Neuroprotection of Adult Human Neurons Against Ischemia by Hypothermia and Alkalinization

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Ischemia of intact dorsal root ganglia (DRG) in situ leads to massive neuron death due to ischemia-triggered secondary events, such as massive release of excitatory amino acids from the neurons, their excessive accumulation and activation of neuron NMDA and other receptors, acidification, and loss of calcium homeostasis. The present experiments tested whether hypothermia and alkalinization, separately or combined, provide neuroprotection against 1-4 hours of ischemia to the neurons within intact DRG acutely removed from organ donors. DRG under hypothermic (20-15°C) or alkaline (pH 8.0 - 9.3) conditions yielded more viable neurons than DRG maintained under physiological conditions (37°C/pH 7.4), 4.1-fold vs. 7.8-fold respectively, but, hypothermia and alkalinization combined (20°C/pH 9.3) increased the yield of viable neurons 26-fold compared to DRG maintained under physiological conditions. These results show that combined hypothermia and alkalinization provide adult human DRG neurons significant neuroprotection against ischemia, and ischemia-induced causes of neuron death.

Key words: Anoxia, Glutamate toxicity, Neurotoxicity, Neurotrauma, Trauma

CNS trauma can lead to ischemia and ischemia-induced secondary events, such as acidification, peroxidation, and loss of calcium homeostasis, excessive accumulation of amino acids, and their activation of NMDA receptors that exacerbate neuronal injury, neuronal death and neurological losses. To minimize trauma-induced neurological losses requires minimizing the number of neurons killed post trauma.

Electrophysiological and other studies on adult rat dorsal root ganglion (DRG) neurons indicate that small changes in their cellular environment (predominantly increases in temperature and decreases in pH), result in dramatic changes in membrane properties that damage and kill neurons (2, 22). Neuron death is also triggered by excitotoxins (1), and trauma-induced inflammation (31, 32), while another major cause of neuron death in the injured spinal cord is massive calcium influx and the disruption of neuron calcium homeostasis (3, 18, 40). Whole body hypothermia (5, 38), and increasing pH, provide neuroprotection to adult rat CNS neurons against infarct-induce ischemia (6). However, clinically there are few data on how to provide neuroprotection against ischemia and secondary causes of neuron death related to trauma.

Clinically whole body hypothermia provides limited neuroprotection, probably because it requires interactions with additional environmental conditions. The temperature that provides neuroprotection varies from mild (33-35°C) (25, 26) to moderate (30-32°C) (25, 41), severe (27-29°C) (25), and extreme (20°C) (35). However, localized hypothermia (32°C) provides good neuroprotection during periods of compromised cerebral blood flow and oxygen delivery (29, 39).

In addition to hypothermia, alkalinization provides neuroprotection against ischemia (16). Alkalinization to pH 8.2 protects adult rat CNS neurons against ischemic effects of infarct (6), and mouse neocortical neurons in primary culture from azide-induced chemical anoxia (17).

It has also been demonstrated that neuroprotection by one mechanism is enhanced when combined with another. The neuroprotection provided by regional hypothermia against 45 minutes of ischemia is increased by the simultaneous infusion with adenosine (14, 21, 29, 30). Adenosine appears to provide neuroprotection by suppressing the neurotoxic GABA-activated current (IGADA) in a majority of the neurons (77%) (15). Similarly, regional hypothermia together with the simultaneous

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infusion of a NMDA receptor antagonist enhances the neuroprotection provided by hypothermia (21). Recently it was reported that hypothermia and alkalinization combined provided greater neuroprotection to ischemic adult rat DRG neurons than either one alone (4).

Trauma-induced secondary insults that cause neuron death include ischemia, lipid peroxidation (23), inflammation (31, 32), and the excessive release of extracellular excitatory amino acids (EAAs) (8-10, 20, 24, 33, 34, 36). The accumulation of EAAs leads to toxic extracellular acidification and toxicity due to the excessive stimulation of a NMDA subtype of glutamate receptor (36), which in turn allows the massive influx of extracellular calcium (9), that disrupts the calcium homeostasis of neurons (3, 18). NMDA-receptor-mediated toxicity is dependent on the influx of extracellular calcium (37). The released glutamate stimulates its own further glutamate release in a positive feedback loop by its interaction with non-NMDA receptor subtypes (36). Calcium-induced calcium release, and a further influx of calcium through voltage-gated calcium channels after glutamate-induced depolarization, contributes to glutamate toxicity.

The tissue and connective tissue coat surrounding isolated intact DRG cause the large and densely packed neurons to become ischemic. DRG neurons contain and release excitatory amino acids, such as glutamate and aspartate (9, 10, 33, 35, 36), and possess receptors to these amino acids (36). Therefore, the neurons within intact DRG should be exposed to all the ischemia-induced secondary causes of neuron death. This makes them a good model on which to study mechanisms for providing protection against ischemia to neurons in situ under conditions that mimic those of DRG and spinal cord neurons following trauma.

Combined hypothermia and alkalinization provided greater neuroprotection to ischemic adult rat DRG neurons within intact DRG than either one alone and when compared to physiological conditions (4). However, the conditions that provide neuroprotection against ischemia for adult human DRG neurons are unknown. The present studies are the first to examine the influences of low temperature and high pH on the viability of adult human DRG subjected to prolonged ischemia.

Experimental Procedures

The methods used are the same as described earlier (35). Briefly, DRG were removed from organ donors (all male) within 1 hour of clamping the aorta. To reduce potential variability in the results we used only cervical DRG. In a process taking about ½ hour, the DRG were isolated together with the surrounding tissue and with their connective tissue capsule intact. When isolated, each DRG was placed on a sterile cloth at room temperature (18°C) in ambient air together with its twin DRG from the same spinal cord level. When all the required DRG were isolated, each pair was placed in a 50 ml tube containing DMEM + F15 (50/50) culture medium (Sigma Chemical, St. Louis), without serum, on ice (4°C). The DRG were taken to the laboratory, which took about ½ hour. Once in the laboratory, one DRG from each pair was placed in hypothermic and alkaline culture medium (20°C/pH 9.3), and its twin was placed in medium at different combinations of hypothermia and alkalinization.

The pH of the medium was adjusted to 8.3 and 9.3 with HEPES buffer. DRG at 37°C were placed in an ambient air incubator, DRG at 20°C were left at room temperatures, and DRG at 15°C were placed on a temperature-controlled stage. Thus, all the DRG received identical treatments, except for the time they were under the different combinations of temperature and pH.

After the specified time under the different combinations of temperature and pH, each DRG was placed in a separate dish containing culture medium at pH 7.2 and 20°C. At that time, the surrounding tissue and connective tissue capsule were removed and the DRG cut into small pieces with irredectomy scissors. All the pieces of a DRG were placed in a siliconized watch glass (Sigma Coat, Sigma Chemical, St. Louis) in culture medium containing the enzymes collagenase P (5 mg/ml), neutral dispase II (8 mg/ml), and DNase (0.3 mg/ml) (Roche Diagnostics, Indianapolis, IN), and the dishes placed in an O_2/CO_2 incubator at 37°C. Every 15 minutes the pieces of DRG were gently triturated 5 times through a 1-ml plastic pipette tip with its end cut to an inner diameter just larger than the pieces of DRG. Complete DRG dissociation was achieved in 1½ hour. Within 1 minute of the watch glass dishes sitting undisturbed, the neurons settled to the bottom of the dish while the cellular debris remained suspended in the medium. The enzymes and cellular debris were removed by five washes with fresh culture medium (pH 7.4).

Cleaned glass cover slips (24x24 mm, Gold Seal, Clay Adams, Lincolnshire, IL) were placed in 35 mm diameter Falcon tissue culture dishes. The cover slips were treated with poly-l-lysine (1 mg/ml) (Sigma Chemical, St. Louis) for 1 hour, washed 2 times with distilled water. They were then treated with laminin (5 mg/ml) (Sigma Chemical, St. Louis) for 1 hour, washed 3 times with normal culture medium, placed in a 35 mm tissue culture dish, and 1½ ml of fresh culture medium was added to each dish.

All neurons from each dissociated DRG were plated on a cover slip using a siliconized micropipette, with a tip with an inner diameter of about 100 im and attached to a mouth sucker. To protect the person plating the neurons from aspirating the neurons or the culture medium, a 0.2

um filter was placed in the line between the glass pipette and the mouthpiece. Viable neurons adhered immediately to the poly-l-lysine/laminin coated cover slips. After 15 minutes heat activated human serum (Sigma Chemical, St. Louis) was added to the medium to a final concentration of 10%. Addition of serum prior to plating the neurons prevented them from adhering to the substrate of the dish. The cultures were placed in an O_2/CO_2 incubator (95/5%) at 37°C, in a saturated water environment. The neurons were cultured without any neurotrophic or other factors.

Assay of viable neurons

The yield of viable neurons was counted 18 hours after plating the neurons. This time point was selected because it was the same at which we analyzed the yield of viable neurons from intact adult rat DRG in similar experiments (4). By using this time point we could compare the data on neuroprotection from the adult rat with those from the human DRG neuron experiments.

Neuron viability was assessed using the Trypan blue dye exclusion test. Trypan blue was added to the culture medium to a final concentration of 0.4%. After 20 minutes, the Trypan blue was washed out and the number of viable unstained neurons counted.

Data Analysis

The data were analyzed using Two-Way Mixed-Design ANOVA.

Analysis of the number of viable neurons from different conditions

DRG at different levels of spinal cord contain different numbers of neurons, while paired DRG from the same level contain the same number of neurons. Therefore, to obtain a quantitative comparison of the extent of neuroprotection provided by the different conditions of temperature and pH, we could only compare the yields of viable neurons from paired DRG from the same spinal cord level.

Preliminary data indicated that hypothermic and alkaline culture medium (20°C/pH 9.3) provided the greatest extent of neuroprotection. Therefore, all the data were normalized against the yield of viable neurons under these most neuroprotective condition.

In a second series of experiments, we examined the differences in the yield of viable neurons from DRG maintained for up to 4 hours at 37°C/pH 7.4 and 20°C/pH 9.3. For these experiments, one DRG of each pair was dissociated and the neurons plated at the time its twin was placed intact in culture medium under the different conditions of temperature and pH where it remained for a specified length of time before being dissociated and the neurons plated. The data for each pair were normalized against the number of viable neurons from the twin that was dissociated immediately. This number represents their largest possible yield of neurons from that pair of DRG without the DRG undergoing any further ischemia.

Due to the limited number of donors from whom to harvest DRG, it was not possible to compare data from several DRG maintained for same length of time under the different conditions of temperature and pH. Therefore, DRG were maintained under the specific conditions of temperature and pH for differing lengths of time and all the data plotted. If the yield of viable neurons over time under the two different conditions fell with a consistent time course these results would indicate that the data were internally consistent and reliable.

Results

Neuroprotection by hypothermia and alkalinization

The yield of viable neurons from DRG maintained at 37°C. while the pH was changed from 7.6 to 8.3, and to 9.3, increased from 3.8 ± 1.1% (N = 5), to 17.0 ± 1.6% (N=2), and to 29.8.0 ± 6.7% (N=4) (Figure 1). The data show a statistically significant increased yield of viable neurons

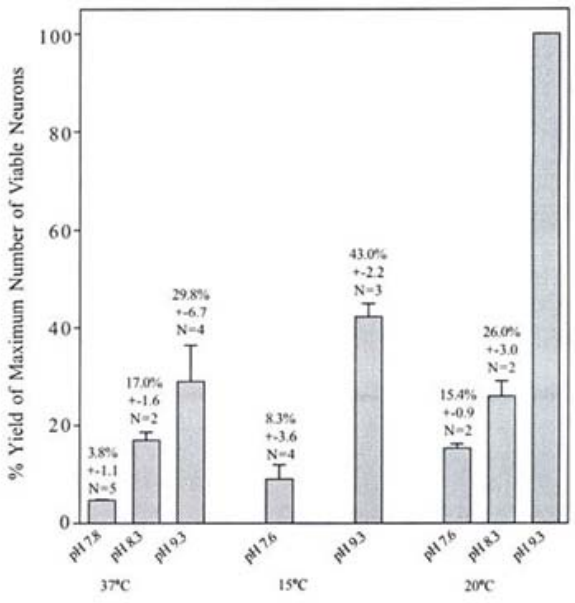

Figure 1. Graph of the yield of viable adult human neurons within intact DRG when maintained for 1 hour under different combinations of temperature and pH. The yields are normalized against that of DRG maintained at 20°C and pH 9.3 which provided the largest yield of viable neurons. Hypothermia and alkalinization each separately increase the yield of viable neurons, but simultaneous hypothermia and alkalinization provided greater neuroprotection than either one alone.

as the pH is changed from 7.6 to 8.3, ($p = 0.001$), but no statistically significant differences when the pH is changed from 8.3 to 9.3 ($p = 0.27$). However, at 37°C changing the pH was from 7.6 to 9.3 provided a statistically significant difference in the yield of viable neurons ($p = 0.003$)

The yield of viable neurons form DRG maintained at 15°C while the pH was changed from 7.6 to 9.3 increased from 8.3% ± 3.5 (N=4), to 43.0% ± 2.2 (N=3). This represents a statistically significant increase in yield of 34.7% ($p = <0.0007$).

The yield of viable neurons from DRG maintained at 20°C while the pH was changed from 7.6 to 8.3, and to 9.3 increased from 15.4% ± 0.9 (N=2), to 26.0% ± 3.0 (N=2), and to 100% (N=5). The change in yield from pH 7.6 to 8.3 was not a statistically significant increase in the yields of viable neurons ($p = 0.76$), while the changes from pH 8.3 to 9.3 and from pH 7.6 and pH 9.2 were statistically significantly different (both $p = < 0000001$). These results show that at each temperature tested, alkalinization produced a statistically significant increase in the yield of viable neurons.

The same data were analyzed to determine the influence of changing the temperature when the pH was maintained constant. The viability of neurons form DRG maintained at pH 7.6, while the temperature was changed from 37°C to 15°C and 20°C increased from 3.8% ±1.1, to 8.3% ±3.6, and 15.4% ±0.9. These represent differences of 4.5% (N=4) and 11.6% respectively (N=2). Although an increase in the yield of neurons is observed, the change from 37°C to 15°C, and from 15°C to 20°C do not represent statistically significant difference in the yields of viable neurons ($p = 0.23$ and 0.27 respectively). However, the change from 37°C and 20°C represents a statistically significant difference ($p = 0.0015$)

The viability of neurons from DRG maintained at pH 8.3 and while the temperature was changed from 37°C to 20°C increased from 17.0% ± 1.6 (N = 2) to 26.0% ± 3.0 (N = 2). This does not represent a statistically significant change ($p = 0.116$).

The viability of neurons from DRG maintained at pH 9.3 while the temperature was changed from 37°C to 15°C, and to 20°C increased from 29.8 ± 6.7 (N = 4) to 43.0% ± 2.2 (N = 3), and to 100%. These change in yield when the temperature changed from 37°C to 15°C is not statistically significantly different ($p = 0.16$). However, the increased yield when the temperature was changed from 15°C to 20°C, and from 37°C to 20°C had a statistically significantly difference ($p = <0.0000001$).

The yield of viable neurons from DRG maintained under physiological conditions (37°C/pH 7.6) was 3.8% ± 1.1 of that from DRGs maintained hypothermic and alkaline (20°C/pH 9.3) (100%). This represents a 26.3-fold increase in the number of viable neurons, a statistically significant difference ($p = < 0.0000001$).

These data show that both hypothermia and alkalinization provide neuroprotection against ischemia to adult human neurons within intact DRG. However when combined, hypothermia and alkalinization provide even greater neuroprotection.

Neuroprotection over time

The first series of experiments established that of the combinations of temperature and pH tested, 20°C and pH 9.3 provided the largest yield of viable neurons from intact DRG after 1 hour of ischemia compared to DRG maintained ischemic under physiological conditions. The second series of experiments examined the neuroprotection against up to 4 hours of ischemia to neurons within intact DRG by combined hypothermia and alkalinization (20°C / pH 9.3) vs. physiological conditions.

For each experiment in this set, one DRG of each pair was dissociated at the time its twin was placed in media under physiological (37°C/pH 7.4) or hypothermic and alkaline (20°C/pH 9.3) conditions. The yield of viable neurons from DRG maintained ischemic for different lengths of time was normalized against that of the twin DRG that was dissociated immediately.

The yield of viable neurons from DRG maintained ischemic for 0.5 hours under physiological conditions (37°C/pH 7.4) decreased to 15%, and after 1 hour to 2.7% (Figure 2). This value is comparable to the 3.8% yield of

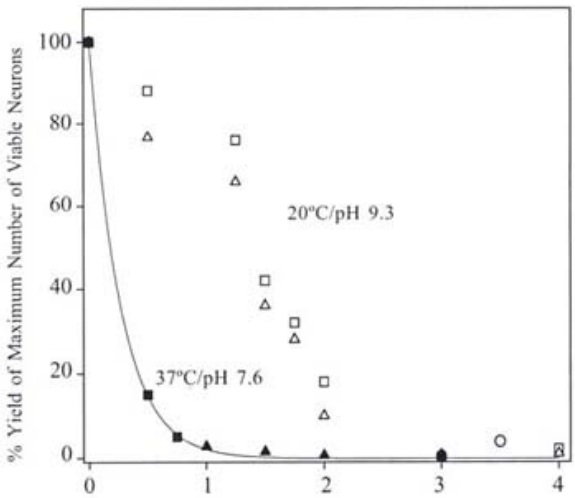

Figure 2. Plot of the decreased yield of viable neurons dissociated from intact adult human DRG maintained ischemic for up to 4 hours under physiological conditions (37°C/pH 7.4) (filled circles, triangles and triangles), vs. hypothermia and alkaline (20°C/pH 9.3) (open circles, triangles and squares). The data for the physiological conditions closely fit a single exponential, 3 parameters regression curve.

viable neurons in the previous set of experiments. After 1.5 hours, the yield of viable neurons was 1.5%, and after 2 hours 0.6%.

After 0.5 hours of ischemia the yield of viable neurons from DRG maintained under hypothermic and alkaline conditions (20°C/pH 9.3) was 82.4%, a 5.5-fold larger yield of viable neurons than from DRG maintained under physiological conditions. After 1.25 hours, the yield decreased to 71%, after 1.5 hours to 39%, 26-fold more than from DRG under physiological conditions, after 2 hours it was 14%, and after 4 hours to 1.5% (Fig. 2). These results show that hypothermia and alkalinization provide significantly greater neuroprotection than is provided by histological conditions at all times tested.

Discussion

Neuroprotection by hypothermia and alkalinization

Hypothermia and alkalinization separately, but to a greater extent when combined, provide neuroprotection to various populations of neuron from animal models (4, 14, 21, 29, 30). Clinical studies have shown that localized and global hypothermia provide neuroprotection for the human CNS (5, 11, 13, 28). However, there are virtually no data for human neurons on the neuroprotection provided by alkalinization alone, or when combined with hypothermia.

One limitation in performing such studies is access to viable adult human neurons on which to work. Another is maintaining the neurons viable long enough to perform such studies. This laboratory developed techniques that allow dissociated adult human DRG neurons to be maintained viable in vitro for up to 4 weeks (35). Therefore, the changes in neuron viability over their first 18 hours in vitro were not the consequence of rapid death due the isolation and maintenance conditions.

The present data show that for neurons maintained ischemic within intact DRG at physiological temperature (37°C), alkalinization to pH 8.3 and to 9.3 increases the yield of viable neurons by 4.5- and 7.8-fold respectively. Further, for DRG maintained ischemic at physiological pH (7.6), decreasing the temperature at which intact DRG are maintained during 1 hour from 37°C to 20°C and 15°C, increases the yield of viable neurons (4.6- and 2.2-fold respectfully).

The greatest degree of neuroprotection against ischemia was provided by combined hypothermia and alkalinization. Compared to DRG maintained under physiological conditions (37°C/pH 7.6), DRG maintained hypothermic and alkaline (15°C/pH 9.3 and 20°C/pH 9.3) had significantly larger yields of 11.3- and 26.3-fold respectively.

Neuroprotection over time

Examination of the viability of neurons within intact ischemic DRG showed that the death of adult human neurons closely follow an exponential decay. Thus, after 0.5 hours the yield of viable neurons was 15%, after 1 hour 3.8%, and after 1.5 hours 1.5%.

For intact DRG maintained ischemic under hypothermic and alkaline conditions (20°C/pH 9.3), the yield of viable neurons decreased far more slowly than for DRG under physiological conditions. Compared to the maximum possible yield of viable neurons, after 0.5 hour the yield of viable neurons was 82.4% (a 5.6-fold larger yield), after 1.5 hour it was 39.1% (a 26-fold larger yield), and after 2 hours it was 14% (a 23.3-fold larger yield). These are the first data showing neuroprotection against ischemia for adult human neurons provided by alkalinization alone, and enhanced neuroprotection provided by alkalinization combined with hypothermia.

The limited number of available adult human donors restricted the number of times we could test each set of conditions, and the combinations of temperatures and pH we could test. However, the results show a dramatic and consistent increase in yield of viable neurons provided by combined hypothermia and alkalinization vs. physiological conditions.HH

Susceptibilities of Adult Human vs. Rat DRG Neurons to Ischemia

In previous experiments we examined the neuroprotection against prolonged ischemia of neurons within intact adult rat DRG provided by hypothermia and alkalinization (4). Similar to the present results from adult human DRG neurons, hypothermia and alkalinization combined provided greater protection against ischemia than either one alone, especially when compared to the viability of neurons within intact DRG maintained at physiological temperature and pH.

Adult rat and human DRG neurons show a strikingly different susceptibility to death due to ischemia. Ischemic adult human neurons die much more rapidly than adult rat DRG neurons. Over 4 hours of ischemia, the number of viable adult rat DRG neurons decreased in a linear manner under both physiological vs. hypothermic and alkaline conditions. For adult human neurons, the death rate was nearly exponential under physiological conditions of temperature and pH. After 1 and 2 hour of ischemia under physiological conditions, adult rat DRG yielded 37% and 27% viable neurons respectively vs. 14% and 1.5% viable neurons respectively from adult human DRG. Hypothermic and alkaline conditions significantly slowed the death rate. After 1 and 2 hour of ischemia, 91% and 84% respectively of the adult rat neurons remained viable, vs. 71% (1.25

hours) and 14% of adult human neurons. Virtually all adult human DRG neurons were dead after 3 hours of ischemia, while after 4 hours of ischemia adult rat DRG yielded 18% viable neurons from DRG maintained under physiological conditions, and 76% from DRG maintained hypothermic and alkaline.

The differences in tolerance to ischemia by adult rat neurons may be an evolutionary adaptation. Such an adaptation would have allowed rats to survive in hostile, low oxygen-content, underground environments, where rats would be relatively safe from predation.

Possible mechanisms of neuroprotection

These experiments did not allow a determination of the mechanisms by which hypothermia and alkalinization provided protection against ischemia. However, based on what is known about ischemia-triggered secondary causes of neuron death, it is possible to speculate on the mechanisms underlying the observed protection.

Ischemia leads to the excessive release of extracellular excitatory amino acids (EAAs) (8-10, 20, 24, 33, 34, 36). DRG neurons contain and release EAAs, such as glutamate and aspartate (12, 27); Mattson et al., 1998), and possess receptors to these amino acids (36). Following isolation of intact DRG, the large and densely packed neurons, surrounded by tissue and a connective tissue coat, become ischemic and therefore exposed to an ischemia-induced excessive accumulation of EAAs and prolonged acidification (pH 7.3 to 6.5) (19, 36, 37) because the EAAs can not diffuse away. Acidification due to the accumulation of EAAs induces both necrotic and apoptotic neuron loss (7). The accumulation of EAAs also leads to excessive activation of NMDA receptors (36), that induces a massive influx of extracellular calcium (9) which in turn leads to a disruption of calcium homeostasis (3, 18), lipid peroxidation (37), and neuron death. Further, the released glutamate stimulates its own release in a positive feedback loop by its interaction with non-NMDA receptor subtypes (36). Calcium-induced calcium release, and a further influx of calcium through voltage-gated calcium channels after glutamate-induced depolarization, contributes to glutamate toxicity.

Mild hypothermia (30-35°C) provides neuroprotection from ischemia-induced excitotoxicity by markedly inhibiting the cellular release of excitatory amino acids, reducing their excessive accumulation, excessive NMDA receptor activation, and development of extracellular acidification. Hypothermia may play a similar role in the present experiments. Similarly, if neuron death were triggered by EAA-induced acidification, extracellular alkalinization would prevent the development of acidification, which would reduce the extent of neuron death. Therefore, it may have been anticipated that simultaneous hypothermia and alkalinization would provide greater neuroprotection than either one alone, by reducing the release and accumulation of EAA release, extracellular acidification, excessive activation of NMDA receptors, and by reducing the loss of calcium homeostasis. The scope of the present study did not allow us to examine the causes of neuron death from which hypothermia and alkalinization provide protection. However, it is still important to separate the potential excitotoxic components of excitatory amino acids from the acidosis brought about by their massive release. Similarly, neuroprotection by hypothermia is not explained solely by blocking or reducing the release of glutamate or other EAAs during ischemia, because hypothermia itself appears to play a pivotal role in neuroprotection.

The present results demonstrate that, consistent with data from experiments on other neuron models, hypothermia and alkalinization separately provide protection to adult human DRG neurons within intact DRG against prolonged ischemia, and ischemic-triggered secondary causes of death,. However, the data also show that hypothermia and alkalinization combined provide significantly greater neuroprotection than either one alone. This demonstration is similar to that for neurons within intact adult rat DRG (4). No previous studies have examined mechanisms for neuroprotection of isolated adult human neurons.

Clinical implications

After 1 hour of ischemia under physiological conditions, the yield of viable neurons from within intact adult human DRG falls to 3.8%. However, combined hypothermia plus alkalinization provides neuroprotection, yielding a 23-fold increase in viable number after 1 hour of ischemia. These results indicate that to preserve neuron viability and neurological functions through a period of ischemia, the cellular environment of the ischemic neurons must be changed to one that is neuroprotective. One approach is the shift the local cellular environment in the region of the trauma and ischemia as rapidly as possible to one that is hypothermic and alkaline.

Resumen

Isquemia "in situ", de ganglios dorsales intactos, induce una muerte masiva de neuronas. Esto se debe a que la isquemia promueve una serie de eventos secundarios como, la liberación excesiva de amino ácidos exitatorios, acumulación excesiva y activación de NMDA y otros neuroreceptores, acidificación y pérdida de la homeostasis de calcio. Estos experimentos se diseñaron para comprobar si hipotermia y alcalinización, separados o en combinación,

podían proveer neuroprotección en contra de 1-4 horas de isquemia a las neuronas dentro de los ganglios dorsales intactos removidos, cuidadosamente, de donantes de órganos. El rendimiento de neuronas aisladas de los ganglios dorsales bajo hipotermia (20-15°C) o bajo alcalinidad (pH 8.0-9.3) fue mayor que en condiciones fisiológicas (37°C/pH 7.4), 4.1 vs. 7.8 respectivamente. La combinación de hipotermia y alcalinidad aumento el rendimiento de neuronas 27 veces más que los ganglios dorsales bajo condiciones fisiológicas. Estos resultados demuestran que la combinación de hipotermia y alcalinización proveen neuroprotección en contra de isquemia a las neuronas de humanos adultos dentro de los ganglios dorsales.

Acknowledgements

We are most grateful to Ms Cristina Castro for her unfailing support of these experiments. We are also most grateful to the families of the organ donors for their generosity to this laboratory. LifeLink of Puerto Rico obtained approval for retrieval of the tissues used in these experiments. These experiments were carried out under an Institutional IRB exemption.

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