MECHANISMS OF HEAVY METAL TOXICITY ON STIMULUS-RESPONSE COUPLING IN SKATE (RAJA ERINACEA) ELECTRIC ORGAN

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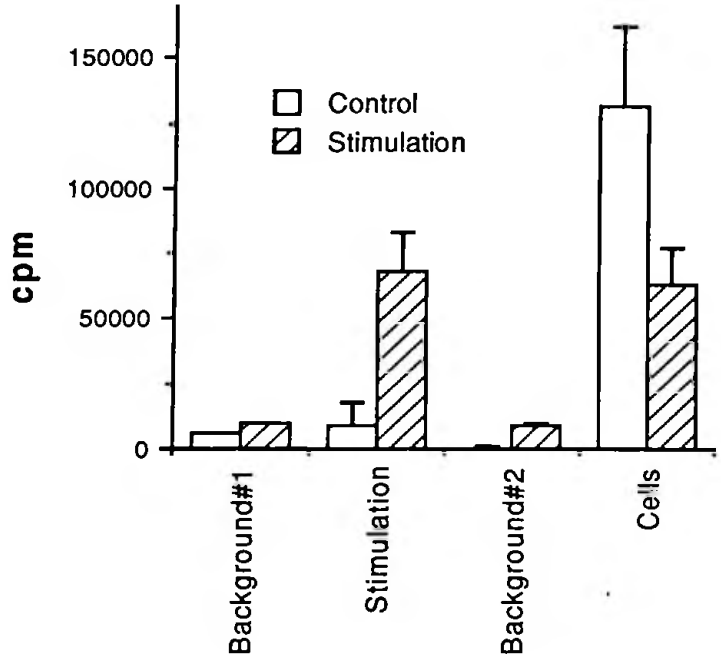
We studied the mechanisms of toxicity of cadmium, mercury, and lead (Cd^{++}, Hg^{++}, and Pb^{++}) on stimulus-response coupling in isolated electric organ sections from the skate, Raja erinacea. The skate has weak electric organs in the tail, one on each side of the spinal cord; the organ probably serves the fish as a system of communication. This organ has been characterized as being a purely cholinergic tissue, utilizing the neurotransmitter, acetylcholine (ACh). This preparation is hardy, stable, and responds robustly to three forms of stimulation: electrical field stimulation, high concentrations of potassium, and applied ACh. Cadmium caused a decrease and a delay in the evoked electrical discharge of the preparation . These effects are concentration-related, and are overcome in part by increasing Ca concentration. Cadmium (100 μM Cd^{++}) also inhibited the evoked (electrical or high-K^+ stimuli) release of ACh.

Electrical field stimulation of the electric organ results in uptake of ^{45}Ca by the post- synaptic electrocytes. We also found that bath-applied ACh (20 μM) stimulated a vigorous uptake of ^{45}Ca. This ACh-stimulated ^{45}Ca uptake was blocked by d-tubocurarine (indicating that this uptake was post-synaptic). Cadmium (100 μM) did not block ^{45}Ca uptake. Our pharmacological characterization of Ca-channels in the electric organ preparation is as follows: pre-synaptic nerve ending channels have T-type properties, since electrically and high-K^+ evoked ACh release is blocked by 10 μM Ni^{++}, but not by nifedipine; and post-synaptic electrocyte channels have L-type characteristics, since ^{45}Ca uptake is blocked by nifedipine, but not by Ni^{++} (5 mM). The above results from our previous efforts serve as the background for the work done during 1992 (see Brown and Andrake, Comp. Biochem. Physiol. 102C:439-446,1992; and our previous reports in MDIBL Bulletins: 28:109,1989; 29:106, 1990; 30:91, 1991; 31:163,1992.).

In addition to expanding on our previously reported studies with Cd++, our recent work included similar experiments with mercury and lead. Hg^{++} (in concentrations of 10 μM or greater) caused a decrease and a delay in the evoked electrical discharge of the electric organ preparation ; whereas, 10μM Pb++ caused a delay in achieving maximal evoked discharge without decreasing the amplitude. Exposure of electric tissue, pre-labeled with ^3H-choline, to mercury or lead (10 μM, Hg^{++} and Pb^{++}) induced release (or increased spontaneous release) of ^3H-ACh. Our preliminary data suggest that, as with cadmium, voltage-dependent ACh release is inhibited by both of these cations. Neither mercury nor lead (10 μM, Hg^{++} and Pb^{++}) inhibited the ACh-stimulated uptake of ^{45}Ca by the post-synaptic electrocytes. From our work to date on isolated electric organ sections we conclude that cadmium, mercury, and lead ions have their major site of action on the pre-synaptic nerve endings in this preparation.

We have begun to isolate individual, innervated electrocytes from skate electric organ by incubation of tissue sections in collagenase. Incubations conditions were: 1% collagenase (Sigma Type 1A) in Raja buffer (Bull. MDIBL 29:106,1990) for 3 hr at 15° C. The cells were then dispersed by repeated aspiration of both tissue and medium into and out of all-plastic transfer pipettes (large orifice diameter "Cell saver" tips, LUX). The isolated cells were then washed several times by resuspending, allowing them to settle, decanting off buffer, and adding fresh (no collagenase) buffer. Microscopic visualization of the isolated electrocytes showed them to be intact and to have attached nerve terminals. Both the pre-synaptic and the post-synaptic elements of these cells are viable.

Isolated electrocytes prepared from tissue pre-labeled with 3H-choline released ^{3}H-ACh when stimulated by incubation in high-K^+ (70 mM) buffer for 15 min (see the figure). Dissociated, innervated electrocyte preparations also vigorously took up ^{45}Ca when incubated with 20 µM ACh for 10 min. Control preparations (no ACh) took up 810 ± 300 cpm ^{45}Ca/100 mg (tissue equivalent), while ACh-stimulated preparations took up 3000 ± 280 cpm ^{45}Ca/100 mg (mean ± SD, n = 2). This preparation could prove to be a powerful system in which to study mechanisms of membrane toxicity of pre- and post-synaptic elements.



Release of ^{3}H-ACh by isolated innervated electrocytes and nerve terminals with high-K^+ stimulation. Means ± SD; experiment was performed twice, with cells equivalent to approximately 100 mg tissue for each sample.

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