MECHANISMS OF CADMIUM TOXICITY IN RAJA ERINACEA ELECTRIC ORGAN

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

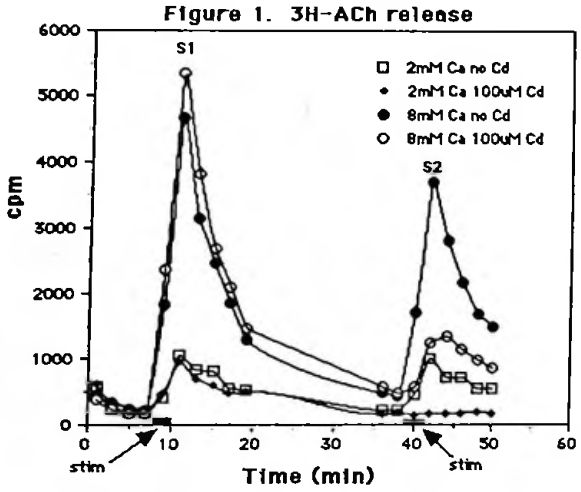
The skate, Raja erinacea, has weak electric organs in the tail, one on each side of the spinal cord. As is the case with other fish electric organs, the Raja organ is a purely cholinergic tissue, utilizing the neurotransmitter, acetylcholine (ACh). We have been studying the effects of cadmium on stimulus-response coupling in the skate electric organ. Our previous reports have described the electrical properties of this preparation (Brown, Bull. MDIBL 27:126-119, 1987-88), and the interaction between Ca and Cd on the evoked electrical discharge (Brown and Andrake, Bull. MDIBL 28:109-111, 1989; FASEB J. 3:A890, 1989; and Bull. MDIBL 29:106-107, 1990). We found that Cd decreases the electrical discharge of the electric organ in a dose related fashion and that this effect can be overcome in part by increasing the Ca concentration. The present report examines the synaptic mechanisms underlying this observation.

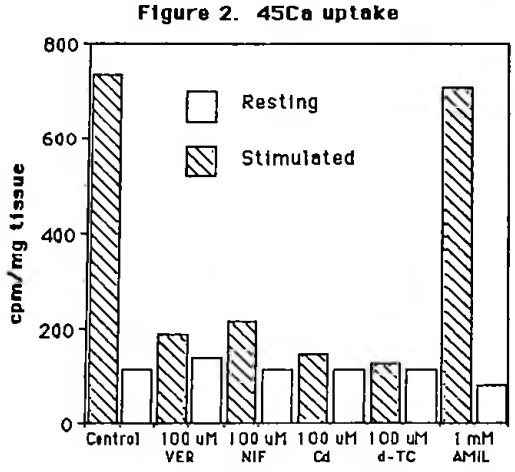
Skates were rapidly sacrificed by pithing and severing of the cervical spinal cord. The electric organs were dissected from both lateral aspects of the tail (approximate organ dimensions are 15 cm length and 3 mm diameter) and placed in Raja buffer. Organs were cut into sections of 2 or 3 cm in length, blotted, weighed, allowed to recover , and incubated in buffer containing various concentrations of cadmium and Ca. Electrical stimulation of tissue sections was performed in either a nerve conduction chamber or a drip-flow superfusion chamber (both chambers are fitted with stainless steel electrodes spaced every 0.5 cm, and were kept at 15-19°C). Tissue sections received end to end supramaximal electrical stimulation; 20 V, 0.1 msec, 10 Hz (Grass stimulator). Electrical events over the central 1 cm were monitored on a Hitachi VC 6025 digital oscilloscope. Electrical stimulation of electric organ sections resulted in an evoked electrical discharge of approximately 150-250 mV per cm.

To evaluate ACh release, sections of electric organ were loaded with ^{3}H-choline (^{3}H-Ch) by incubating and stimulating the tissue in 2 ml buffer containing 5 μCi ^{3}H-Ch . After washing the tissue, it was placed in the superfusion chamber and superfusate fractions were collected every 2 min during cycles of electrical stimulation and rest. A control superfusion buffer was used during the first stimulation period (S1). The buffer was then switched to one containing 100 μm Cd (or another a test compound, see Summary Table), which flowed over the tissue for at least 15 min before a second stimulation (S2). Electrical stimulation of tissue for 2 min at the times indicated by the bars in Fig. 1 resulted in the release of ^{3}H-ACh (S1 and S2) When the control superfusion buffer was switched to one containing 100 μm Cd, the evoked release of ^{3}H-ACh was greatly decreased (S2) as compared to release in control buffer (S1). Cd completely blocked ^{3}H-ACh release in 2 mM Ca, this effect was partially overcome by increasing the Ca concentration to 8 mM. Results are expressed as a ratio of counts released, S2:S1, as follows:

	S2:S1 ratios	
	Control	100 μm Cd
2 mM Ca	0.972	0
8 mM Ca	0.884	0.211

The effects of Cd on electric tissue and its interaction with Ca may involve Cd blocking Ca entry through Ca channels. To further characterize this interaction, we looked at the effects of other known Ca channel blockers on mV output, ^{45}Ca uptake, and ^{3}H-ACh release. For Ca uptake





experiments, sections of electric organ were pre-incubated for 1 hr in Raja buffer (4 mM Ca) with (Experimental) or without (Control) test compound (Cd, Ni, verapamil, amiloride, nifedipine, and d-tubocurarine). Sections were then incubated for 10 min in identical media with the addition of 2 μCi/ml ^{45}Ca. Half of the sections were electrically stimulated (20 V, 0.1 msec, 10 Hz) for 5 min, and the other half (Resting) were left unstimulated. The sections were then washed for 30 min each in 3 successive changes of Ca-free Raja buffer containing 2 mM EGTA, and the sections were digested in 1 ml 1 N NaOH for 1 hr at 60°C and neutralized in 1.5 ml 0.067 N HCl with 150 μl H_2O_2 for decolorizing. 100 μl aliquots were counted by liquid scintillation. In previously reported experiments (Brown and Andrake, 1990) using a 50 V stimulus, we demonstrated a vigorous voltage-dependent ^{45}Ca uptake but were unable to show inhibition by Cd or verapamil. The present experiments employ a stimulation voltage of 20 V and show that ^{45}Ca uptake is blocked by several agents, including: Cd, verapamil (Ver), nifedipine (Nif), Ni, and d-tubocurarine (d-TC), but not amiloride (Amil) (Figure 2).

Some insight into synaptic mechanisms may be gained by considering together our findings on evoked potential, calcium uptake, and neurotransmitter release. Using the superfusion technique, we examined the effects of several agents on ^{3}H-ACh release. These results are summarized in the Summary Table (below) along with the effects of the various test compounds on mV discharge and ^{45}Ca uptake. d-Tubocurarine is an antagonist of nicotinic ACh receptors and should block those receptors on the post-synaptic electrocytes. d-Tubocurarine does not affect ACh release, although it does block evoked discharge and Ca uptake. This fact suggests that most of the Ca uptake measured in our experiments is post-synaptic (electrocytes). The L Ca-channel antagonist, nifedipine, also blocks Ca uptake and evoked potential without affecting ^{3}H-ACh release. This indicates that nifedipine is also acting post-synaptically and does not interfere with pre-synaptic Ca-dependant ACh release. In contrast, Ni, which is characterized as a T Ca-channel antagonist, does block ACh release. These properties are shared by Cd and verapamil, suggesting that the effects of Cd, Ni, and verapamil on electric organ are (at least in part) to block pre-synaptic T Ca-channels. In some systems amiloride has been shown to block T Ca-channels; however, it had no effect on either ACh release or Ca uptake, while inexplicably preventing evoked discharge in electric tissue.

The present 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. Sections of this organ allow physiological changes and neurochemical changes to be measured in the same preparation. Our results demonstrate that Cd inhibits the electrical discharge of skate electric organ by blocking evoked neurotransmitter (ACh)

release. Further, the evoked release of ACh from Raja nerve endings is dependent on Ca uptake through channels with T properties. Voltage-dependent Ca uptake through these channels is blocked by Cd, Ni, and verapamil. Electrical discharge by Raja electrocytes results from nicotinic ACh receptor-activated Ca uptake through channels with L characteristics.

Summary Table

	Discharge	Ca-uptake	ACh Release
d-Tubocurarine (100 μM)	Blocks	Blocks	No effect
Nifedipine (100 μM)	Blocks	Blocks	No effect
Verapamil (100 μM)	Blocks	Blocks	Blocks
Cadmium (100 μM)	Blocks	Blocks	Blocks
Nickel (5 mM)	Blocks	Blocks	Blocks
Amiloride (1 mM)	Blocks	No effect	No effect

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