Protection against spinal cord ischemia with insulin-induced hypoglycemia

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The effect of insulin-induced reduction in blood glucose to 65 ± 20 mg/dl (mean ± standard deviation) on recovery of electrophysiological function and extracellular lactate concentration was studied in a rabbit model of spinal cord ischemia. These results were compared to findings in animals with spinal cord ischemia that either were fasted overnight (fasted group: blood glucose 97 ± 26 mg/dl) or had no pretreatment (control group: blood glucose 172 ± 65 mg/dl). The aorta was occluded until the postsynaptic waves of the spinal somatosensory evoked potentials (SSEP's) had been absent for 20 minutes, a period of ischemia that produces paraplegia in 100% of untreated rabbits. The total aortic occlusion time was not significantly different in the three groups. Recovery of the SSEP's was significantly better in the insulin-treated animals than in the fasted or control animals. The N3 wave of the SSEP's, which has been found to correlate best with neurological recovery, returned to 65% ± 48% of the preischemia amplitude in the insulin-treated animals, compared to 40% ± 34% in the fasted group and 26% ± 24% in the control animals. Extracellular lactate concentration in the spinal cord increased immediately after occlusion of the aorta, reached a plateau as the postsynaptic waves disappeared from the SSEP's, and then increased a second time during the first 15 minutes of reperfusion. The peak lactate concentration during ischemia and during reperfusion correlated with the preischemia glucose concentration (r = 0.60336 and r = 0.76930, respectively). Lactate concentration in the spinal cord was higher during ischemia and throughout the first 2 hours of reperfusion in the control and fasted animals than in the insulin-treated animals. During the 2nd hour of reperfusion, lactate concentration was significantly higher in the control animals than in the fasted animals. Reduction in blood glucose with insulin improves recovery of electrophysiological function after spinal cord ischemia, probably because of reduced lactic acid production, especially during the early reperfusion period.

KEY WORDS - spinal cord · ischemia · hypoglycemia · lactic acid · rabbit

THE type and amount of energy substrates available to the central nervous system can alter the neurological sequelae of ischemia. Provision of excess glucose can temporarily compensate for oxygen deprivation. The duration of ischemia prior to potassium efflux from neurons is proportional to the blood glucose concentration at the onset of ischemia. The delay of potassium efflux and of membrane depolarization provided by hyperglycemia is due to the energy obtained from the anaerobic metabolism of glucose. However, the additional ischemia time gained with hyperglycemia is relatively short and is achieved at the expense of developing a more severe lactic acidosis. The late neurological sequelae of cerebral ischemia are consistently worse when the blood glucose level is elevated during ischemia, suggesting that lactic acidosis or other metabolic consequences of glucose metabolism damages nervous tissue.3,8,10,12

It therefore appears that reduction of blood glucose levels during ischemia might afford neuronal protection. Blocking glucose metabolism with 2-deoxyglucose in rats has reduced mortality and seizures after cerebral ischemia; however, insulin-induced reduction of blood glucose concentration to 20 mg/dl has not been protective during cerebral ischemia.4,12 Despite this negative observation, reduction of glucose concentration to 60 mg/dl has been shown to preserve in vitro measurements of mitochondrial function during cerebral ischemia.13 The protective effect of a moderate reduction in glucose concentration on recovery of neurological function has not been studied in detail.

The purpose of the present study was to compare the effects of two levels of moderate hypoglycemia, produced by insulin infusion and by fasting, on the recovery of neurological function after spinal ischemia.

Materials and Methods

Surgical Preparation

New Zealand albino rabbits, each weighing 3 to 5 kg, were anesthetized with intravenous methohexital so-
dium (Brevital, up to 10 mg/kg total dose) during the surgical preparation. Anesthesia was maintained with intramuscular ketamine (44 mg/kg followed by 25 mg/kg/hr) for the duration of the study. At least 90 minutes elapsed between the last dose of methohexital and the induction of ischemia.

All rabbits were intubated and ventilated to maintain normal arterial blood gas levels on a volume-cycled ventilator, with oxygen supplement if necessary. Arterial blood gas levels were monitored periodically on a blood gas analyzer.* Whole-blood glucose concentrations were measured with a YSI analyzer prior to occlusion of the aorta.

For monitoring spinal somatosensory evoked potentials (SSEP's), a bipolar cuff stimulating electrode was placed on the left sciatic nerve, and three needle recording electrodes† were inserted along the spinous processes of the L-4, L-5, and L-6 vertebrae, with the tips of the electrodes resting on the laminae. The animals were paralyzed with pancuronium bromide (Pavulon) during the SSEP recordings to avoid obtaining a muscle twitch artifact from the sciatic nerve stimulation. The sciatic nerve was stimulated with square-wave pulses of 0.1-msec duration and 0.5-mA intensity delivered at 3.1 Hz. The SSEP's were recorded in a bipolar fashion from the L-6 to the L-5 and from the L-5 to the L-4 vertebral bodies, averaging 50 repetitions. The SSEP's were displayed so that a negative potential at L-6 with respect to L-5 was an upgoing deflection. Amplitudes of the individual SSEP component waves were measured from the preshock baseline.

For measuring extracellular lactate concentration in the spinal cord, a dialysis probe was constructed as described by Ungerstedt of a loop of dialysis tubing, 0.2 mm in diameter, with a molecular weight cut-off of 6000. This was inserted into the spinal cord through a 0.5-cm diameter laminotomy of the L-5 vertebra. The probe was positioned so that the permeable segment passed through the entire cross section of the spinal cord just to the right of the midline. The probe was perfused with buffered Ringer's solution at a rate of 10

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* Blood gas analyzer, Model 165/2, manufactured by Corning Medical Instruments, Medfield, Massachusetts.
† Analyzer, Model 23A, manufactured by Yellow Springs Instrument Co., Yellow Springs, Ohio.
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### Experimental Protocol

The experimental protocol is depicted in Fig. 1. After surgical preparation and drug pretreatment as described above, baseline SSEP’s, spinal cord lactate concentration, blood glucose levels, and systemic physiological parameters were obtained. The Swan-Ganz catheter balloon was then inflated to occlude the abdominal aorta. The SSEP’s were recorded at 1- to 2-minute intervals until the postsynaptic waves of the SSEP’s were no longer evoked. The balloon of the catheter was then kept inflated until the postsynaptic SSEP waves had been absent for 20 minutes. When the catheter balloon was deflated, the systemic acidosis was corrected with intravenous sodium bicarbonate, the amount determined by arterial blood gas measurements. The SSEP’s were recorded at 15- to 30-minute intervals for 2 hours after deflation of the balloon so as to monitor the recovery of the SSEP waves. The final SSEP recording at 2 hours after reperfusion was compared to the preischemia recording to estimate functional neurological recovery. In previous studies, recovery of the amplitude of the N1 wave to less than 70% of the preischemia amplitude uniformly predicted an abnormal motor neurological examination at 48 hours after the ischemia.

### Results

#### Systemic Physiological Parameters

Preischemia rectal temperature and arterial blood gas levels were not significantly different in the experimental and control groups (Table 1). Systemic blood pressure was slightly higher in the control group than in the fasted animals. The preischemia blood glucose level was 172 ± 65 mg/dl in the control group, 97 ± 26 mg/dl in the fasted group, and 65 ± 20 mg/dl in the insulin-treated group.

The systemic response to inflation of the balloon in the abdominal aorta was similar in all groups. An abrupt increase in arterial blood pressure developed above the balloon, and the pressure fell to and remained near zero below the balloon. The elevated blood pressure above the balloon gradually returned toward normal. When the balloon was deflated, the systolic arterial pressure abruptly fell, only gradually returning to normal, and a systemic metabolic acidosis developed. There was no difference in any of the three groups in the lowest systolic blood pressure during reperfusion, in the time required for the blood pressure to recover to 90 mm Hg, or in the amount of bicarbonate required to correct the systemic acidosis (Table 2).

#### SSEP’s During Ischemia and Reperfusion

The SSEP’s of the rabbit have been described in detail in previous publications. In these studies, the...
SSEP’s recorded from L-6 to L-5 and from L-5 to L-4 normally consisted of an initial positive wave, four negative waves, and a late long-duration positive wave (Fig. 1). The first two negative waves, N1 and N2, were present even at low stimulus intensities and remained when stimulus frequencies were high, suggesting a presynaptic origin. The N1 and N2 waves were relatively resistant to ischemia and frequently returned to an almost normal appearance during reperfusion, even in the animals that became paralyzed. In contrast, the late negative waves, N3 and N4, and the late positive wave, P2, were not maximally evoked unless stimulus intensity was at least 0.5 mA; they rapidly attenuated at stimulus frequencies greater than 14 Hz, consistent with a postsynaptic origin. The N1 and N2 waves were generated in the dorsal interneurons of the lumbosacral cord, and P2 is probably extracellularly recorded field potential generated by hyperpolarizing inhibitory postsynaptic potentials. These late waves were quite sensitive to ischemia, and recovered to a variable degree during the reperfusion period. The degree to which the N3, N4, and P2 waves recovered by 2 hours after reperfusion predicted the neurological recovery at 48 hours, and correlated with the size of ischemic injury estimated by histological examination.1,11

In the present study, the SSEP’s were not affected by anesthesia and were resistant to reductions in blood glucose levels, even to as low as 25 mg/dl. The doses of insulin given to the treated group did not alter the amplitudes or latencies of the SSEP waves.

After inflation of the catheter balloon, the SSEP waves decreased in amplitude and finally disappeared in the following order of sensitivity: P2, N4, N3, N2, and then N1. The time required for the postsynaptic SSEP waves to fail varied from animal to animal, and tended to be shorter in the insulin-treated group (Fig. 2), although the difference was not significant. The N1 wave disappeared from the SSEP at 8.4 ± 6.1 minutes, 8.7 ± 4.9 minutes in the fasted animals, and 5.9 ± 3.8 minutes after inflation of the catheter balloon in the control, fasted, and insulin-treated groups, respectively.

The length of time that the aortic balloon was kept inflated after failure of the postsynaptic SSEP waves was maintained at 20 minutes in each animal. In previous studies, this duration of aortic occlusion has resulted in paraplegia in 100% of control animals.13 When blood flow was restored by deflating the aortic catheter balloon, the SSEP waves recovered to a variable degree, typically in the reverse order of their disappearance. The recovery of the SSEP waves at 2 hours after reperfusion, which in previous studies predicted the extent of neurological recovery at 48 hours, is summarized in Table 3 and shown in detail in Fig. 3. Recovery of the N2, N3, and N4 waves was significantly better in the insulin-treated animals than in the fasted or control animals. Five animals in each group had no SSEP recovery; however, of those remaining animals whose spinal cord recovered some ability to generate evoked potentials, the recovery was near normal in the insulin-treated animals but poor in the control group. Recovery of the SSEP’s was intermediate in the fasted animals. The best SSEP recovery (> 90% of the control amplitude) occurred when the preischemia blood glucose
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level was 55 to 100 mg/dl, regardless of the experimental group.

**Spinal Cord Lactate Concentration**

Lactate concentration in the spinal cord dialysate increased immediately after inflation of the balloon in the aorta, and tended to plateau or even decrease slightly after the postsynaptic SSEP waves could not be evoked (Fig. 4). After the balloon was deflated, lactate concentration increased again to even higher levels than were recorded during aortic occlusion. This second peak in lactate concentration occurred at about 15 minutes after reperfusion and gradually subsided during the 2nd hour following reperfusion. The highest dialysate lactate concentration during ischemia and the peak lactate level during early reperfusion were closely related to the arterial glucose concentration (r = 0.60336 and r = 0.76930, respectively), but not to the reperfusion blood pressure, or to the duration of hypotension after deflation of the balloon, or to systemic lactic acidosis. During both ischemia and reperfusion the mean lactate concentration was higher in the control group than in the insulin-treated or fasted groups, and during the 2nd hour of reperfusion it was higher in the fasted animals than in the insulin-treated group.

**Discussion**

There is evidence from models of cerebral ischemia that the adverse effect of posts ischemic lactic acidosis, and possibly other metabolic products, exceeds the beneficial effect of additional energy provided by the anaerobic metabolism of glucose during ischemia. The present study extends these observations to the spinal cord. The protective effect of reduction in blood glucose levels was smaller than that obtained in previous studies in which pretreatment with hypothermia and thiopental was used. In those studies, all animals recovered normal neurological function and the N3 wave of the SSEP recovered to 90% of the preischemia amplitude after a similar duration of ischemia.

In the present study, a small number of animals in each group developed an irreversible ischemic injury, without recovery of the postsynaptic waves after reperfusion. The number of these animals (five in each group) was not altered by the treatment to reduce blood glucose concentration. However, in the animals that regained the ability to generate an evoked potential, the ultimate recovery of the postsynaptic waves was inversely related to the preischemia blood glucose concentration.

A consistent finding in this model of ischemia was that higher lactic acid concentrations were recorded in the spinal cord during the early reperfusion period than during ischemia. The peak spinal cord lactate concentration occurred during the time that the systemic circulation was recovering from the aortic occlusion, and may have been due in part to hypoperfusion of the spinal cord secondary to the systemic hypotension. However, it was not possible to demonstrate a relationship between the peak lactate concentration in the spinal cord and systemic hypotension. A similar increase in lactic acid concentration during reperfusion has been reported in other models of central nervous system ischemia, in which systemic hypotension is not a factor. It seems more likely that aerobic metabolism requires some time to recover after a period of ischemia, and that the lactic acidosis during reperfusion is due to the increased availability of glucose. The peak lactic acid concentration during reperfusion correlated even more closely with the blood glucose concentration than did the lactic acid concentration during the ischemia period.

**Fig. 3.** Recovery of the individual spinal somatosensory evoked potential (SSEP) waves at 120 minutes after reperfusion, expressed as a percentage of their control amplitude.

**Fig. 4.** Lactate concentration in the spinal cord dialysate (μmol/ml) during ischemia and the first 2 hours of reperfusion in the three experimental groups. * = different from control group, and + = different from fasted group (p < 0.05). SSEP = spinal somatosensory evoked potentials.
As indicated above, a correlation between lactic acid production and neurological injury does not prove that lactic acid is the causative agent; other metabolic products may be involved. Similar caution must be expressed about ascribing the effect of hypoglycemia only to hypoglycemia per se. It is possible that insulin, which has a variety of metabolic effects such as increasing the cellular influx of potassium, might exert protective effects independent of its hypoglycemic action.

In the present study, neurological recovery was measured by short-term recovery of electrophysiological parameters. Past experience with this model has shown that neurological function is accurately predicted by recovery of SSEP waves N3, N4, and P2, which are generated locally in the gray matter of the lumbosacral spinal cord. However, only studies with long-term testing of motor and sensory function will indicate whether a moderate reduction of blood glucose levels with insulin might have application in protection against ischemia.

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References


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