MECHANISMS OF HEAVY METAL ION TOXICITY TO SYNAPTIC TRANSMISSION IN RAJA ERINACEA ELECTRIC ORGAN

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We are studying the mechanisms of evoked neurotransmitter release and the mechanisms of action of heavy metal ions on excitable membranes. As a model synaptic system, we are using sections and isolated, innervated electrocytes from the electric organ of the skate, Raja erinacea. These preparations allow us to quantitate and correlate changes in physiology, neurotransmitter release, and ion movement in the same experiment. Further, this unique system responds robustly to three forms of stimulation: electrical field stimulation, high concentrations of K^+ (70 mM) (High K), and applied acetylcholine (ACh, 20 μM).

The weak electric organ in the tail of the skate probably serves the fish as a system of communication. The organ is a purely cholinergic tissue, utilizing a single neurotransmitter, ACh. Our previous studies indicate that the electrophysiological and biochemical properties of Raja electric organ provide a unique and useful system with which to examine synaptic events and the mechanisms of heavy metal ion toxicity. We continue to study the effects of Cd^{++} and Hg^{++} and other agents on in vitro sections and isolated cells from this homogeneous model nervous system (for background, please see our reports in the previous five MDIBL Bulletins, and Comp. Biochem. Physiol. 102C: 439-446, 1992). The evoked electrical discharge of the tissue was measured as a means of assessing its physiological status. Raja tissue was stimulated (electrically or with 70 mM K^+) and incubated in buffer containing tritiated choline; ^{3}H-Ch was readily taken up and converted to ^{3}H-ACh. Labeled ACh was released upon subsequent electrical or High K stimulation (in the presence of a cholinesterase inhibitor, 100 μM neostigmine) and measured by liquid scintillation counting. Labeled Ch and ACh remaining in tissue sections or cells were also measured. The identity of the stimulation-released tritium as ^{3}H-ACh was verified by thin layer chromatography.

We have previously reported that Cd^{++} inhibited evoked electrical discharge and evoked release of ^{3}H-ACh in a concentration-related fashion (Brown and Andrake, MDIBL Bull. 29:106, 1990; 30:91, 1991; 31: 163, 1992; 32:149, 1993). This summer we confirmed these effects on evoked release of ^{3}H-ACh in parallel experiments performed on isolated, innervated electrocytes prepared by collagenase (1% for 12 hours at 4° C) treatment of electric organ. Hg^{++} (10 μM to 100 μM) also inhibited evoked electrical discharge and evoked release of ^{3}H-ACh. Additionally, Hg^{++} (unlike Cd^{++}) induced the spontaneous release of ^{3}H-ACh (with both intact tissue sections and isolated, innervated electrocytes). These effects of Hg^{++} on ^{3}H-ACh released from intact tissue sections, evoked with electrical and High K^+ stimulation, are shown Figures 1 and 2. Unlike with Cd^{++}, the effects of Hg^{++} do not appear to be calcium-dependent. Both spontaneous release and inhibition of evoked release were not altered when the concentration of calcium was varied. ^{3}H-ACh release from tissue sections, both spontaneous and evoked by High K, are expressed as percent of total counts in each sample (means $\pm$ SD, n = 3-7). Bkgd-1 and Bkgd-2 are buffer washes before and after incubation in High K (Stimulation) buffer for 20 min. Tissues were stimulated with High K alone, High K with Hg^{++}, or Hg^{++} without High K.

Figure 2 shows the results of a superfusion experiment, wherein sections of electric organ, previously loaded with ^{3}H-Ch and washed with cold buffer, were electrically stimulated while superfusate fractions were collected (one sample every two minutes) and counted. Electrical stimulation of tissue for 2 min at the times indicated by the arrows in Fig. 2 resulted in the release

of ^{3}H-ACh (S_1 and S_2). When the control superfusion buffer was switched to one containing 100 μM Hg^{++} (at 20 min), not only was the evoked release of ^{3}H-ACh greatly decreased (S_2) as compared to release in the control buffer (S_1), but spontaneous release of ^{3}H-ACh was greatly enhanced - note the elevated baseline before the second stimulus.

We conclude that cadmium and mercury ions have their sites of action on the pre-synaptic nerve endings in this preparation. This conclusion was corroborated by similar results in experiments with isolated, innervated cells dissociated from electric organ by collagenase incubation.

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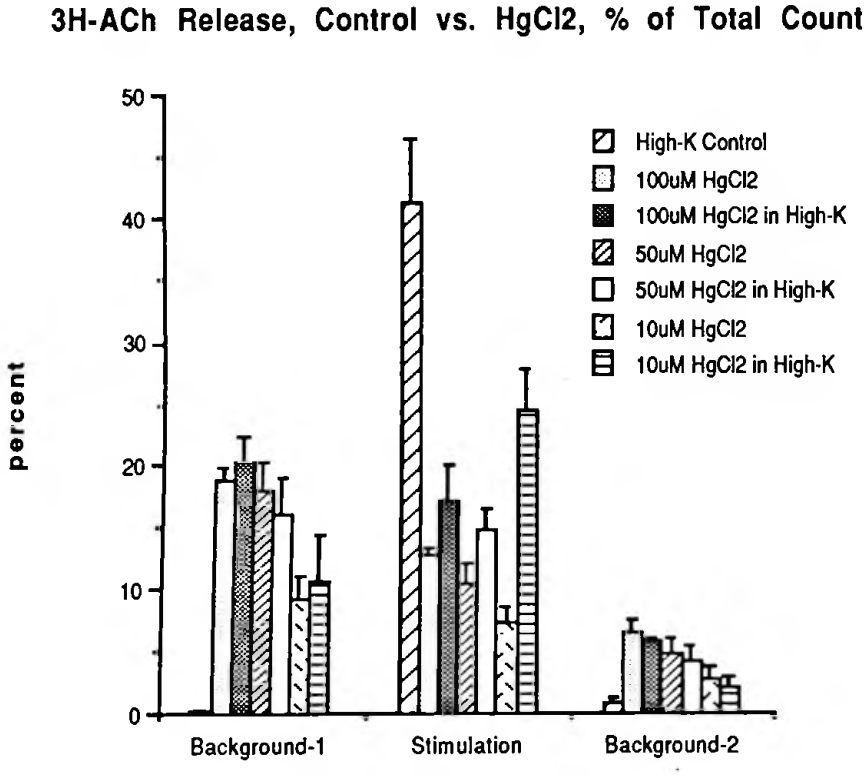

Fig.1 ^{3}H-ACh release with High-K^+ stimulation in the presence of 100 μM, 50 μM, and 10 μM $HgCl_2$. Results are expressed as the percent of the total counts in each sample.

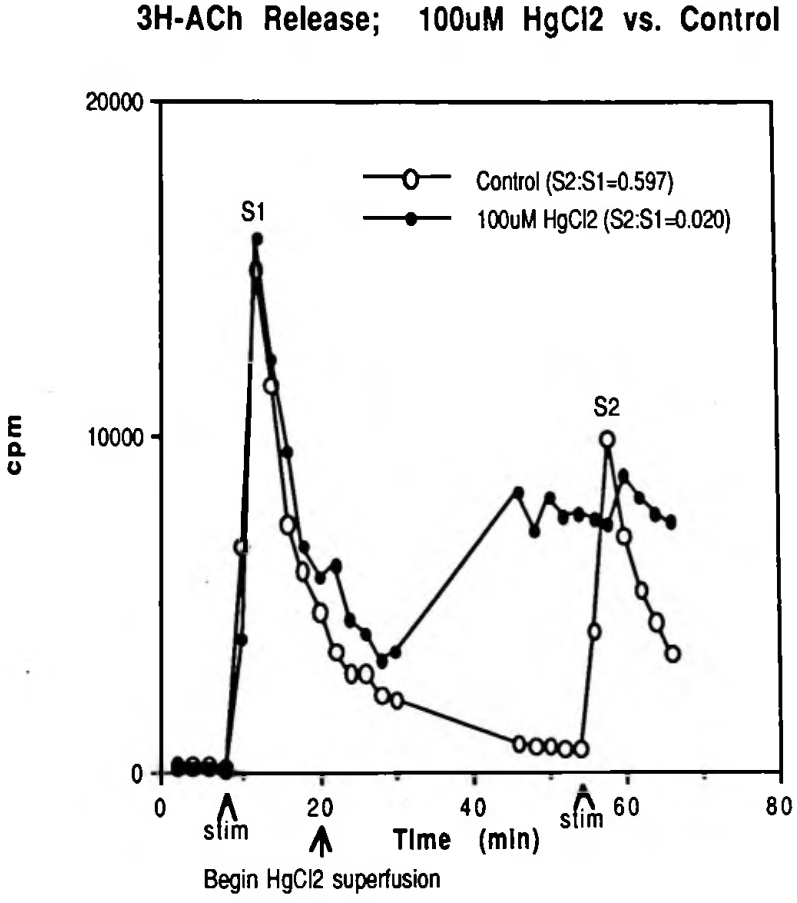

Fig.2 ^{3}H-ACh release with electrical stimulation. Superfusate is collected and measured with liquid scintillation counting. The tissue is stimulated (20 V, 0.1 ms, 10 Hz) for 2 min. at the times indicated. S_2:S_1 ratios were calculated. The tissue superfused with 100 μM Hg^{++} demonstrates spontaneous release and inhibition of evoked release.