Effect of GM1 Ganglioside
After Focal Cerebral Ischemia
in Halothane-Anesthetized Cats

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The effect of the ganglioside GM1 was studied in a focal cerebral ischemia model in 30 cats consisting of 2 hours of middle cerebral artery occlusion followed by 4 hours of recirculation. The cerebrocortical electrical activity, extracellular potassium activity, and microcirculation indicated by NAD/NADH fluorescence were measured during occlusion as well as during recirculation in the core of the middle cerebral artery territory, while the cerebral metabolic rate for glucose (ICMRgl) was measured at the end of recirculation. The cats were classified into either mildly or moderately severe stroke groups based on the depression of the cerebrocortical electrical activity on the occluded side. Of 12 cats with only a mild stroke, six were administered GM1 intravenously 30 minutes after occlusion, while six cats were not treated. Of 12 cats with a moderate stroke, six were treated and six were left untreated. In six additional cats, only a sham insult was undertaken. In the cats with mild stroke, GM1 treatment significantly increased ICMRgl in the peripheral middle cerebral artery territory compared with the untreated cats; for the six treated cats, ICMRgl was normalized toward the control level, whereas it was depressed in the six untreated cats. There were no other significant effects of GM1 treatment on the other measured parameters. A potential protective effect of anesthesia is discussed. (Stroke 1989;20:795–802)

Integrity of the cell membrane is essential for the maintenance of normal brain function. Relatively small stores of high-energy substances make the brain very vulnerable to an interruption of the oxygen and substrate supply. Impaired energy production leads to disintegration of membrane function, and if the ischemic insult is extended the morphologic structure of the neural membranes can be irreversibly damaged. The extracellular potassium activity ([K+]c) measured continuously with ion-selective microelectrodes, is a good indicator of neural membrane damage.

Gangliosides are present in mammalian tissues, and their concentration in the neural cell membranes of the brain cortex in particular is very high. Gangliosides increase the activity of Na⁺,K⁺-ATPase, facilitate recovery in the hippocampus after lesioning, and facilitate dopaminergic neuronal regeneration in the central nervous system. These properties suggest that gangliosides have the potential for aiding the recovery of neurons affected by prolonged ischemia.

Previous work in our laboratory has shown that GM1 ganglioside significantly reduces the elevated local cerebral metabolic rate for glucose (ICMRgl) in the peripheral middle cerebral artery (MCA) territory (P MCA) in the moderate stroke group. Histologic damage was also attenuated by GM1, suggesting that this natural membrane constituent may have a protective effect against ischemic brain damage. However, since in the above-mentioned study ICMRgl values even in the sham-operated group were low due to barbiturate anesthesia, it seemed reasonable to measure ICMRgl using halothane/N₂O anesthesia. Halothane does not decrease cerebral glucose metabolism and blood flow, so one may expect less interference regarding potential protective effects of GM1.

In addition to the variables measured in our previous study (electrocortical activity [ECoG],

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Received March 9, 1988; accepted December 16, 1988.
cerebrocortical blood flow (CBF), nicotinamide adenine dinucleotide [NAD/NADH] redox state changes), in the present study we also measured $[K^+]_e$, a sensitive indicator of neuronal membrane damage, in the central MCA territory (C MCA) of the brain cortex of cats during 120 minutes of focal cerebral ischemia as well as during the first 240 minutes of reperfusion. We also studied the changes of ICMRgl at the end of recirculation. To avoid any protective effect from barbiturates, cats were anesthetized with halothane/N$_2$O.

**Materials and Methods**

Thirty male cats weighing 2.5–3.5 kg were initially anesthetized with 4% halothane in O$_2$. The anesthetic mixture was quickly changed to 1% halothane, 30% O$_2$, and 69% N$_2$O and maintained during the surgical preparation. After completion of surgery, the halothane concentration was reduced to 0.5%. Both femoral arteries, a femoral vein, and the left lingual artery were cannulated. After tracheostomy, the cats were immobilized with 5 mg/kg gallamine triethiodide (American Cyanamid, Pearl River, New York) and ventilated (Harvard Apparatus, South Natick, Massachusetts). Blood gases and pH were checked periodically and corrected by intravenously adjusting the rate and volume of the respirator and/or by infusing NaHCO$_3$ if necessary to keep the pH in the physiological range; plasma glucose concentration was also determined from these blood samples. Arterial blood pressure and the temperature of the cat were continuously monitored; the temperature was maintained at 37° C by a thermostatically controlled heating lamp. The head of the cat was mounted in a stereotactic holder, and the skin and muscles were removed from both sides of the skull. A 12-mm-diameter disk over the middle ectosylvian gyrus was removed and after each experiment with solutions of known K$^+$ concentration to which NaCl was added to maintain a constant ionic strength. Electrodes showing 48–56-mV change for a 10-fold change in K$^+$ concentration were used. The signal was fed into a differential amplifier (10$^{14}$ Ω input impedance) and registered on a chart recorder.

Cerebrocortical NADH fluorescence and reflectance (microregional blood flow), intracranial pressure, arterial blood pressure, and expired CO$_2$ concentration. One hundred ninety-five minutes after the initiation of reperfusion, 250 μCi of $[^1^4^C]$-2-deoxyglucose ($[^1^4^C]$-2DG, New England Nuclear, Boston, Massachusetts) was injected as an intravenous bolus for the determination of ICMRgl. Arterial blood samples were taken during the next 45 minutes for the measurement of the time course of both plasma $[^1^4^C]$-2DG activity and glucose concentration.

The cats were killed with an intravenous bolus of a saturated KCl solution, and the brains were quickly removed and frozen in Freon 22. Twenty-micrometer-thick sections were cut in a cryostat (American Optical, Buffalo, New York), dried on a hot plate at 60° C for 5 minutes, and then placed on x-ray film for 1 week together with calibrated carbon-14 standards. Quantitative densitometry of the autoradiograms was performed using a computer-assisted digital scanner (Onptronics International, Inc., Chelmsford, Massachusetts) attached to an image processor (Grinnell Systems, San Jose, California). The operational equation of Sokoloff et al$^{11}$ was employed for the calculations of ICMRgl.

Whereas ICMRgl was measured in several territories and evaluated according to the territories' relations to the blood supply of the MCA (explained in detail below), ECoG, $[K^+]_e$, cerebrocortical reflectance, and NAD/NADH redox state changes were measured only in the core of the MCA territory. $[K^+]_e$ was measured using double-barreled ion-selective microelectrodes, prepared with modification as described by Lux and Neher,$^{12}$ and using a “potassium cocktail” (Fluka Chemical Corp., Ronkonkoma, New York). The tip of the electrode was 1–3 μm. The electrodes were calibrated before and after each experiment with solutions of known K$^+$ concentration, and their response was checked and corrected periodically. Electrodes showing 48–56-mV change for a 10-fold change in K$^+$ concentration were used. The signal was fed into a differential amplifier (10$^{14}$ Ω input impedance) and registered on a chart recorder.
alterations in NADH fluorescence were calculated using the corrected factor.

The reflected light was used to determine the changes in cerebrocortical vascular volume (CVV), mean transit time of cortical blood flow (tm), and CBF. Baseline reflectance, measured during the control period, was regarded as representing 100% CVV. To determine 0% CVV, the blood was washed from the monitored cerebrocortical region via the lingual artery with 2–2.5 ml oxygenated isosmotic dextran solution during the control period. The difference in cortical reflectance obtained between the blood-perfused (100% CVV) and blood-free (0% CVV) brain was linearly divided to calculate CVV changes. tm was calculated from the dextran-induced reflectance reactions using the area=height analysis, with the reference value of tm set to 100%. Finally, relative microregional CBF changes were calculated by dividing the percentage values of CVV by the percentage values of tm. The application of this technique for the measurement of CBF in pathologic states such as epilepsy, severe arterial hypotension, hypoxia, ischemia, and reperfusion has been used by several groups of investigators.

ICMRgl was determined in 15 structures in the left and right hemispheres and classified according to their relation with the blood supply of the MCA. The caudate nucleus and the middle ectosylvian anterior, middle ectosylvian posterior, and anterior sylvian gyri formed the C MCA. The P MCA consisted of the anterior ectosylvian, anterior suprasylvian, and middle suprasylvian gyri. The sigmoid, anterior, middle and posterior cingulate, and para-hippocampal gyri were considered non-MCA structures (N MCA). ICMRgl was also determined in the cerebellar cortex.

In 24 cats the MCA was occluded for 120 minutes, while in six sham-operated cats used as controls, the MCA was only lightly touched with a glass rod. MCA occlusion in cats produces different hemodynamic and metabolic alterations depending on individual variations in vasculature and the age of the cat, and accordingly produces different severities of stroke. To better match the treated and untreated cats, we classified them based on the ECoG depression after 30 minutes of MCA occlusion. If the mean amplitude of the ECoG in the ischemic territory divided by that of the nonischemic side (ECoG ratio) was >0.7, the cat was classified as having a mild stroke; when the ECoG ratio was 0.2–0.7, the cat was considered as having a moderate stroke, and if the ECoG ratio was <0.2, the cat was classified as having a severe stroke. Due to the few cats with severe strokes when halothane/\textsubscript{N}_2\textsubscript{O} was used as the anesthetic (approximately 8%), no severe cats were included in this report. Twelve MCA-occluded cats were treated with 30 mg/kg i.v. GM1 ganglioside, and the other 12 were given a saline injection 30 minutes after the occlusion since by this time the severity of the stroke could be assessed from the ECoG. The cats treated with GM1 were alternately selected (i.e., every second cat in a severity group was treated). The treated and untreated mild and moderate subgroups comprised six cats each. All cats were monitored for an additional 240 minutes.

All stroke subgroups were compared with the sham-operated group. The treated and corresponding untreated subgroups were also compared. For statistical evaluation of the data, analysis of variance and the Mann-Whitney U test was used.

**Results**

Control mean±SD arterial blood pressure of all cats was 111.8±15.8 mm Hg. No significant changes were observed during the experiment, nor were there differences among groups. Control blood gases were PCO\textsubscript{2}=11.4±14.9 mm Hg, PCO\textsubscript{2}=33.1±4.3 mm Hg, and pH 7.359±0.039.

Control plasma glucose concentration was 132.2±4.0 mg/dl in the sham-operated group and 124.8±13.4 mg/dl in the mild untreated, 116.2±10.0 mg/dl in the mild treated, 88.6±4.8 mg/dl in the moderate untreated, and 119.3±12.5 mg/dl in the moderate treated subgroups. There were no significant differences between the treated and the corresponding untreated subgroups. After 2 hours of occlusion, plasma glucose concentration increased to 135.4±3.7, 149.8±23.1, 138.0±7.4, 106.4±12.3, and 145.2±14.4 mg/dl in the sham-operated group and the mild untreated, mild treated, moderate untreated, and moderate treated subgroups, respectively. There were no significant differences between the treated and the corresponding untreated subgroups at this time nor at the end of recirculation.

The ECoG ratio in the sham-operated group remained close to 1.0 throughout the experiment (Figure 1) but was significantly depressed immediately after MCA occlusion in all subgroups. The treated and untreated mild subgroups returned to the control level after 30 minutes of occlusion; in the moderate subgroups the ECoG ratio remained depressed during the entire occlusion period. After release of the occlusion at 120 minutes, ECoG in the untreated moderate subgroup recovered more rapidly than in the treated moderate subgroup, the difference being significant between 120 and 240 minutes, although there was no significant difference at the end of the experiment (360 minutes).

Control mean±SEM [K\textsuperscript{+}] was in the normal range in all groups and subgroups (3.19±0.04 mM, Figure 2). Upon MCA occlusion, before treatment, [K\textsuperscript{+}] increased significantly more in the mild treated subgroup than in the mild untreated sub-
erate treated subgroup during the first part of occlusion, while the increase in the moderate untreated subgroup was more pronounced during the second part of the occlusion period. Although there were significant differences between the moderate treated and untreated subgroups immediately following occlusion, this difference disappeared by the end of occlusion. In two cats from the moderate treated subgroup, spreading depression occurred during the first part of occlusion while spreading depression was observed in two cats in the moderate untreated subgroup during the second half of the ischemic period; this largely influenced the subgroups' mean values. After release of the MCA occlusion, \( [K^+]_e \) in the moderate group decreased rapidly but remained significantly higher than that in the sham-operated group.

Microregional CBF showed an initial large reduction following MCA occlusion in all groups (Figure 3). Subsequently, CBF increased steadily, and by the end of occlusion the mean±SEM value in the mild untreated and mild treated subgroups was 67.3±11.8% and 77.8±16.9%, respectively; corresponding values in the moderate untreated and moderate treated subgroups were 49.7±4.3% and 63.8±8.0%, respectively. There was no significant effect of GM1 treatment on CBF during occlusion. Release of the MCA was followed by reactive hyperemia; thereafter, CBF gradually returned to the control level by the end of recirculation in all cats.

**Figure 1.** Graph of mean±SEM changes of electrocorti-cogram (ECoG) ratio (ratio of amplitude of ECoG on ipsilateral side to that on contralateral side) during middle cerebral artery occlusion and recirculation in cats. Significant differences between treated (filled symbols) and untreated (open symbols) subgroups are indicated (*p<0.05). Moderate stroke groups (squares) were significantly different from sham-operated group (O) during occlusion and reperfusion. ECoG ratios in mild stroke groups (triangles) were significantly depressed immediately following occlusion but returned to control level 30 minutes after initiation of stroke.

**Figure 2.** Graph of mean±SEM extracellular potassium activity ([K+] e) changes on logarithmic scale in center of middle cerebral artery territory during occlusion and recirculation in cats. Increase was significant in all groups following occlusion and remained significantly elevated throughout reperfusion except for both mild stroke subgroups (triangles), which were not significantly different from control 120 minutes after release of occlusion (at 240 minutes). Significant differences between treated (filled symbols) and untreated (open symbols) moderate stroke (squares) subgroups are indicated (*p<0.05). O, Sham-operated group.
groups and subgroups except the moderate treated subgroup. CBF remained elevated during the entire reperfusion period in this subgroup although no significant treatment effects on CBF were found.

The NAD/NADH redox state became reduced in both stroke groups upon occlusion of the MCA and showed no significant recovery even after release of the occlusion (Figure 4). NADH fluorescence in the sham-operated group remained close to the initial level during the entire study.

Table 1 lists the mean±SEM ICMRgl values in the three groups, and Figure 5 depicts the data as a percentage of the corresponding values in the sham-operated group. In the C MCA, in both hemispheres ICMRgl in all subgroups was less than that in the sham-operated group. This decrease was not significant on the right (unoccluded) side, but on the left side the decrease in ICMRgl was significant in all except the mild treated subgroup. In the P MCA, on the left side the mild treated subgroup showed a significantly higher ICMRgl than the mild untreated subgroup (p<0.05). Other regions examined also showed an increase in ICMRgl in the mild treated subgroup, although these increases did not reach the level of significance. The moderate treated and moderate untreated subgroups showed no differences in ICMRgl in any region.

Discussion

We examined the effect of GM1 ganglioside on ECoG, [K⁺], CBF, NAD/NADH redox state, and...
TABLE 1. Local Cerebral Metabolic Rates for Glucose in Cats Subjected to 2 Hours of MCA Occlusion

<table>
<thead>
<tr>
<th>Groups</th>
<th>MCA territories</th>
<th>Central</th>
<th>Peripheral</th>
<th>Non-MCA</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Sham-operated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild stroke</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td>57.2±8.86</td>
<td>55.8±8.13</td>
<td>51.6±6.69</td>
<td>51.6±6.01</td>
</tr>
<tr>
<td>Treated</td>
<td></td>
<td>50.2±2.98</td>
<td>52.3±3.48</td>
<td>53.0±3.66</td>
<td>55.5±4.02</td>
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<tr>
<td>Moderate stroke</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td>39.1±4.88</td>
<td>45.0±4.64</td>
<td>43.7±3.51</td>
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<td>50.2±2.98</td>
<td>52.3±3.48</td>
<td>53.0±3.66</td>
<td>55.5±4.02</td>
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</tbody>
</table>

Data are mean±SEM μM/100 g/min. MCA, middle cerebral artery.

*Significantly less than sham-operated.
†Significantly greater than mild untreated.

ICMRgl in a focal cerebral ischemia model in cats. To avoid the potential beneficial effects of barbiturates we used light halothane/N₂O anesthesia. Upon MCA occlusion, ECoG was depressed in all subgroups (Figure 1). Its recovery in the mild treated and mild untreated subgroups at 60 minutes after occlusion (i.e., during the stroke period) was identical; however, [K⁺], remained significantly elevated in these subgroups (Figure 2). The moderate treated subgroup showed a slower ECoG ratio recovery between 120 and 240 minutes than the moderate untreated subgroup, but by the end of recirculation there was no significant difference between subgroups. The more pronounced restoration of the ECoG ratio in the moderate untreated subgroup can probably be attributed to a transient overshoot of CBF and, hence, tissue oxygen supply upon removal of the MCA clip. ECoG and [K⁺]e in the mild treated and mild untreated subgroups at the end of recirculation were not different from those of the sham-operated group, but ICMRgl of C MCA and P MCA (except for the mild treated subgroup) was depressed even after 240 minutes of recirculation. The mild treated subgroup showed an increase of ICMRgl in all territories compared with the mild untreated subgroup, and in the P MCA the increase was significant. The CBF and NAD/NADH changes were similar in all four subgroups (Figures 3 and 4), except for the transient hyperemia in the moderate untreated subgroup.

The changes in [K⁺]e did not follow the well-described pattern of changes observed in complete cerebral ischemia, in which after a slow initial increase a rapid secondary increase occurs. In the focal ischemic model of cats, ischemia is never complete, especially in cats with moderate and mild strokes, as indicated by the relatively high CBF and ECoG ratios. [K⁺]e indicates an absence of "complete membrane failure," which is usually accompanied by excessive K⁺ leakage to the extracellular space. In previous experiments we studied the effect of GM1 on glucose metabolism, and a decrease in ICMRgl was found in the P MCA of the moderate treated cats, with a significant morphologic improvement, perhaps due to a reduction of glycolytic activity in the periphery of the focal ischemic zone, in this region. During focal ischemia, a region of increased glucose utilization frequently surrounds the central ischemic core, due most likely to the presence of anaerobic glycolysis. Anaerobic glycolysis persists even during recirculation, although...
to a lesser degree. The decreased glycolysis seen in the GM1-treated cats with barbiturate anesthesia probably leads to less lactic acid accumulation, diminishing the acidotic pH shift that has been indicated as one of the significant detrimental factors in ischemic cell damage.

The overall metabolic activity in the sham-operated group in this light halothane/N₂O-anesthetized series was approximately 30% higher than that in our previous study in which pentobarbital was used as the anesthetic. This is in agreement with other data in the literature showing that barbiturates cause a decrease in ICMRgl. Even at doses 1/5 of the usual anesthetic doses, barbiturates can substantially depress ICMRgl. In contrast to the depression seen with barbiturates, halothane anesthesia produces either no change or only a slight decrease in ICMRgl.

ICMRgl in the C MCA in these experiments was severely depressed following occlusion, with no regions showing an increase. In the territories surrounding the ischemic core, we did not find any region that showed higher metabolism than the sham-operated group, suggesting more severe damage since the cells surrounding the C MCA were not capable of switching to anaerobic glycolysis. This interpretation is supported by the higher residual [K⁺], during recirculation compared with that of cats anesthetized with barbiturates, in which [K⁺] returns to the control level 60 minutes after the release of the MCA occlusion. It may seem contradictory that both the decrease (pentobarbital anesthesia) and an increase (halothane anesthesia, present study) of ICMRgl in the P MCA produced by GM1 is considered beneficial. This is actually not a contradiction since excessive lactic acidosis and diminished adenosine 5'-triphosphate synthesis due to a low ICMRgl (present study) may equally result in brain damage. In both barbiturate- and halothane-anesthetized and halothane-anesthetized cats, the ICMRgl in the P MCA of GM1-treated cats tended to be shifted toward control. This shift may be related to the membrane stabilization properties of gangliosides. Exogenously administered gangliosides facilitate ganglioside synthesis and speed up ganglioside recycling, leading to a restoration of the membrane.

Halothane increases CBF. The less depressed ICMRgl and the increased CBF due to anesthesia might be responsible for the small percentage of cats with severe strokes we observed (approximately 8%). This does not mean, however, that the final outcome following stroke under halothane/N₂O anesthesia is better. In two moderate untreated cats, tissue blocks through the center of the MCA territory were immersion-fixed in formalin and examined for histologic damage by light microscopy. The damage seen in these two cats was comparable to that seen in barbiturate-anesthetized cats that were classified as having severe strokes as part of a previous study.

NAD/NADH redox state is a complex parameter of cellular metabolism. The redox state depends on the supply of reducing equivalents, on the rate of mitochondrial electron transport and oxidative phosphorylation, and finally, on the oxygen supply. Accordingly, the significant reduction of NAD during MCA occlusion can be attributed to tissue hypoxia and enhanced anaerobic glycolysis. At the same time, the lack of significant oxidation of NAD upon recirculation and during the later phases of reperfusion indicates that the majority of optically monitored cells in the brain cortex may have been irreversibly damaged and that mitochondrial electron transport had already shut down during MCA occlusion. This may correspond to the marked decrease of ICMRgl in the core of the MCA territory observed in most of the cats in the mild and moderate groups. In this context, the recovery of CBF could be misleading since irreversibly damaged cells will not respond to improvement of the tissue oxygen supply.

In conclusion, in this focal ischemia model with halothane/N₂O anesthesia we found that GM1 significantly elevated ICMRgl in the P MCA in cats with mild stroke. In cats with moderate stroke, we did not find any effect of GM1 on ICMRgl. The other parameters measured ([K⁺], CBF, NAD/NADH redox state, and ECoG) were not altered significantly by treatment with GM1 in any group.

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KEY WORDS • anesthesia • cerebral ischemia • gangliosides • cats
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Stroke. 1989;20:795-802
doi: 10.1161/01.STR.20.6.795

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