ratio suggests that the administration of dextromethorphan affects the cellular metabolism of NAA or Cr. To explore the effect of dextromethorphan administration on metabolism in nonischemic brains, we performed $^1$H and $^{31}$P MRS before and after the administration of this agent in an additional series of six rats. We could not detect any influence of dextromethorphan on the NAA/Cr ratio or other metabolites.

The $^{31}$P spectra showed a low cellular energy level during ischemia. Although no difference in cellular energy state between groups could be demonstrated, we cannot exclude different time courses during the early phase of ischemia since the first measurements were made at the interval of 5–10 minutes of ischemia. It has been shown that the significant drop in the concentrations of PCR and other high-energy metabolites occurs within the first few minutes after the induction of ischemia. Furthermore, the PCR/PI ratio gives information about the energy content but not the energy turnover of the cell. Therefore, it is possible that in the untreated group energy turnover was increased, as indicated by the more pronounced lactic acidosis.

In this rat model of near-complete forebrain ischemia, MRS shows that dextromethorphan, when administered before the induction of low-flow ischemia, can significantly decrease the formation of Lac and the development of tissue acidosis. Histologic and survival rates also point to a beneficial effect of this agent in this model. Future clinical application of these findings could be prophylactic treatment with dextromethorphan to prevent the sequelae of developing cerebral ischemia in cases of trauma, subarachnoid hemorrhage, shock, high-risk surgery, or intracranial expanding tumors. Recent animal studies also suggest a beneficial effect when dextromethorphan is administered after the ischemic insult.

The tolerance of humans to high doses of dextromethorphan is currently under investigation.

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