Mild Hypothermia and Mg++ Protect Against Irreversible Damage During CNS Ischemia

FRANCIS X. VACANTI, M.D., AND ADELBERT AMES III, M.D.

SUMMARY Spinal cord ischemia was produced in rabbits by temporary occlusion of the abdominal aorta just distal to the renal arteries; and recovery, or failure to recover, was assessed by examining the rabbits for permanent loss of sensory and motor function in the hind limbs. A temperature reduction of 3°C during the period of circulatory impairment caused a doubling of the duration of ischemia that could be reversibly sustained. Intravenous administration of 5 mmoles/kg of MgCl₂ before the ischemia (a dose sufficient to produce neuromuscular blockade) caused a 50% increase in the tolerable duration. The combination of the 3°C reduction in temperature and the elevated Mg++ increased by about 3 fold the duration of ischemia that could be sustained before irreversible damage occurred. These results may have implications for the care of patients subjected to marginal degrees of CNS ischemia.

IRREVERSIBLE DAMAGE to CNS is frequently observed clinically following ischemic insults that are only slightly more severe or slightly longer than can be reversibly sustained. Under these circumstances, measures that provide even modest protection may preserve tissue that would otherwise be lost. To be most useful the protective measure should not interfere with natural restorative processes such as the development of collateral circulation, the fibrinolysis of an embolus, or the resorption of edema. Severe hypothermia is the best established protective measure to date.1-3 However, it can be expected to slow markedly the natural restorative processes, and it may therefore offer few advantages under circumstances in which these processes are essential to recovery.

In the present study, we have examined the possible protective effects of small reductions in temperature; and also the possible protective effects of an elevated level of Mg++, administered either alone or in conjunction with the hypothermia. Several interrelated effects of Mg++ recommend it as a possible protective agent. It stabilizes plasma membranes;4 it competes with Ca++ to reduce Ca++ entry into cells;5 it inhibits the release of neurotransmitter;6 it inhibits contraction of smooth muscle and acts as a vasodilator.5

The testing of measures designed to protect against CNS infarction has been notoriously difficult because of the difficulty in creating reproducibly a marginal CNS ischemia in an experimental animal. We used a modification of the rabbit spinal cord infarction model, previously described by Zivin and DeGirolami.7 A snare was placed around the aorta just below the renal arteries; and recovery, or failure to recover sensory or motor function in the hind limbs.

Methods

Experiments were performed on 51 New Zealand White rabbits weighing from 1.2 to 3.0 kg. In a preliminary operation under 1.0-1.5% halothane and 50% nitrous oxide anesthesia, the abdominal aorta was exposed through a midline incision, and a snare made of 14 gauge polyethylene tubing surrounding a nylon ligature was placed around the aorta just distal to the renal arteries. The ends of the snare were brought out through the skin of the back lateral to the vertebral bodies. During the same operation, a 7 cm cylindrical segment of 7 mm O.D. polypropylene, closed at one end, was inserted through the posterolateral portion of the abdominal wall to permit the introduction of an electronic temperature probe, and a cystostomy tube was placed in the bladder. The abdominal incision was closed in 2 layers. The animals were then allowed 24 or 48 hr of recovery before being subjected to a specified period of aortic occlusion.

In preparation for the aortic occlusion, the rabbits were temporarily anesthetized with 1.0-1.5% halothane and 50% nitrous oxide (the only exceptions being 5 rabbits that were made ischemic at 38.0–38.9°C and that were not treated with Mg++). This made it easier to reduce core temperature by reducing ambient temperature. It also permitted endotracheal intubation and facilitated the introduction of recording devices. The animals were allowed to emerge from the halothane and nitrous oxide anesthesia before being subjected to the ischemia. Some animals received MgCl₂, 5 mmoles/kg, administered as a 40% (w/v) solution of MgCl₂·6H₂O in Ringer’s solution containing 28 mM lactate. This was delivered over a 30 to 60 min period by intravenous infusion (2.5 ml/kg) just prior to the ischemia. The Mg++ caused a generalized neuromuscular blockade requiring artificial respiration, but did not seriously affect the electrocardiogram. Ventilation was set, as determined by preliminary studies, to result in the rapid resumption of spontaneous ventilation upon discontinuation of controlled respiration in the rabbit. In agreement with previous reports,5 the Mg++ also caused a drop in arterial pressure as monitored via a cannula in the central artery of the rabbit’s ear. Pre-
liminary studies showed that the arterial pressure could be restored by the intravenous infusion of 30 ml/kg of the polysaccharide plasma expander, Hesperan®, so the animals receiving Mg++ were also given 30 ml/kg of Hesperan, administered over a 5 to 10 min period just prior to the aortic occlusion. (The only exceptions to this were 7 Mg++ treated rabbits subjected to 30 min of ischemia that were given 80 ml/kg of lactated Ringer’s solution instead of 30 ml/kg of Hesperan as a volume expander.) In order to control for possible effects of hemodilution on recovery from the circulatory arrest, the animals not treated with Mg++ were given the same dose of Hesperan as the Mg++ treated animals. The core temperature of the animals, which had been adjusted as described above by modifying ambient temperature, was monitored throughout the period of aortic occlusion by the electronic probe whose tip was located in the abdominal peritoneal cavity. At the end of the specified period of ischemia, the snare was removed from the aorta and the animals were allowed 24 hr to recover before being examined for irreversible damage.

The extent of residual damage following the ischemic episode was assessed by neurological testing of spinal cord function performed after 24 hr of recovery, the 24 hr period being selected on the basis of preliminary studies that showed no significant further improvement after this time. The rabbits were rated as having a good functional recovery or as having a poor functional recovery. The animals in the poor recovery group had deficits ranging from complete loss of sensory and motor function in the hind extremities to less complete deficits consisting of areas of diminished sensation and inability to stand and ambulate normally. Animals judged to be in the good recovery group had little or no apparent deficits.

There were 21 rabbits studied without an elevated Mg++ level and 30 rabbits with an elevated Mg++ level. Of the rabbits without an elevated Mg++ level, 7 were subjected to 12.5 minutes of ischemia at a core temperature ranging from 37.0°C to 38.9°C, and 14 rabbits were subjected to 25 minutes of ischemia over the temperature range of 34.0°C to 36.8°C. Of the Mg++ treated rabbits, there were 3 rabbits at 12.5 min of ischemia, 6 at 20 minutes, 10 at 25 minutes, 7 at 30 minutes, and 4 at 35 minutes. Temperatures ranged from 32.8°C to 39.0°C.

The various groups of rabbits were statistically analyzed using the Fisher exact test or by the test for a linear trend in proportions.

**Results**

Figure 1 shows data for 21 untreated rabbits, 7 subjected to 12.5 min of spinal cord ischemia at temperatures from 37.0 to 38.9°C and 14 subjected to 25 min of ischemia at temperatures from 34.0 to 36.8°C. It is evident that for each duration of ischemia the cooler animals showed uniformly good recovery (as indicated by the closed circles) whereas the warmer animals generally showed poor recovery (as indicated by the open circles). The critical temperatures, separating good recovery from poor for each duration of ischemia, are indicated by the asterisks. These points were calculated, by the Fisher exact test, as having the highest probability of dividing the rabbits with good recoveries from those with poor recoveries, as determined by Fisher exact test analysis of all possible intervals.

The rabbits that had been pretreated with Mg++ were similar to the untreated animals with respect to the effect of temperature on the reversibility of the ischemic insult (fig. 2). At each of the 5 durations of ischemia tested the cooler animals recovered better than the warmer animals, as demonstrated in Fig. 2 by the fact that the filled symbols (with only 2 exceptions among the 30 rabbits) fall beneath the open symbols. The critical temperatures, calculated for each of the 5

*The data were analyzed by dividing the animals into groups corresponding to one degree temperature intervals (34.0-34.9; 35.0-35.9; 36.0-36.9°C) and evaluating the numbers of good vs. poor recoveries in each group according to the test for a linear trend in proportions. This analysis yielded a Z of 2.12 (p < 0.05).
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FIGURE 2. Effect of temperature on recovery from ischemia of rabbits given an intravenous infusion of 5 mmole/kg of MgCl₂ prior to the ischemic insult. Otherwise like figure 1.

The critical temperature data for both the untreated and the Mg++ treated animals are presented together in figure 3. The data for Mg++ treated rabbits suggest that there was a fairly linear relationship between temperature and duration of ischemia tolerated over the 4°C range studied (from 38.5 to 34.5°C). Regression analysis of the data for critical temperatures generated the solid line shown in figure 3 and showed that there was significant (p < 0.01) negative correlation between the temperature and the duration of ischemia that could be survived. Over the range studied, each 1°C reduction in temperature increased the survivable duration by 5.5 min. Since only 2 durations of ischemia were studied in the rabbits not treated with Mg++, regression analysis of the data was not possible. The similarity of the slopes of the 2 lines in figure 3 indicates that there was a similar response to temperature under the two conditions. The displacement of the data for the Mg++ treated animals to the right and upwards indicates that, at a given temperature, the Mg++ treated animals had a longer survival time; or that, for a given duration, the treated animals could tolerate a higher temperature. The protective effect of Mg++ was statistically significant. There were 7 animals that were subjected to 12.5 min of ischemia at temperatures that were higher than the critical temperature for untreated animals (37.5°C) and lower than the critical temperature for Mg++ treated animals (38.9°C). Of these, all of the untreated animals (5) exhibited poor recovery and all of the treated animals (2) exhibited good recovery (p < 0.05).

Discussion

The procedure of Zivin and DeGirolami for producing ischemia of the spinal cord of the rabbit was modified somewhat for the present experiments. A preliminary operation was performed to position the aortic snare, and the rabbit was then allowed to recover from the surgery before occluding the aorta. This made it possible to examine the response to ischemia with the animal free of anesthesia and with the blood pressure and core temperature well stabilized. Provision was also made for recording core temperature during the period of ischemia — clearly an important feature of the present preparation.

The rabbit spinal cord preparation, as used in these experiments, adequately satisfies a number of criteria for an experimental model of ischemia that have often been difficult to meet: 1) The investigator is able easily to introduce a period of ischemia of precisely predetermined duration. 2) The region of CNS affected is not critical for the animal's physiological homeostasis, which is therefore well maintained during and after the ischemia, even if the ischemia leads to edema and infarction. 3) The response to the ischemia is readily monitored by neurological examination of the lower limbs, which has been shown to correlate well with the extent of infarction as assessed histologically. 4) The experimentally induced reduction in CNS blood flow appears to be quite reproducible, owing apparently to a reproducible pattern of the principal vessels and collaterals in this region. This last feature is of particular importance. It presumably accounts for the reproducibility of the responses to the vascular occlusion (as indicated by the symbols in figures 1 and 2), which was sufficient to permit detection of rather small effects of experimental variables without using large numbers of animals.

Both the untreated and the Mg++ treated animals demonstrated a considerable degree of protection from small reductions in temperature during the period of

FIGURE 3. Data for critical temperatures reproduced from figures 1 and 2 to compare recovery of untreated rabbits (.....) with recovery of Mg++ treated rabbits (-----).
ischemia. These results emphasize the importance of controlling or, at least of carefully monitoring the temperature of the CNS at risk during experiments on ischemia. This is particularly true since many types of intervention that have been tested for possible protection against ischemia may also facilitate the lowering of body temperature; e.g., anesthetics, Ca++ antagonists that cause peripheral vascular dilatation, various neurotransmitter antagonists.

The effects of temperature reported here may have clinical implications. They provide further evidence of the hazard that even a small amount of fever presents to the patient with marginal circulation to some portion of the CNS. They also suggest that a modest degree of hypothermia may provide useful protection under circumstances in which a temporary reduction in circulation can be anticipated, as in the surgical repair of intracranial aneurysms and arterio-venous malformations and in the repair of an abdominal aortic aneurysm. The latter procedure duplicates closely the conditions imposed by the present experiments, and it is associated with a 0.2–2% risk of spinal cord infarction. A question of considerable clinical importance is whether a continued, modest reduction in temperature would provide long range protection under circumstances in which the circulation is only slightly less than would be tolerable at normal temperature. The latter situation presumably pertains at the margin of the tissue affected by a stroke, as well as in other clinical situations. Evidence is accumulating that the development of an infarct depends, over a wide range, on both the extent to which the blood flow is reduced and the duration of the reduction. It therefore seems quite possible that a protective measure (such as hypothermia), which extends survivability during marked hypoperfusion by 10 or 20 min, might extend survivability indefinitely during mild hypoperfusion. If this were the case, and if the protective measure did not interfere with natural recuperative mechanisms such as collateral enlargement or clot lysis, it might play a useful role in our clinical armamentarium.

The protective effects of Mg++ were additive to those of hypothermia, so that the combination extended the survivable period of ischemia by almost 3 fold. An additional advantage of the combination was the ease with which hypothermia could be introduced in the presence of increased Mg++. The mechanism of action of the elevated Mg++ is unclear and may be quite complex. Elevations in plasma Mg++ have been shown to be associated with elevations in CSF Mg++,

but of lesser degree. It is not known to what extent an increase in plasma Mg++ is reflected by increases in the level of Mg++ at other sites such as the walls of CNS vessels or the interstitial fluid of the brain parenchyma. Possible mechanisms of action include reduction of neurotransmitter release; blockade of other Ca++ mediated functions (such as vascular contractions); or its ability to act as a Ca++ channel blocker to prevent an ischemia-induced fall in extracellular Ca++. References

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F X Vacanti and A Ames, 3rd

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