The Effects of Cadmium on Hemodynamics and Transport of Ammonia Across the Gill of the Spiny Dogfish, Squalus acanthias

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The passive and active steps involved in the mechanisms of solute transport across the fish gill mimic those present in the mammalian nephron and other epithelia (e.g. Andreoli et al., Physiology of Membrane Disorders, Plenum Medical Book Co., New York, 1066 pgs., 1986). In addition, gill function is dependent upon the complex branchial vasculature which is thought to be the evolutionary precursor of the coronary vessels of the terrestrial vertebrates (Keys & Bateman, Biol. Bull. 63:327-336, 1932). Thus, the fish gill provides an animal model system for investigating the effects of heavy metals on both epithelial transport and vascular hemodynamics, two known sites of action of these environmental contaminants (e.g., Samarawickrama, In: The Chemistry, Biochemistry and Biology of Cadmium, M. Webb. Ed., Elsevier/North Holland, Amsterdam, pgs. 341-421, 1979).

Dogfish pup heads were perfused as previously described (Evans & Claiborne, J. Exp. Biol. 105:363-371, 1983; Evans & Robbins, Bull. MDIBL 25:166-167, 1985). After control periods for the determination of either afferent pressures or total ammonia effluxes, cadmium was added to either the perfusate or irrigate as $CdCl_2$. In some experiments, dithiothreitol (DTT; Cleland's reagent) was added to determine if the response to the heavy metal was reversible, and in others the alpha-adrenergic antagonist phentolamine or muscarinic cholonergic antagonist atropine was added to examine the role of these receptors in the hemodynamic response to Cd^{2+}. In most experiments, erioglaucine was added to the perfusate for a final experimental period in order to estimate structural leaks. Erioglaucine concentrations in the irrigate were measured at 632 nm.

Fig. 1 demonstrates that addition of even relatively low concentrations of $CdCl_2$ to the perfusate produces measurable vasoconstriction of the branchial vasculature of the dogfish pup; the effect is statistically significant ($p = 0.016$ compared to control) at perfusate concentrations as low as 0.1 μM or only 11 ppb. However, it is clear that 10 μM and 100 μM Cd^{2+} produce much more significant changes than lower concentrations. Whether this increased responsiveness at higher Cd^{2+} concentrations is due to a critical, cumulative sensitivity, or merely breakdown of the vascular tone is unknown. All responses to acute doses of Cd^{2+} were seen within 10 min after the addition of the metal.

Since earlier studies had demonstrated that vasoconstriction of the elasmobranch branchial vasculature can be produced by stimulation of either alpha-adrenergic receptors or muscarinic, cholinergic receptors (Evans & Claiborne, Bull. MDIBL 21:9-11, 1981; J. Exp. Biol. 105:363-371, 1983), we also examined the ability of phentolamine and atropine to inhibit the vasoconstrictory response to 10 μM Cd^{2+}. In addition, we tested the ability of dithiothreitol (DTT) to reverse the vasoconstrictive action of Cd^{2+}. The results are presented in Tables 1 and 2.

Table 1. The effects of perfusate phentolamine (0.1 mM) and DTT (1 mM) on the hemodynamic effects of 10 μM Cd^{2+} on the shark gill. All pressures are expressed as percent of initial, control pressure in the presence of DTT. Each fish was its own control. The mean afferent pressure of the control fish was 24.7 ± 4.3 torr (N = 6).

	Control	-->	Phen.	-->	Phen. + Cd	-->	DTT
Pressures:	100	-->	120 ± 11.3	-->	141 ± 9.6	-->	121 ± 13

0.1 mM phentolamine alone in the Ringer's perfusate produced significant vasoconstriction ($p = 0.03$ compared to control, N = 6), but subsequent addition of Cd^{2+} (in the presence of phentolamine) still produced further vasoconstriction ($p < 0.001$ compared to control) indicating that the Cd^{2+} effect was not via stimulation of alpha receptors. Washing with Ringer's containing DTT (1 mM) reduced the afferent pressure ($p < 0.02$ compared to phentolamine + Cd^{2+} period), but we have no explanation for the finding that washing with DTT did not reduce the afferent pressure to control levels.

Table 2. The effects of perfusate atropine (1 mM) and DTT (1 mM) on the hemodynamic effects of 10 μM Cd^{2+} on the shark gill. All pressures are expressed as percentage of initial, control pressure in the presence of DTT. Each fish was its own control. The mean afferent pressure of the control fish was 29.4 ± 4.2 torr (N = 6).

	Control	-->	Atr.	-->	Atr. + Cd	-->	DTT
Pressures:	100	-->	105 ± 6.0	-->	113 ± 8.2	-->	94.7 + 6.1

Perfusion with Ringer's containing 1 mM atropine did not increase pressure significantly above that in the previous DTT wash ($p = 0.1$ compared to control, N = 6). Addition of 10 μM Cd^{2+} (in the continued presence of atropine) increased the pressure slightly, but significantly ($p < 0.02$ compared to control period), indicating an apparently reduced stimulation (compare with stimulation in the presence of phenotolamine, Table 1) by the Cd^{2+} when muscarinic receptors were blocked. A final wash with DTT reduced the pressure to control levels ($p = 0.7$ compared to control). The present data do not allow a definitive statement about the role of muscarinic receptors in Cd^{2+}-induced vasoconstriction, but they do raise the interesting possibility that they may be involved.

A second series of experiments examined the effect of addition of Cd^{2+} to either the perfusate (serosal side) or irrigate (mucosal side) on the efflux of ammonia across the branchial epithelium of the perfused pup head. An initial control experiment determined that during three, sequential 20 min efflux periods the ammonia efflux (sum of both NH_3 and NH_4^+) declined by 17% during the second period, but remained stable during the third period (see Evans et al., this volume). The erioglaucine efflux at the end of the third experimental period was only 0.6 ± 0.8% (N = 9), indicating that structural leaks (and permeability to this molecule) were minimal. Other experiments have demonstrated that the erioglaucine leak is 0.84 ± 1.12% (64) under a variety of experimental conditions, exclusive of experiments using Cd^{2+} (see below). Addition of 1 μM Cd^{2+} to the perfusate after initial control period had no effect on the efflux of ammonia during either the second or third experimental periods, and the erioglaucine leak was 0.84 ± 1.1% (7). Addition of 100 μM Cd^{2+} to the perfusate did inhibit the ammonia efflux by some 25% (N

= 9, $p < 0.05$), but the efflux during the third period (second Cd^{2+} period) returned to control levels ($p > 0.3$). The erioglaucine leak under these conditions was 5.4 ± 1.0%, indicating that either structural leaks or increased permeability to erioglaucine were produced by these relatively high concentrations of Cd^{2+} in the perfusate, presumably because of significant increase in branchial pressure (see Fig. 1). However, either structural leaks or increased general epithelial permeability would have increased, rather than decreased, apparent total ammonia efflux. Thus is appears that 0.1 mM Cd^{2+} applied to the serosal surface is capable of at least transiently inhibiting ammonia efflux. Concurrent experiments on the mechanisms of ammonia efflux across the pup gill epithelium (Evans et al., this volume) indicate that a basolateral, butemanide-sensitive step is involved (presumably via NH_4^+ interaction with a Na-K-2Cl transporter), as well as paracellular diffusion of both NH_3 and NH_4^+ (Evans and More, in preparation), so future experiments will attempt to delineate the site of action of serosal Cd^{2+}.

Addition of 100 μM Cd^{2+} to the irrigate also produced significant inhibition of ammonia efflux (39%, N = 5, $p = 0.02$), which remained reduced (39%) during the second Cd^{2+} period, without any effect on either a final erioglaucine leak (0.88 ± 0.69%) or branchial pressure in either experimental period ($p = 0.25$, $p = 0.18$, respectively, compared to the initial, control period). Thus, it appears clear that addition of Cd^{2+} to the mucosal surface inhibits ammonia efflux. Since other experiments have demonstrated that addition of even 1 mM amiloride to the irrigate does not inhibit ammonia efflux across the pup gill it is apparent that the Cd^{2+} inhibition is not via inhibition of an apical Na^+/NH_4^+ exchange, but presumably via interaction with paracellular, diffusive pathways accessible from the mucosal surface. Future experiments will determine if the mode of action of mucosal Cd^{2+} is the same as serosal Cd^{2+}.

In summary, our experiments demonstrate that Cd^{2+} is vasoactive with the shark branchial vasculature, producing significant construction at ppb concentrations. Addition of 1 μM Cd^{2+} to the serosal side of the epithelium does not affect ammonia efflux, despite significant hemodynamic effects, but addition of 0.1 mM Cd^{2+} to either the serosal or mucosal side significantly inhibits ammonia efflux, possibly via interactions with the paracellular, diffusive pathways for NH_3 and/or NH_4^+, or basolateral carriers. (Supported by NIEHS grant 1 P50 ES 03828-01 to the Center for Membrane Toxicity Studies.)