**Effect of the Ganglioside GM1, on Cerebral Metabolism, Microcirculation, Recovery Kinetics of ECoG and Histology, During the Recovery Period Following Focal Ischemia in Cats**

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**SUMMARY** The effect of the ganglioside GM1 on the recovery of local cerebral glucose metabolism (ICMRgl), recovery kinetics of cerebrocortical electrical activity, cerebral blood flow and redox state as well as histological changes following focal ischemia has been studied in the cat. Ischemia was produced by occlusion of the left middle cerebral artery (MCA), and GM1 (30 mg/kg) was injected intravenously at 30 min after the MCA occlusion or at the time of release of the occlusion, at 120 min. Another group of animals were subjected to the same ischemic insult, but without GM1 treatment, and sham-operated treated and not treated cats were also studied. The animals of both GM1-treated and non-treated stroke groups were classified into 2 groups (severe and moderate) depending on the depression of electrocortical activity in the ischemic hemisphere at 30 min of the ischemia. There was a significant increase in local cerebral blood flow in the ischemic area in the treated animals. Additionally there was a significant treatment effect on the left peripheral MCA territory for ICMRgl in the 30 min treated moderate group, (p < .05). This group of animals showed decreased ICMRgl accompanied by less severe histological damage suggesting that GM1 may produce metabolic depression so as to maintain a normal flow-metabolism couple and prevent ischemic structural damage. The possible mechanism of metabolic depression induced by GM1 is briefly discussed.

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GANGLIOSIDES, sialic acid-containing glycosphingolipids, are natural components of cellular membrane, localized in the outerleaflet of the lipid bilayer of the plasma membrane, and particularly abundant in nerve tissue. Current studies have suggested the possible functional role of gangliosides in neuronal development, synaptogenesis and synaptic transmission. Additionally, exogenous gangliosides have been shown to facilitate the regeneration of either damaged peripheral nerves or the central nervous system. There is also clinical evidence that gangliosides are capable of promoting the process of reinnervation in patients suffering from diabetic or alcoholic polyneuropathy. GM1 (nomenclature according to Svennerholm) is one of the major gangliosides in the mammalian brain, and it is exogenously administered is known to penetrate the blood-brain-barrier and to be actively incorporated into the neuronal membrane. In fact, the interaction of the gangliosides with brain membranes is reportedly accompanied by various metabolic effects including increased adenylate cyclase, increased phosphodiesterase activity, enhanced dopamine release and modification of (Na⁺, K⁺) ATPase activity.

Cerebral ischemia induces a wide spectrum of metabolic derangements, ranging from severely depressed glucose utilization along with a depletion of high-energy phosphates to a persistent activation of anaerobic glycolysis which is closely associated with histological damage (unpublished observation). Damage to plasma membranes is one of the major phenomena consistently characterizing irreversible ischemic cellular injury. Indeed, it is generally accepted that membrane failure triggers the processes causing irreversible structural damage during the ischemic event. There is also growing evidence that irreversible cellular damage is manifested during the recovery period following ischemia. A postischemic decline in the activities of the (Na⁺, K⁺) ATPase and adenylate cyclase, which are critically dependent on the integrity of the membrane, notably on its lipid-layer composition, has been demonstrated.

It is therefore reasonable to hypothesize that postischemic brain can be protected by intervention that either prevents or delays the development of membrane failure. In the present study, we have examined the effect of ganglioside GM1 on cerebral glucose metabolism, local cerebral blood flow (ICBF), recovery of ECoG, NAD/NADH redox state, and histologic alterations at the end of a 4 hr recovery period following 2 hr of MCA occlusion in cats.

**Methods**

**Preparation of Animals**

The experiments were carried out on 28 male cats (weighing 2.5–3.5 kg) anesthetized with 40 mg/kg sodium pentobarbital. The animals were immobilized with 5 mg/kg gallamine triethiodide (Flaxedil, American Cyanamid) and this dose was repeated every hour. The trachea, the femoral arteries and veins and the left
lingual artery were cannulated. The animals were ventilated with a Harvard respirator, the volume and rate of respiration set to obtain an arterial pO2 and pCO2 at approximately 100 mm Hg and 32 mm Hg, respectively. When arterial pH was below 7.35, an appropriate amount of sodium bicarbonate solution (2.5%) was infused into the femoral vein.

The heads of the animals were mounted in stereotaxic holders and the skin and muscles were removed from both sides of the skull. A 12-mm diameter hole was drilled in the left parietal bone above the middle ectosylvian gyrus (MEG). The dura was carefully opened with a fine needle and scissors, and the bleeding from dura and bone was stopped with gelfoam (Upjohn) and bone wax (Ethicon). A cranial window was fixed into the burr hole with dental cement, as described previously. Electrical activity of the exposed brain cortex was measured with silver electrodes 2 mm apart built into the plastic ring of the cranial window. EEG of the contralateral side was measured via a transorbital approach. The MCA was occluded with a fine needle and scissors, and the bleeding from dura and bone was stopped with gelfoam (Upjohn) and bone wax (Ethicon). A cranial window was fixed into the burr hole with dental cement, as described previously. Electrical activity of the exposed brain cortex was measured with silver electrodes 2 mm apart built into the plastic ring of the cranial window. EEG of the contralateral side was measured with copper screws fixed into the right parietal bone. The intracranial pressure was measured from metal taxic holders and the skin and muscles were removed from both sides of the skull. A 12-mm diameter hole was drilled in the left parietal bone above the middle ectosylvian gyrus (MEG). The dura was carefully opened with a fine needle and scissors, and the bleeding from dura and bone was stopped with gelfoam (Upjohn) and bone wax (Ethicon). A cranial window was fixed into the burr hole with dental cement, as described previously. Electrical activity of the exposed brain cortex was measured with silver electrodes 2 mm apart built into the plastic ring of the cranial window. EEG of the contralateral side was measured with copper screws fixed into the right parietal bone. The intracranial pressure was measured from metal tubes sealed into the plastic ring of the cranial window.

The left middle cerebral artery (MCA) was exposed via a transorbital approach. The MCA was occluded in 19 animals in close proximity to its branching from the internal carotid artery with a miniature Mayfield clip. After two hours of occlusion the clip was removed and the blood flow through the MCA was reinstated. In 5 of 19 animals with MCA occlusion GM1 (30 mg/kg) was injected intravenously when the occlusion was released, i.e. after 120 min of occlusion (GM1/120 group). In 9 animals, GM1 was injected 30 minutes after the occlusion (GM1/30 group), (in 3 preliminary experiments the lCMRgl and histological changes were not studied) while 5 animals were occluded but not treated (Untreated stroke group). In 9 animals a sham insult was initiated by lightly touching the completely prepared MCA with a glass rod; 5 were treated with GM1 at 120 min (Sham treated group), while the remaining 4 received a saline injection (Sham untreated group).

**Measurement of Cerebral Glucose Metabolism**

At 3 hr and 15 min after the release of the MCA occlusion, 250 μCi of 14-C-2-deoxyglucose (14C-2DG, New England Nuclear) was injected intravenously as a bolus for the determination of the local cerebral metabolic rate for glucose (lCMRgl). Arterial blood samples were taken during the next 45 min, initially at 15 to 30 sec intervals and later at 1 to 10 min intervals. Plasma aliquots of these samples were assayed for 14C concentration by liquid scintillation counting (Packard Instruments). Blood samples were also drawn at 10 to 15 min intervals for the determination of the plasma glucose level using a glucose analyzer (Beckman Instruments).

After 4 hours of recirculation, the animals were sacrificed with an intravenous administration of potassium chloride solution, and the brains were quickly removed and cut into 4 mm thick coronal blocks. Alternate blocks were immersion-fixed with 10% formalin for 10 days and then processed for histological evaluation by light microscopy. The sections cut from paraffin-embedded blocks were stained alternately with Nissl and hematoxylin-eosin. The remainder of the blocks were frozen in Freon-22 (DuPont), and 20 μm thick sections were cut in a cryostat (American Optical) from each of these blocks for autoradiography. The cut sections were placed on glass coverslips and dried on a hot plate (60°C). The dried sections were then placed on x-ray film (SB-5, Eastman Kodak) together with calibrated 14-C embedded methylmethacrylate standards for 10 days.

Quantitative densitometric analysis of the autoradiograms was performed by using a computerized scanning system composed of a rotating drum scanner (Optronics) and an image processor (Grinnell Systems) interfaced to a digital computer (Digital Equipment Corp.). For the calculation of lCMRgl, the operational equation of Sokoloff et al was modified for changing plasma glucose level by Savaki et al. A lumped constant of normal anesthetized cats (0.411) was used. The values of the rate constants of glucose and deoxyglucose obtained from the rat were used, since the similarity of values between rat and monkey suggests that the rate constants for the cat will also be similar (see Discussion).

**Histological Evaluation**

Histological changes were graded into four categories: histologically normal (Grade 0), slight ischemic changes with only a few scattered affected neurons (eg. shrunken cell bodies with triangular, darkly stained cytoplasm and a loss of discrete Nissl substance) (Grade 1), moderate changes with a large portion of affected neurons (Grade 2), a large portion of affected neurons which are often accompanied by edematous neuropil (Grade 3).

**Reflectofluorometric Measurements**

Cerebrocortical NADH fluorescence and reflectance (sum of the scattered and reflected light) were measured as described previously. Briefly, an area 1 mm in diameter in the middle ectosylvian gyrus, where the diameter of the pial vessels was less than 50 μm, was excited at 366 nm through the internal lens of the Ultropak objective (6.5; Wetzlar, F.R.G.). The emitted NADH fluorescence (450 nm) and the reflected light (sum of scattered and reflected light at 366 nm) were measured by photomultipliers (EMI, England) using appropriate optical filters. To avoid the changes in NADH fluorescence caused by the alterations in tissue blood content, a correction method based on artificial hemodilution was used. For this purpose, 0.1–0.3 ml isosmotic and oxygenated dextran solution was injected into the lingual artery and the NADH concentration-dependent alterations in NADH fluorescence were calculated using the measured correction factor.

Reflected light, measured at 366 nm, was used to...
determine the changes in cerebrocortical vascular volume (CVV), mean transit time of cortical blood flow (tm), and blood flow (CBF). The reflectance base line, measured at the beginning of the experiments during the control period, was regarded as representing 100% CVV. In order to determine the reference value of tm, 0.1–0.3 ml isosmotic and oxygenated dextran solution was injected into the lingual artery. Mean transit time was calculated from the hemodilution-induced reflectance reactions by the area over height analysis. The reference value of tm was regarded as 100%. In order to determine 0% CVV, the blood was washed out from the brain via the lingual artery. 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. Finally, CBF changes were calculated by dividing the percentage values of CVV with the percentage values of tm. Physiological Measurements

Since there is a considerable variability in the severity of ischemia in the MCA occlusion model, experimental animals were classified into 3 groups based on the severity of the depression in electrocortical activity (ECoG) of the ischemic hemisphere at 30 minutes after occlusion. When the ratio of the ECoG mean amplitude of the ischemic side over that of the non-ischemic side was less than 0.2, the animals were considered part of the severe group; when this ratio was between 0.2 and 0.7, the animals were classified as part of the moderate group. Animals in which the EEG ratio was greater than 0.7 were classified as mild and were not included in the study. The 28 animals described in this communication do not include these mildly stroked cats. Previous studies indicate that the NAD/NADH redox state and ICNRgl are different in these three groups during the recovery period after 2 hrs of ischemia (unpublished observations). The GM1/120 group consisted of three moderate and two severe animals while the GM1/30 group consisted of three moderate and six severe. All groups were compared to the corresponding not treated MCA occluded animals (three moderate, two severe) (See table 1).

The pH, pCO2, and pO2 values of the arterial blood samples were determined during the control period, at the 60th min of MCA occlusion (MCAO), and at every hour during the recovery period (BMS3 Mk2 blood gas analyzer; Radiometer, Denmark). At the same time serum glucose concentration was also measured in the arterial blood samples (glucose analyzer, Beckman). The rectal temperature of the animals was maintained at 37 degrees C with an infrared lamp and a temperature regulator (75/A, Yellow Springs). The following variables were recorded on an eight channel Hewlett-Packard polygraph: cerebrocortical uncorrected and corrected NADH fluorescence, reflectance, arterial blood pressure and intracranial pressure (Statham P 23/d electromanometers), electrocorticograms, and tidal CO2 (LB-2, Beckman Instruments).

Results

Physiological Data

As shown in table 2 arterial blood gases and blood pressure were within the normal range and were maintained throughout the study in all groups of animals. There was no statistical difference in these parameters among the sham group, non-treated and GM1-treated stroke groups. However, the plasma glucose level in the GM1/120 group was statistically higher than that in other groups during and after ischemia (p < 0.05). This difference in plasma glucose level cannot be attributed to the GM1 treatment, since it had existed prior to the treatment. Additionally, intracranial pressure from the 180th minute after the release of occlusion was higher in the GM1/120 treated group.

Electroencephalography

In the sham control group, the mean amplitude both of ipsi- and contralateral ECoG were increased during the 6 hour period of observation; the ECoG ratio remained close to unity. There was no difference between the treated and non-treated animals (treatment: 30 mg/kg GM1 iv at 120 min immediately after MCA release) and we therefore used the average of these two groups as the basis of comparison for the MCA occlusion study. The mean amplitude of ipsilateral ECoG as well as the ECoG ratio were similary depressed during MCAO in all stroke groups (fig. 1/A). Within 1 hour after the release of the MCAO, the mean amplitude of the ipsilateral ECoG and the ECoG ratio showed slight but similar increases in both the treated and untreated stroke groups. There was no significant difference between the untreated and treated (either at 30 nor at 120 min) groups. All were significantly different from the sham operated groups. The time of the treatment didn't change the EEG activity and the GM1/30 and GM1/120 severe as well as the moderate cases are
TABLE 2  Control Values in Experimental Groups (mean ± SD)

<table>
<thead>
<tr>
<th>Measured Parameters</th>
<th>Sham Operated</th>
<th>Stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>136.2</td>
<td>128.0</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>16.6</td>
</tr>
<tr>
<td>ICP (mm Hg)</td>
<td>4.0</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>pO2 (mm Hg)</td>
<td>106.7</td>
<td>93.8</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>4.7</td>
</tr>
<tr>
<td>pCO2 (mm Hg)</td>
<td>32.2</td>
<td>30.8</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>2.95</td>
</tr>
<tr>
<td>pH</td>
<td>7.378</td>
<td>7.376</td>
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<tr>
<td></td>
<td>0.009</td>
<td>0.032</td>
</tr>
<tr>
<td>SEgl (mg%)</td>
<td>130.7</td>
<td>109.5</td>
</tr>
<tr>
<td></td>
<td>21.0</td>
<td>19.7</td>
</tr>
</tbody>
</table>

lumped together in figure 1/A. These were not significantly different from the untreated stroke groups. At the end of the recovery period, the ECoG ratio (mean ± SEM) in the untreated and ganglioside treated stroke groups were: Severe not treated: 0.075 ± 0.035; Severe GM1/30, 120: 0.09 ± 0.05; Moderate not treated: 0.54 ± 0.056; Moderate GM1/30, 120: 0.53 ± 0.15.

Reflectofluorometry

The treated and non-treated sham operated animals showed similar changes in cerebrocortical vascular volume, mean transit time of cortical blood flow and cortical blood flow during the experiment. At the end of the MCA occlusion the flow values in the stroked groups were: Severe not treated: 4.9 ± 3.1%, Severe treated GM1/30, 120: 41.06 ± 26.42%, Moderate not treated: 20.96 ± 9.5%, Moderate treated GM1/30, 120: 55.83 ± 41.9% of the preischemic reference values (fig. 1/B). Following the release of MCAO, a significant CBF increase occurred in the ganglioside treated stroke groups, whereas CBF was slightly lower than its preischemic reference value in the untreated stroke groups. During the later phases of recovery, CBF remained approximately at the same high level in the ganglioside treated stroke groups, while the untreated stroke groups slowly recovered to the preischemic reference level (but remained significantly lower than the flow in the sham operated group).

The NAD/NADH redox state changes were similar

FIGURE 1. Changes in ECoG (A), CBF (B) and NAD/NADH redox state (C) in untreated cats and in cats treated with GM1 during the occlusion and the recovery period. Significant differences between the stroked and sham operated animals are indicated for the NAD/NADH data. Differences between the treated and untreated stroked animals are indicated for the CBF and ECoG data. Note that the NAD/NADH in the treated severe group is not significantly different from the sham 2 hours after the release of the occlusion.
The regions analyzed in each animal were grouped into four territories: central MCA territory, peripheral MCA territory, non-MCA territory and cerebellar gray matter. The classification of the first three is based on the blood supply of the MCA.38

There was no significant difference between both hemispheres in each region of the sham group although some regional differences existed. The absolute values of ICMRgl of the sham operated, treated and the non-treated stroked groups are shown in the table 3 while table 4 expresses the ICMRgl data as a percentage of the corresponding sham data in each anatomical structure. For each region an analysis of variance was performed with two factors: treatment (Non treated; GM1/30, GM1/120 treated) and severity (severe, moderate). Regions were divided into two groups, those where an effect of the drug was expected and those where there was no hypothesized effect. This hypothesis was based on the observation that the GM1 treatment didn't affect the EEG, cerebral blood flow and NAD/NADH changes in the sham operated animals, i.e. did not affect the function of the normal cerebral cortex. The left central and peripheral MCA territory (occluded side) were placed into the first group; all the other regions were placed into the second group. A Bonferroni adjustment of the alpha level was done separately for each group. To be significant at the .05 level, an ANOVA for the a priori group had to be significant at the .025 level; for the second group, the alpha level was set at .0083. The only region where either a significant treatment effect or interaction occurred was the left peripheral MCA territory in the moderate group (fig. 3). For this region a significant treatment effect was found (p < .025). Dunnett's t-tests were done to compare the GM1/30 and GM1/120 groups against the controls. A significant difference was found between the control and the GM1/30 group (p < .05), the metabolism being lower in the treated group.

Figure 2 depicts the ICMRgl in the stroked hemisphere in the severe groups. The left (postischemic) hemisphere of both the treated and the non-treated
Table 4  Local Cerebral Metabolic Rate of Glucose (ICMRgl) in Different Territories. All Values are Expressed as the % of the Mean of the Corresponding Sham Untreated Values, ± SEM

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Central MCA</th>
<th>Peripheral MCA</th>
<th>Non MCA</th>
<th>Cerebellar GM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>severe</td>
<td>63.5</td>
<td>88.9</td>
<td>93.2</td>
<td>101.2</td>
</tr>
<tr>
<td>moderate</td>
<td>1.9</td>
<td>5.9</td>
<td>8.4</td>
<td>5.9</td>
</tr>
<tr>
<td>Treated, GM1/30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>severe</td>
<td>123.6</td>
<td>94.3</td>
<td>117.6</td>
<td>100.7</td>
</tr>
<tr>
<td>moderate</td>
<td>11.0</td>
<td>7.5</td>
<td>3.9</td>
<td>6.8</td>
</tr>
<tr>
<td>Treated, GM1/120</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>severe</td>
<td>75.8</td>
<td>74.8</td>
<td>77.9</td>
<td>75.8</td>
</tr>
<tr>
<td>moderate</td>
<td>6.1</td>
<td>12.3</td>
<td>9.2</td>
<td>13.1</td>
</tr>
<tr>
<td>Central</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>91.8</td>
<td>136.3</td>
<td>68.9</td>
<td>84.9</td>
</tr>
<tr>
<td>Right</td>
<td>8.7</td>
<td>14.7</td>
<td>7.5</td>
<td>9.5</td>
</tr>
<tr>
<td>MCA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>64.0</td>
<td>95.9</td>
<td>67.5</td>
<td>87.6</td>
</tr>
<tr>
<td>Right</td>
<td>9.7</td>
<td>1.8</td>
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</tr>
<tr>
<td>Left</td>
<td>103.0</td>
<td>108.9</td>
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</tr>
<tr>
<td>Right</td>
<td>10.9</td>
<td>16.6</td>
<td>8.6</td>
<td>13.0</td>
</tr>
</tbody>
</table>

ICMRgl as a percentage of sham.

result demonstrated a statistically significant depression in the central MCA territory as compared to either the sham value or to the right hemisphere. There was no difference, however, between the treated and the non-treated groups. In the moderate groups, (fig. 3) the left hemisphere of the treated group had a near normal ICMRgl, while the corresponding area of the non-treated group exhibited a statistically significant activation of ICMRgl compared to the control level. There was no statistical difference, however, in either hemisphere between GM1/30 and GM1/120 groups. In addition, for the peripheral MCA territory, a trend towards a treatment by severity interaction was found (p < .10) in the severe group. This trend was explored using the Scheffe procedure. Four simple effects contrasts were examined: 120 min and 30 min treatment groups versus controls within the severe and moderate groups separately. A significant (p < .05) difference was found between the GM1/30 and controls within the moderate group with metabolism being lower in the treated group (fig. 3).

Histology

We followed the same procedure in the statistical evaluation of histological changes as in the evaluation of metabolic data. We also employed a two-tailed Student's t-test for comparing the treated and untreated groups. No significant differences were found between the GM1/30 and GM1/120 groups.
of cerebral glucose metabolism. In the severe group, there was no statistically significant difference in the histological damage in the central MCA territory between the treated and non-treated groups, although the treated group showed a less homogeneous pattern (fig. 4). In the non-treated animals the histological damage was almost always grade 3, while in the treated group some of the tissue was only moderately damaged. A similar pattern was observed in the central and peripheral MCA territories of the moderately stroked animals. In the non-MCA territory and the cerebellar gray matter, no histological damage was observed in either the treated or the non-treated animals.

Discussion

The severity of ischemia in these studies was assessed by the degree of depression of the mean ECoG amplitude of the affected brain cortex and by the ratio of the mean amplitudes of ipsi- and contralateral ECoG (ECoG ratio). We used ECoG amplitude rather than a frequency index, since Shima et al. revealed a better correlation between ECoG amplitude and CBF than between ECoG frequency index and CBF during MCAO. The correlation between ECoG depression and the histological and metabolic parameters was excellent.

The severity of ischemia (before the injection of ganglioside) was identical in the untreated and treated stroke groups (see fig. 1). Although ganglioside treatment increased CBF significantly, the ECoG ratios of the treated and untreated stroke groups during the recovery period were not significantly different. This suggests that GM1 may not have the protective effect against ischemic neuronal damage that has been described for damaged peripheral nerves or hippocampal lesions. It should be noted, however, that the recovery process may not have been followed for a sufficient period of time and that single dose treatment may not have the optimal effect. The beneficial effect of GM1 on hippocampal damage as well as the axonal sprouting effect was found after longlasting treatment with repeated doses. Only two treatment times were chosen as part of this investigation — 30 min and 120 min after the MCA occlusion; these may not have been the optimal times.

The ganglioside treatment in our studies enhanced and extended the reactive hyperemia which, per se, could have induced secondary impairment of microcirculation, increased formation of edema, and, hence, further disintegration of the neurons. Another potentially 'beneficial' agent, nimodipine, is capable of enhancing reactive hyperemia with the potential of worsening neurological outcome of ischemia.

The effect of GM1 on CBF is significant. Furthermore, GM1 does not decrease arterial blood pressure as do other cerebral vasodilators. The increase in CBF is due partly to the ability of GM1 to prevent the constriction of cerebrocortical vessels observed during the initial 120 min of recirculation in the untreated stroke group (fig. 1/B). Since GM1 did not alter the mean arterial blood pressure it can only be assumed that the decrease in mean transit time was primarily due to dilatation of large upstream arterial vessels (some major branches of the MCA or collateral vessels) that indirectly supply the cortical area monitored by the reflectometer. Since GM1 does not increase CBF in sham control animals its CBF enhancing effect may have clinical significance under ischemic conditions, i.e. it may only dilate vessels in the ischemic area and therefore not pose the threat of shunting blood away from the ischemic zone by dilating surrounding normal vessels.

Cerebral glucose metabolism was calculated using the lumped constant for the normal animal, since preliminary data indicate a normal LC during the recovery period following cerebral ischemia. In the ischemic hemisphere, there was a statistically significant difference in ICMRgl between the treated and the non-treated groups in the peripheral MCA territory. The treated animals of the moderate group demonstrated a significant decrease of ICNRgl, whereas the average ICMRgl value of the corresponding non-treated animals was higher than the sham values (fig. 3). Histological findings in this region suggested that there was less damage to the treated (GM1/30) animals in the moderate group than that of the non-treated animals (fig. 4). Previous observations in our laboratory indicate that the brain tissue may have the capability of reducing its metabolic demand during ischemia so as to maintain a normal flow-metabolism couple as well as structural integrity (unpublished data). These data suggest that the depression of ICMRgl accompanied by less severe damage in the treated group may be due to a protective mechanism that has been triggered by GM1. In support of this hypothesis, gangliosides are known to inhibit membrane-bound enzymes such as (Na+, K+) ATPase or K+-dependent nitrophenylphosphatase under certain in vitro conditions, and have also been reported to inhibit endogenous respiration of isolated brain mitochondria.

Such a protective mechanism was not observed in the central MCA territory, due possibly to damage
occurring during the 2 hours of ischemia, without sufficient time to repair any tissue damage. This may also be related to the higher plasma glucose levels in the treated animals: 209 ± 40.7 mg/dl in the treated GM1/120 and 126 ± 13.6 in the nontreated animals, 60 min after occlusion (p < 0.05). High plasma glucose levels have been shown to aggravate ischemic tissue damage.48

A previous histopathological study has shown that ischemic brain tissue may exhibit structural alterations as early as 15 min after MCA occlusion,49 and that these changes mature and progress during the recirculation period.23, 50 It is reasonable, therefore, to assume that GM1 may exert some beneficial effects if it is administered during the early phase of ischemia before tissue damage has become irreversible. Since gangliosides are known to facilitate the recovery of the lesioned central nervous system over a period of 2 weeks or more,10, 12 the effect of GM1 treatment should also be examined with a chronic stroke model.

Although the ganglioside GM1 significantly increases CBF in the recovery period, it does not significantly alter the recovery kinetics of cortical NAD/NADH redox state. In those experiments where the ECoG became isoelectric (severe stroke group) or moderately depressed (ECoG ratio between 0.2–0.7; moderate stroke group) during the MCAO, a transient NADH hyperoxidation and secondary NAD reduction occurred respectively, after recirculation. The pattern didn’t change after the GM1 treatment although the degree of hyperoxidation and reduction diminished in the severe and moderate treated groups respectively. The treated severe group reached its preischemic level 240 min after the MCA release and subsequently did not differ significantly from the sham operated animals, while the untreated severe group remained oxidized even 480 min after the release of the MCA occlusion remaining significantly different from the sham during the entire observation period (fig. 2/C).

A comparable hyperoxidation of NADH, and cytochrome a, a3, following a severe ischemic insult was also observed by Kogure et al51 and Duckrow et al.52 They interpreted the hyperoxidation to be a consequence of an insufficient supply of reducing equivalents (lack of substrates) into the mitochondrial respiratory chain, or an uncoupling of oxidative phosphorylation.

Recent experimental data22, 53, 54 suggest that free radicals are produced during ischemia with a vastly increased production during recirculation (availability of oxygen is increased). Since NADH became spontaneously oxidized in the severe stroke group during the MCAO (CBF was maintained constant at the same low level) and was hyperoxidized upon recirculation, we hypothesize that the direct oxidizing effect of free radicals may have contributed to the oxidation and hyperoxidation of NADH during ischemia and upon recirculation.

The secondary reduction of NADH that occurred in the late recovery phase in the moderate stroke group was not significantly diminished by the ganglioside treatment. This suggests that the secondary NAD reduction does not depend principally on the supply of oxygen since ganglioside treatment doubled CBF. Because the impairment of cortical microcirculation is very heterogenous, even at the microcirculatory level,47, 48 the role of tissue hypoxia in the secondary NADH reduction cannot be excluded even though these measurements were made in a relatively large area, i.e. approximately 1 mm in diameter. We also must consider the possibility that the mitochondrial electron transport in dying cells is shut down and glycolysis and glycogenolysis are stimulated in some of the surviving cells. Cessation of mitochondrial electron transport and increased substrate supply can lead to NADH reduction.35, 55, 56

In summary, these results show that the ganglioside GM1 injected as a single dose at 30 min after MCA occlusion, or at the time of MCAO release, exerts no facilitatory effect on the recovery of the electrical activity of the affected brain cortex. Furthermore, it does not alter significantly the recovery kinetics of cortical NAD/NADH redox state in the recirculatory period following 120 min occlusion of the middle cerebral artery, although the NAD reduction in the moderate and the hyperoxidation in the severe group decreased. GM1 does significantly increase CBF in the ischemic area and produces a decrease of ICVRgI accompanied by less severe histologic damage in the periphery of the ischemic region, especially in the case of early treatment (30 min after the MCA occlusion). These potentially protective effects must be examined further before any beneficial action of this substance during ischemia can be adduced.

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