CADMIUM TOXICITY IN RAJA ERINACEA ELECTRIC ORGAN

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We have been studying the effects of cadmium (Cd) on synaptic transmission in the electric organ of the skate, Raja erinacea. This unique tissue is serving as a model system to help determine the mechanism of action of Cd toxicity on excitable membranes. Our previous reports have described the electrical properties of this preparation (Brown, Bull. MDIBL 27:126-119, 1987-88), and the decrease in evoked electrical discharge resulting from exposure to Cd (Brown and Andrake, Bull. MDIBL 28:109-111, 1989; and FASEB J. 3:A890, 1989). The present report expands upon these initial observations.

Electric organs were dissected from the tail of the skate as described previously (Brown, op. cit.). Tissue sections were incubated in oxygenated Raja buffer (see below) containing Ca chloride (2-10 mM) and Cd chloride 0, 1-100 μM). Tissue sections (2.3 cm x 3 mm) were kept at 15-19°C and received end to end supramaximal electrical stimulation; 20-50 V, 0.1 msec, 10 Hz (Grass stimulator). Electrical events over the central 1 cm were monitored on a Hitachi VC 6025 digital oscilloscope. Higher concentrations of Ca and Cd resulted in precipitate formation (presumed to be carbonates) in shark Ringer's buffer. Thus, we developed the following Raja buffer which is based upon HEPES rather than bicarbonate, and reflects the various components of Raja plasma as outlined by Marin (In: Sharks, Skates, and Rays, 287-292, 1967): (in mM) urea 350, NaCl 270, TMAO 70, HEPES 10, KCl 6, glucose 5, $CaCl_2$ 4, $MgCl_2$ 3, NaH_2PO_4 1, Na_2SO_4 0.5; pH = 7.6. The osmolality of this buffer was 981 mOsm, which is similar to that measured in skate plasma; 1002 ±15 mOsm (x ± SD, n = 5, by freezing point depression). Electrical stimulation of electric organ sections resulted in an evoked electrical discharge of approximately 150-250 mv per cm. Cadmium (1 to 100 μM) caused a decrease and a delay in the maximum discharge amplitude; effects which were concentration-related and were overcome in part by increasing the extracellular Ca concentration. Both of these phenomena are shown in Figure 1, which plots the maximum discharge amplitude (normalized) for tissue sections (n = 4-8) incubated for 1 hr in buffer containing various concentrations of Cd and Ca. The concentration-dependent inhibition by Cd is clearly shown, as is the ability of Ca to partially antagonize this inhibition. The IC_{50} for this effect of Cd is 30 μM (in 4 mM Ca). Other experiments on tissue pre-loaded with ^{3}H-choline confirmed our earlier observations that 100 μM Cd blocked the evoked release of acetylcholine (ACh).

These studies indicate that Cd inhibits both evoked ACh release and electrical discharge of skate electric organ. It is possible that these effects of Cd result from a block of Ca entry, as they can be partially antagonized by increasing the concentration of extracellular Ca. To explore this, we conducted Ca uptake experiments, using the following sequential protocol: a) tissue sections were prepared as described above; b) sections were pre-incubated for 1 hr in Raja buffer (4 mM Ca) with (Experimental) or without (Control) test compound (Cd, verapamil, d-tubocurarine); c) sections were then incubated for 10 min in identical media with the addition of 2 μCi/ml ^{45}Ca; d) half of the sections were electrically stimulated (50 V, 0.1 msec, 10 Hz) for 5 min (other half = Resting); e) sections were then washed for 30 min each in 3 successive changes of Ca-free Raja buffer containing 2 mM EGTA; f) sections were digested (in 1 ml 1 N NaOH for 1 hr at 60°C) and neutralized (1.5 ml 0.067 N HCl with 150 μl H_2O_2 for decolorizing); and g) aliquots were counted by liquid scintillation.

Figure 2 shows the results of several Ca uptake experiments, presented as CPM ^{45}Ca/mg for Resting and Stimulated tissue. Electrical stimulation increased Ca uptake several-fold; however, neither Cd (30 and 100 μM) nor the Ca-channel blocker, verapamil (100 μM), effectively inhibited this voltage-dependent Ca uptake. The nicotinic ACh receptor blocker, d-tubocurarine, was also ineffective, suggesting that the observed Ca uptake was presynaptic. Since Cd did not block Ca uptake, but did block ACh release, the presynaptic site for Cd toxicity may be the Ca-dependent neurotransmitter release mechanism. All three of the tested agents (at 100 μM), Cd, verapamil, and d-tubocurarine, completely blocked evoked discharge in this preparation. Ca "L" channels are reported to be sensitive to verapamil and Cd, whereas Ca "T" and "N" channels are not so. These facts would indicate that the voltage-sensitive Ca channels in the Raja electric organ are not of the L type. To characterize the Ca channels in this tissue, future Ca uptake experiments will be conducted with antagonists (e.g., nickel and amiloride) of the T and N Ca channel.

Cd inhibits the physiological response (electrical discharge) of the Raja electric organ by preventing ACh release. This effect on ACh transmission appears to involve Ca, but the exact mechanism has yet to be determined.

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Figure 1.

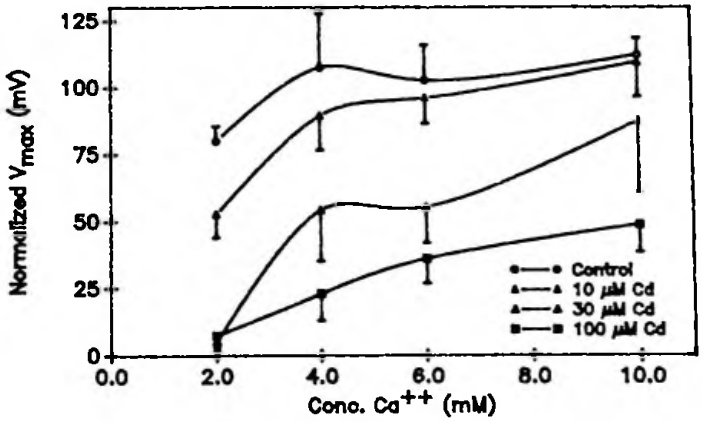

Figure 2.