ELECTRICAL DISCHARGE AND ACETYLCHOLINE OUTPUT FROM *RAJA ERINACEA* ELECTRIC ORGAN ARE DECREASED BY CADMIUM

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The electrical organ in the tail of the skate, *Raja erinacea*, is a promising model system for the study of neurotransmitter dynamics and release mechanisms. The uptake of labeled choline (Ch), the synthesis and release of acetylcholine (ACh), and the electrical properties of this tissue have been recently described (Brown, Bull. MDIBL 27:116-119, 1987-1988). Preliminary experiments further indicated that the stimulus-response coupling in this organ was inhibited by exposure to low concentrations of cadmium. Thus, it may be productive to study the interactions of cadmium with this system.

Electric tissue was prepared for *in vitro* experiments as described previously (Brown, op. cit.). Tissue sections were incubated in oxygenated shark Ringer's buffer (Sawyer and Beyenbach, Am. J. Physiol. 249:F884-F890, 1985) containing various concentrations of calcium chloride (1-10 mM) and cadmium chloride (0, 1-100 μM). Tissue sections (approximately 2 cm x 3 mm) were kept at 15-19°C and were stimulated from end to end with a Grass stimulator, using 20 v pulses of 0.1 msec duration at 10 Hz. Electrical events over the central 1 cm were monitored on a Tectronix oscilloscope. For Ch uptake and ACh release experiments, tissue sections were pre-stimulated and incubated in buffer containing tritium-labeled Ch (specific activity 80 Ci/mmol). Tritiated samples were counted by liquid scintillation.

Stimulation of *Raja* electric organ sections (20 v, 0.1 msec at 10 Hz) resulted in an electrical discharge, the amplitude of which fatigued with time. This exponential fatigue is shown in Figures 1 and 2, also shown are the effects of incubation in cadmium for 1 hr on the electrical discharge (incubation was followed by 1/2 hr wash in control buffer before stimulation). Concentrations of cadmium from 1 to 100 μM both decreased and delayed the maximum discharge amplitude in a dose-related fashion. These effects of cadmium were partially overcome by increasing the extracellular calcium concentration. Figure 1 plots the electrical discharge amplitude with time for a representative tissue section incubated in buffer containing 2 mM calcium and increasing concentrations of cadmium; the line for 100 μM cadmium was not drawn since it was 0 mv at all times. Figure 2 is from a tissue section incubated in buffer containing 6 mM calcium, and clearly shows the cadmium effect being partially antagonized by the higher calcium concentration. In 4 mM calcium (the approximate *Raja* plasma concentration), cadmium inhibited the electrical discharge amplitude with an IC_{50} of 30 μM. This concentration of cadmium (30 μM) also caused a delay in the maximum discharge amplitude by 2.2 min in 4 mM calcium.

Incubations in buffer containing ^{3}H-Ch showed that cadmium (0.1 to 100 μM) had no effect on the uptake of labeled Ch into electric tissue. However, cadmium did inhibit the evoked release of ACh, an effect that could also be partially overcome by increasing the calcium concentration. Tissue sections pre-loaded with ^{3}H-Ch were washed in unlabeled buffer (with or without cadmium) for 1 hr, placed in a drip-flow chamber, and superfused with buffer (with or without cadmium). Superfusate was collected (for scintillation counting) for 2 min periods during several cycles of electrical stimulation and rest. The results of a typical experiment with 1 and 8 mM calcium are shown in Figure 3. Plotted are the cpm for each 2 min superfusate collection against time. Electrical stimuli of 20 v at 10 Hz were applied for 2 min at the times indicated by the bars on the graph (8-10 min and 22-24 min). Stimulation resulted in the release of ^{3}H-ACh. 100 μM cadmium completely inhibited

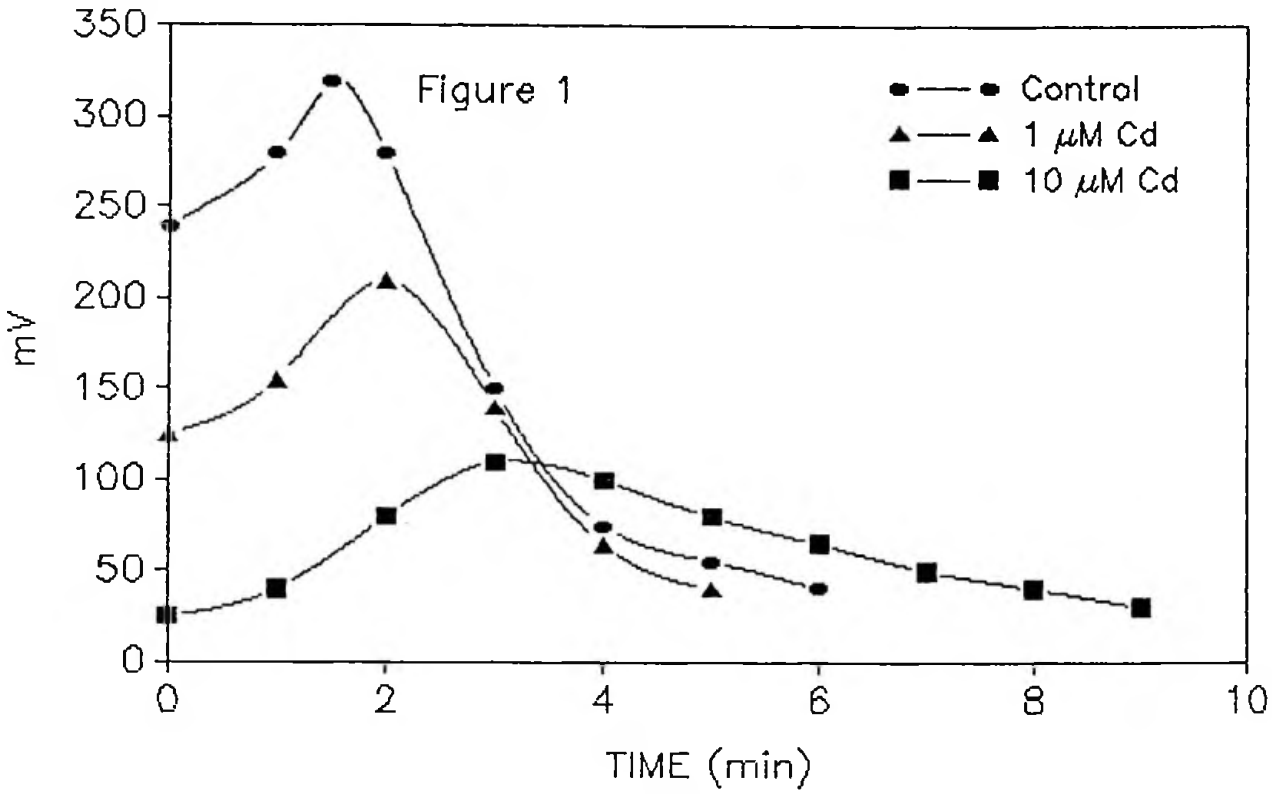


Figure 1
Control
1 μM Cd
10 μM Cd
mV
350
300
250
200
150
100
50
0
0
2
4
6
8
10
TIME (min)

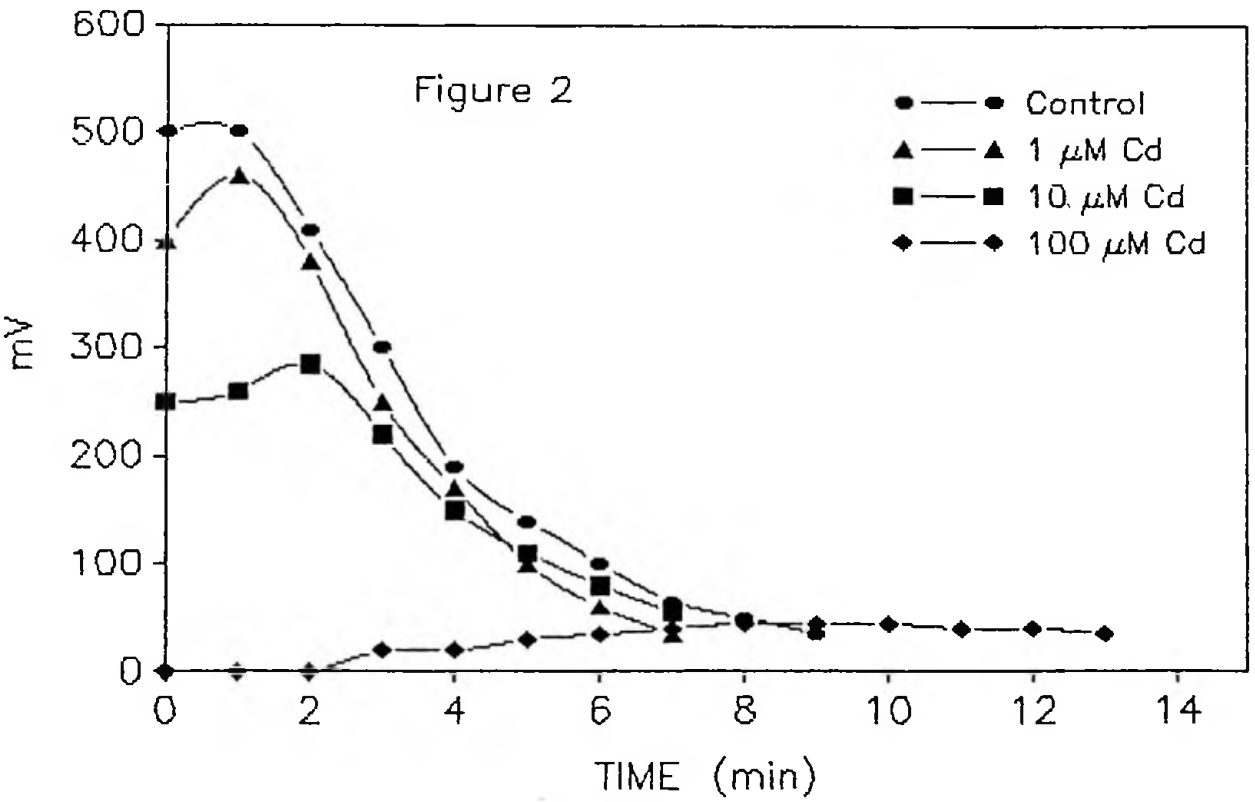
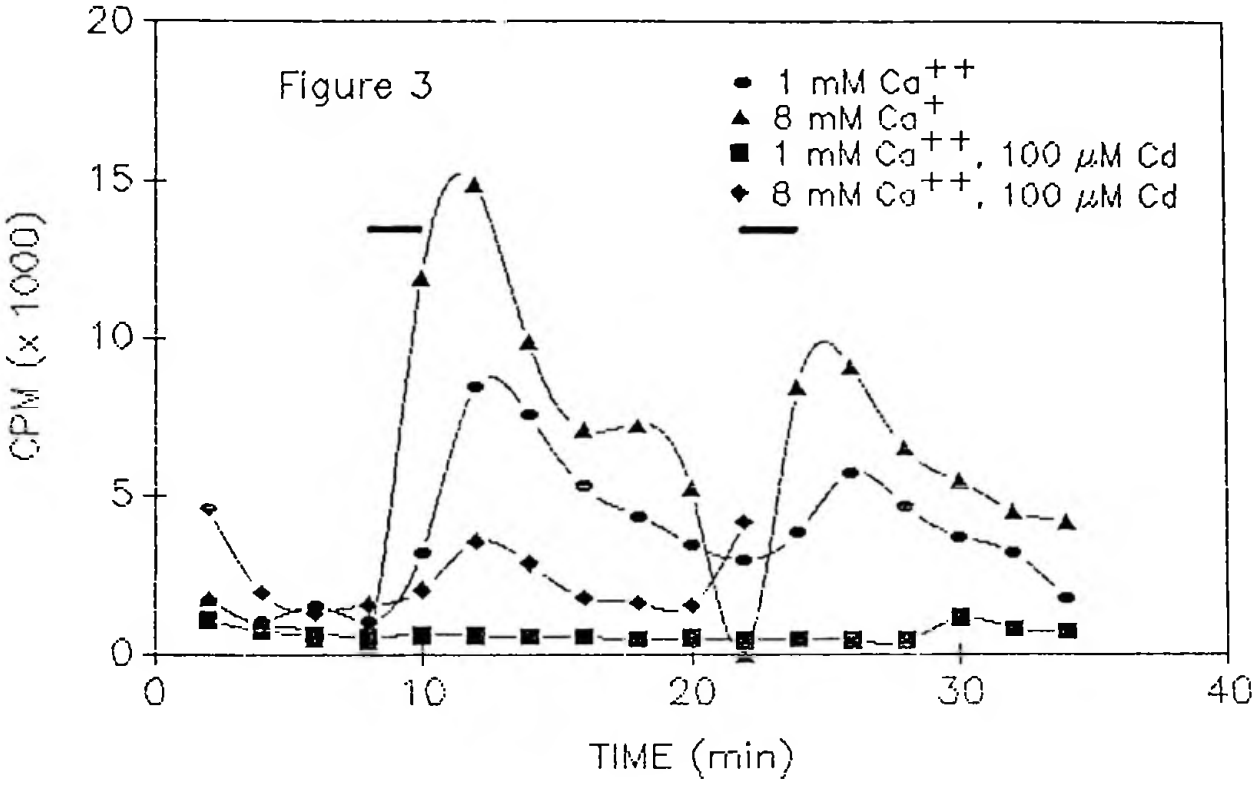


Figure 2
Control
1 μM Cd
10 μM Cd
100 μM Cd
mV
600
500
400
300
200
100
0
0
2
4
6
8
10
12
14
TIME (min)



^{3}H-ACh release from tissue incubated in 1 mM calcium, and caused approximately 85% inhibition in 8 mM calcium.

The release of a neurotransmitter is triggered by the entry of calcium into the nerve terminal through membrane channels. The present experiments demonstrate that cadmium inhibits both evoked ACh release and the electrical discharge of Raja erinacea electric organ. It is likely that these effects of cadmium result from a block of calcium entry, as they can be partially antagonized by increasing the concentration of extracellular calcium. Cadmium not only decreased the amplitude of the electrical discharge, but it also caused delay. This finding suggests that cadmium may act as an antagonist at intracellular calcium binding sites, in addition to its expected action of blocking calcium entry (Guan, et al., Can. J. Physiol. Pharmacol. 65:2131-2136, 1987).

The Raja electric organ appears to be a sensitive system in which to study the mechanisms of cadmium toxicity in the nervous system.

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