MECHANISMS OF CADMIUM TOXICITY IN RAJA ERINACEA ELECTRIC ORGAN

Oliver M. Brown and John S. Andrake
Departments of Pharmacology and Pediatrics
State University of New York Health Science Center
Syracuse, New York 13210

We have been studying the mechanisms of evoked neurotransmitter release and the mechanisms of action of cadmium (Cd) on excitable membranes. As a model system in this effort, we are using the electric organ of the skate. This preparation allows us to quantitate and correlate changes in physiology, neurotransmitter release, and ion movement in the same preparation.

The skate, Raja erinacea, 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). Our previous studies indicate that the electrophysiological and biochemical properties of Raja electric organ provide for a unique and useful system with which to examine synaptic events and the mechanisms of cadmium toxicity. We continue to study the effects of Cd and other agents on in vitro sections of this unique and homogeneous model nervous system (for background, please see our reports in the previous four MDIBL Bulletins, and FASEB J. 3:A890, 1989). The evoked electrical discharge of the tissue was monitored as a means of assessing its physiological status. Following loading with ^{3}H-choline (^{3}H-Ch), synaptic release of ^{3}H-ACh was evoked by electrical stimulation or by high concentrations of potassium (K). Ch and ACh in the tissue and released from the tissue were measured by liquid scintillation counting of tritium-labeled samples and by thin layer chromatography. Calcium uptake by the tissue was stimulated by electrical pulses, by high concentrations of K, or by application of ACh, and was measured by liquid scintillation counting of ^{45}Ca.

Raja tissue, stimulated and incubated in buffer containing tritiated choline, readily took up ^{3}H-Ch and converted it to ^{3}H-ACh, which was released upon subsequent electrical stimulation. We recently found that ^{3}H-ACh release was also stimulated by brief incubation of loaded tissue in buffer containing 70 mM K (high K) (Fig. 1). Also, we found that our ability to measure ACh release was markedly enhanced by treating the tissue with the cholinesterase inhibitor, neostigmine (100 μM). Further, we verified by thin layer chromatography the identity of the stimulation-released tritium as ^{3}H-ACh. Cadmium inhibited the release of ^{3}H-ACh evoked by both electrical pulses and high K in a concentration-related fashion. This effect of Cd, as well as its inhibition of electrical discharge, was overcome in part by increasing extracellular Ca concentration. These findings are consistent with those of others, and with the hypothesis that Cd blocks cell membrane Ca channels or other Ca mechanisms.

To further test the interactions between Cd and Ca we examined the effects of Cd and other agents on voltage-dependant uptake of ^{45}Ca in the isolated electric organ (see Andrake, et al., MDIBL 30:91-93, 1991). The robust ^{45}Ca uptake seen in electrically or high K stimulated Raja tissue was eliminated in the presence of 100 μM Cd. We also found that d-tubocurarine, an antagonist of nicotinic ACh receptors, blocked Ca uptake (and evoked discharge), but did not affect ^{3}H-ACh release, suggesting that most of the ^{45}Ca uptake measured is post-synaptic. The L-type Ca channel antagonist, nifedipine, blocked ^{45}Ca uptake and evoked potential without affecting ^{3}H-ACh release, indicating that the post-synaptic, but not the pre-synaptic Ca channels have L-type properties. In contrast, nickel, which is characterized as a T-type Ca channel antagonist, did block ACh release. This property is shared by cadmium and verapamil, suggesting that the effects of nickel, cadmium, and verapamil on electric organ are (at least in part) to block pre-synaptic T-type Ca channels. The block of ACh release by cadmium, nickel, and verapamil would be expected to result in and an inhibition of post-synaptic ^{45}Ca uptake by preventing stimulus-response coupling;

although an additional direct effect of these agents on post-synaptic Ca channels cannot be excluded by these data. However, our preliminary experiments with bath-applied ACh (10 μM) demonstrated a vigorous uptake of ^{45}Ca with this stimulus (Fig. 2). This ACh-stimulated ^{45}Ca uptake was blocked by d-tubocurarine, but not by 100 μM Cd, which is evidence that Cd does not act post-synaptically, via L-type Ca channels, in this Raja electric organ.

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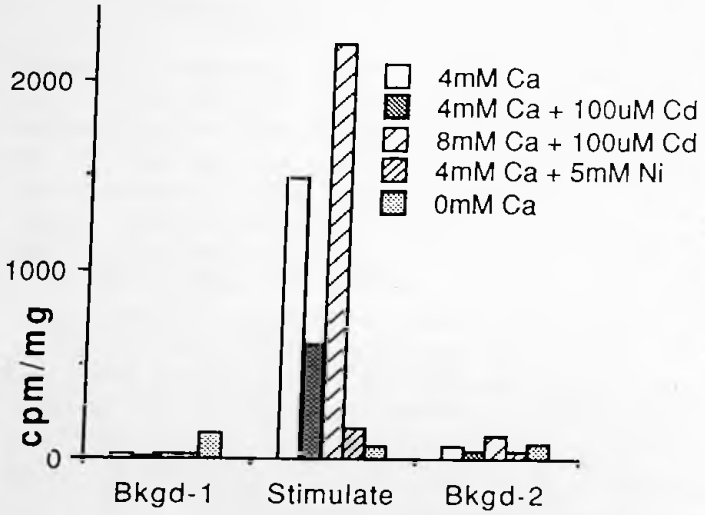

Figure 1 - High K release of ^{3}H-ACh, expressed as cpm ^{3}H per mg tissue. Bkgd-1 and Bkgd-2 are washes before and after high K incubation. In 4 mM Ca buffer, ^{3}H-ACh release was inhibited by 100 μM Cd and 5 mM Ni. Cd inhibition was antagonized by increasing Ca to 8 mM. High-K = incubation in 70 mM K buffer.

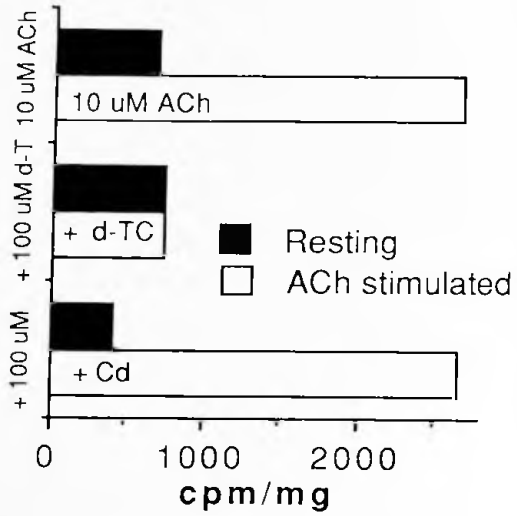

Figure 2 - ACh-stimulated ^{45}Ca uptake, expressed as cpm ^{45}Ca per mg tissue. Ca uptake was blocked by tubocurarine (d-TC, 100 μM) but not by 100 μM Cd. ACh Stim = incubation in 10 μM ACh.