Anesthesiology
V 75, No 3A, Sep 1991

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ISCHEMIC-HYPOXIC INJURY IN CULTURED NEURONS AND ASTROCYTES MEASURED BY LDH RELEASE.

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In recent years a considerable amount of data has suggested that different types of neurons in vitro vary in their sensitivity to hypoxia, substrate deprivation or ischemia (combined hypoxia and substrate deprivation). In addition, in vitro experiments have suggested that homogenous cultures of neurons are more sensitive to hypoxia than cocultures of neurons and astrocytes grown together (to simulate in vivo conditions). Finally, astrocytes are said to be relatively resistant to hypoxia. Neurons make up somewhat more than one half of the brain, while astrocytes comprise most of the rest.

Methods: Primary cultures of cerebral neurons were obtained from 15-day-old mouse fetuses by preparing a cell suspension from the neopallium. Cells were seeded on a polylysine coated culture dishes in tissue culture medium. Astrocyte cell proliferation was inhibited with 40μM cytosine arabinoside. This step was excluded in the preparation of neuronal-astrocytic cocultures. Cerebellar granule cells were obtained from 7-day-old postnatal mice and grown in a manner similar to cerebral neurons except that these cultures were grown at 24.5 mM potassium, a precondition for development of normal transmitter function in cerebellar granule cells. Astrocytes were grown on non-treated Falcon culture dishes in a similar manner, except that the glucose concentration was 7.5 mM. Neuronal cultures and co-cultures were studied after at least nine days and astrocytic cultures after at least three weeks in cultures. For the ischemic/hypoxic insult cultures were exposed to one of the following four conditions: 1) control, i.e., normal medium and air; 2) severe hypoxia (1-3%$O_2$) and normal medium; 3) normoxic conditions and culture medium deprived of glucose and glutamine; and 4) hypoxia and substrate deprived medium. The air phase always contained 5% $CO_2$.

LDH (lactate dehydrogenase) release into the medium was measured spectophotometrically in the medium after the insult period and after "recovery" in a normoxic control medium. Release of this high-molecular cytosolic enzyme is an indication of severe injury.

Results: LDH production was expressed as a percentage of LDH production under control conditions in the same type of cultures. Neither cerebral cortical neurons, nor cerebellar granule cells showed any significant insult induced release after 3 hrs of incubation. After 9 hrs of insult, LDH was increased (2 fold) in both types of neurons. There was no difference between hypoxia and ischemia, but substrate deprivation alone had no effect. Cocultures and isolated neurons showed identical sensitivity. In astrocytes, hypoxia had no deleterious effect as long as any substrates remained for anaerobic glycolysis. This confirms previous results by Yu et al. (J. Cereb. Blood Flow & Metab. 9, 20, 1989). The most serious insult to astrocytes therefore was ischemia, which caused extensive (up to 12 fold) increase in LDH release after >12 hrs.

Discussion: The above results demonstrate that hypoxia leads to increased LDH release in both types of neurons regardless whether substrate is present or not. Hence, neurons are equally sensitive to hypoxia or ischemia but the response in remarkably slow. There was a similar response in co-cultures. Astrocyte cultures appeared quite resistant to hypoxia, moderately sensitive to substrate deprivation but as sensitive to ischemia as neurons. The different response by neurons and astrocytes to hypoxia may explain "neuronal specific" damage of hypoxia alone (e.g. in anaesthesia accidents and near-drowning) and the more widespread brain damage which is induced by ischemia. Moreover, LDH release in astrocytes coincides with cell death estimated by dye methods, whereas LDH release in neurons appears to occur well after cell death estimated by dye methods.

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TITLE: A SCORPION VENOM THAT ACTIVATES NORMAL AND INHIBITS MALIGNANT HYPERTHERMIA SUSCEPTIBLE RYANODINE RECEPTORS

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The venom of the scorpion *Buthotus hottentota* selectively enhances [$^3$H]ryanodine binding to the ≈500 KDa ryanodine receptor of rabbit skeletal muscle,(1) and activates the calcium release channel associated with this protein. Because the radioligand binding characteristics and single channel gating properties are altered in MH (2), we compared the effect of the scorpion venom on ryanodine receptor calcium release channels isolated from normal (N) and MH susceptible (MHS) pigs.

Methods. Crude venom was centrifuged at 10,000 g for 3 min to remove insoluble mucopolysaccharides. Sarcoplasmic reticulum (SR) vesicles were isolated from skeletal muscle of 6 N and 6 MHS pigs by a modified microsome fractionation procedure.(3) Solubilization of SR vesicles was achieved by resuspending vesicles (10 mg/ml) in an equal volume of 1% CHAPS, 40 mM Tris-maleate pH 7.2 and incubating 40 min on ice. [$^3$H]Ryanodine binding assays were carried out for 90 min at 36°C in 0.1 ml aliquots containing 30-40 μg of solubilized SR vesicles in 0.15 M KCl, 10 mM Na-Pipes pH 7.2, 0.1% CHAPS and 10 μM free [$Ca^{2+}$]. Reconstitution of ryanodine receptors in planar lipid bilayers was conducted as previously described.(4)

Results. In N receptors, *Buthotus* venom (80 μg/ml) enhanced the binding of [$^3$H]ryanodine 2.6-fold, from 0.3±0.08 to 0.78±0.10 pmols/mg protein (Fig. 1) and increased open probability (Po) of calcium release channels reconstituted in lipid bilayers from 0.21±0.05 to 0.48±0.08. The increase in Po was due to an increment in the frequency of open events and the appearance of a ≈180-ps long-lived subconductance state. These results indicate that *Buthotus* venom affected a domain within the channel that controls gating. The same venom concentration decreased [$^3$H]ryanodine binding to MH receptors by 60%, from 0.35±0.05 to 0.14±0.04 pmols/mg protein and inhibited open probability of MH channels from 0.19±0.04 to 0.06±0.02. The differential effect of *Buthotus* venom on MHS muscle demonstrates that the porcine MH receptor has a structurally altered gating domain which may be responsible for the hypersensitivity of the channel to $Ca^{2+}$, caffeine and halothane.

References

1. Biophys J 1991; 59:63a.; 2. Biophys J 1990; 50:471.; 3. J Gen Physiol 1990; 95:1.; 4. Biophys J 1990; 58:471.