TITLE: RYANODINE BINDING IN SWINE CORRELATES WITH STRAIN DIFFERENCES, NOT MALIGNANT HYPERTHERMIA (MH) SUSCEPTIBILITY

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The MH syndrome is a hypermetabolic episode elicited by potent inhalation anesthetics and/or succinylcholine that is believed to result from enhanced sensitivity of Ca^{2+} release from the terminal cisternae (TC) of the sarcoplasmic reticulum. Binding of ryanodine to the Ca^{2+} release channel (ryanodine receptor) of TC has been reported to be altered in MH muscle under specific conditions (no ATP, Ca^{2+} 6 μM).[1] However, the TC in these studies were derived from control and MH swine from different strains (Yorkshire and Pietrain, respectively). The present study examines ryanodine binding in TC from control and MH swine of the same strain (a Duroc/Yorkshire cross) under the specific conditions differentiating "MH susceptibility" in the previous studies. The pigs were confirmed as either MH susceptible or control by all tests employed (barnyard challenge, H blood typing, *in vitro* contracture test, CK values). Highly enriched TC fractions were isolated on a discontinuous sucrose gradient[2] from *longissimus dorsi* removed during nontriggering anesthesia (N_2O, ketamine). ^{3}H-Ryanodine was obtained from New England Nuclear (99% purity, 95 μCi/pmol). The binding conditions originally used to discriminate MH susceptible from control swine (37°C, 90 min, pH 7.0, 0.2 mg TC/ml) were used, with a PIPES buffer containing (in mM): (PIPES 10, KCl 100, $CaCl_2$ 2, EGTA 3.7, nitrilotriacetic acid 3.7) to buffer Ca^{2+} at 6 μM.[1] The samples were filtered and Scatchard analysis performed, as described in the original studies.[1] The results of pilot studies determining specific binding and K_d by methods in the original studies,[1] were qualitatively similar (no difference between MH and control) to those obtained by more standardized methodologies[3] reported in the Table. The B_{max} values (Table) were in agreement with other investigators,[1] suggesting the TC fractions were of similar purity. Also in agreement with other investigators,[1] there were no differences in B_{max} related to MH susceptibility (Table). In contrast to the 3.5-fold difference in K_d in previous studies,[1] there was no difference in the K_d value related to MH susceptibility (Table). We did not corroborate that ryanodine binding is altered in MH. The reported differences in ryanodine binding most likely relate to the use of different control and MH strains, not MH susceptibility.

References

1 J biol Chem 263 9310 1988; Am J Physiol 257 C787 1989

2 J Cell Biol 99 875 1984

3 Neurotransmitter Receptor Binding (Yamamura HI et al., eds) Raven Press, NY 1978

TABLE. ^{3}H-Ryanodine binding to TC fractions (X±SEM)

	n	K_d (nM)	B_{max} (pmol/mg)	
Control	3	20 ± 4	11 ± 0.2	no significant
MH Susceptible	4	17 ± 3	11 ± 2.9	differences

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TITLE: ELEVATION OF CSF CONCENTRATIONS OF GLUTAMATE DURING SPINAL CORD ISCHEMIA

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Current evidence strongly suggests that glutamate and aspartate are important excitatory neurotransmitters in mammalian brain and spinal cord. Dysfunction of these neurotransmitters may be important etiological factors in the development of nerve cell damage following cerebral and spinal cord ischemia. In this study, we measured the CSF concentrations of glutamate, aspartate and other amino acids during and after induced spinal cord ischemia (SCI).

Methods: Six rabbits were anesthetized with 1-2% halothane/O_2, intubated and ventilated to maintain normal $PaCO_2$ and pH. Normal body temperature was maintained and N saline administered at 15 ml/kg/hr. A 4 Fr balloon catheter was advanced into the abdominal aorta and the tip positioned distal to the renal artery. The rabbits were placed in a sitting position and MAP maintained with a phenylephrine infusion, if necessary. A 22 G spinal needle was inserted in the lumbar region and continuous CSF samples obtained by gravity drainage. Following control samples, the balloon was inflated for 30 min to induce SCI. CSF samples (0.2 ml) were obtained before balloon deflation and 30, 60 and 90 min post reperfusion. Collected CSF was immediately frozen and stored for amino acid analysis by HPLC. Concentrations (μM) were computed from peak areas by comparison to external standard.

Results: The control values are comparable to those found in other studies. Following 30 min of SCI, there were elevations in most amino acid levels, but only glutamate reached statistical significance ($p<0.05$). Upon reperfusion of the spinal cord, the highest level of glutamate was attained in the 30 min sample ($p<0.01$) followed by elevations in subsequent samples ($p<0.02$) at 60 and 90 min. Of the other amino acids, only GABA showed a significant elevation at 30 min post-reperfusion ($p<0.05$).

Amino Acid Concentrations in Rabbit CSF During and After Spinal Cord Ischemia

Amino Acid	Control	Ischemia	Post-Ischemia		
μM		*30*	*30*	*60*	*90 min*
Aspartate	1.76 ±0.59	2.37 ±0.82	2.46 ±1.01	2.43 ±0.96	2.37 ±0.30
Glutamate	3.39 ±0.43	6.69* ±3.18	6.47*** ±1.97	5.13** ±1.25	4.85** ±0.69
Taurine	5.96 ±2.21	7.19 ±2.54	7.66 ±2.36	6.54 ±2.25	6.02 ±2.39
GABA	0.69 ±0.29	0.90 ±0.25	1.08* ±0.33	0.99 ±0.30	0.70 ±0.09

*Results are averages ±SD. Asterisks indicate significant changes (*p<0.05, **p<0.02, ***p<0.01)*

Conclusion: We observed an increase in the CSF concentration of glutamate during spinal ischemia in-vivo which is consistent with those results obtained in various in-vitro preparations. These findings support the concept that glutamate is an important etiologic factor in CNS ischemic damage.