VOLUME REGULATORY MECHANISMS IN HEPATOCYTES FROM RAJA ERINACEA: IMPAIRMENT BY MERCURY

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Skate hepatocytes exposed to hypotonic media swell in proportion to the decrease in extracellular osmolarity, but subsequently exhibit a regulatory volume decrease (RVD). In contrast to hepatocytes from other vertebrate species, RVD in skate hepatocytes is associated with the release of only a small fraction of intracellular K^+, but a substantial fraction of intracellular taurine, an amino acid found in relatively high concentrations (65 mM) in skate hepatocytes (Ballatori and Boyer, Am. J. Physiol., in press). RVD in skate hepatocytes is essentially abolished by pretreatment with 50 μM mercuric chloride (Ballatori et al. Toxicol. Appl. Pharmacol. 95:279, 1988). To further characterize the mechanisms involved in RVD and in mercury's ability to impair volume recovery, the present study used primary suspension cultures of skate hepatocytes (Smith et al., J. Exp. Zool. 241:291, 1987). Intracellular water space was determined as the difference between the ^{3}HOH and ^{14}C-inulin distribution spaces, and ^{14}C-taurine fluxes measured by a rapid centrifugation procedure (Ballatori and Boyer, Am. J. Physiol. 254:R801, 1988).

The effects of metabolic poisons and sulfhydryl reagents on RVD is shown in Table 1. As previously observed with mercury, 2,4-dinitrophenol, iodoacetate plus KCN, N-ethylmaleimide and diamide all inhibited RVD in skate hepatocytes. In contrast, 2 mM ouabain had no effect on volume recovery (Table 1). This concentration of ouabain has previously been shown to effectively inhibit $^{86}Rb^+$ uptake into skate hepatocytes (Ballatori et al., Biochim. Biophys. Acta 946:261, 1988). Thus, RVD is not directly influenced by Na-K-ATPase activity, but appears to be dependent on metabolic energy and reduced sulfhydryl groups.

Table 1. Effects of metabolic inhibitors, sulfhydryl reagents and ouabain on RVD.

	Relative Volume (%)				
	Minutes after dilution with 40% H_2O				
	1	5	20	40	60
Control	157±4	148±4	136±2	134±2	129±3
2,4-Dinitrophenol	156±6	154±6	149±6	151±7*	153±6*
Iodoacetate + KCN	158±9	160±8	155±8*	159±8*	160±8*
N-Ethylmaleimide	158±8	159±9	162±8*	164±7*	167±7*
Diamide	154±7	154±6	150±7	149±12	153±11*
Control	154±4	146±8	128±6	123±5	120±4
Ouabain	153±5	147±5	132±7	128±4	124±2

Cells were preincubated for 30 min with 0.5 mM 2,4-dinitrophenol (n=6), 1 mM iodoacetate plus 1 mM KCN (n=4), 1 mM N-ethylmaleimide (n=4) or untreated (n=6), before dilution with 40% H_2O. Osmolarity was decreased from ~940 to 564 by addition of H_2O. Ouabain (2 mM, n=7) was added 2h before hypotonic challenge. Values are means ±SE. *Significantly different from control, $p<0.05$, with Student t test.

Because both K^+ and taurine contribute to RVD, additional studies examined the effects of mercury on intracellular K^+ content and taurine fluxes. Skate hepatocytes treated with 50 µM mercury lost 42% of intracellular K^+ over 2h (from 0.416 to 0.242 µEq/mg protein), whereas intracellular Na^+ increased from 0.156 to 0.571 µEq/mg protein over the same time interval. Because the gain in Na^+ is more than double the K^+ lost, Na^+ entry probably contributes to both the mercury-induced cell swelling and its inhibition of RVD. In addition, mercury pretreatment inhibited the rapid initial release of taurine normally observed after cell swelling. The inability to release this osmolyte may also contribute to the observed inhibition of RVD.

The reversibility of mercury's effects on RVD was examined by adding the sulfhydryl-containing compounds glutathione (GSH) and dithiothreitol (DTT) at various times after mercury addition (Table 2). DTT was able to nearly completely reverse the effects on RVD even when added as late as 3 min after mercury administration. In contrast, GSH blocked the effects of mercury only when added simultaneously with the toxic metal. GSH addition at 1 or 3 min post mercury only partially reversed the inhibition of RVD (Table 2). Because DTT enters cells relatively easily, whereas exogenous GSH is largely excluded from the intracellular space, these observations indicate that mercury is interacting with intracellular components to elicit its effects on RVD.

Table 2. Dithiothreitol (DTT) and glutathione (GSH) reverse the mercurial inhibition of RVD.

	Relative Volume (%)			
	Minutes after dilution with 40% H_2O			
	1	10	30	60
Control	156±1	141±2	126±4	118±4
DTT, 0.5 mM	159±3	144±2	128±3	128±4
$HgCl_2$, 0.05 mM	159±2	165±7	175±13	188±20
$HgCl_2$ plus DTT at t=0 min	158±5	142±4	129±7	120±4
$HgCl_2$ plus DTT at t=1 min	162±3	146±2	129±3	120±2
$HgCl_2$ plus DTT at t=3 min	163±5	150±5	138±2	124±3
Control	157±1	143±3	125±2	118±2
GSH, 0.5 mM	156±3	140±3	124±3	115±1
$HgCl_2$, 0.05 mM	159±1	163±5	173±10	186±15
$HgCl_2$ plus GSH at t=0 min	155±3	143±3	129±4	117±6
$HgCl_2$ plus GSH at t=1 min	163±2	162±2	159±5	157±5
$HgCl_2$ plus GSH at t=3 min	161±2	162±5	159±4	158±9

Cells were diluted with 40% H_2O at time zero. Where indicated, this H_2O also contained $HgCl_2$ to give a final concentration in the cell suspension of 50 µM. DTT (n=3) and GSH (n=4) were added at either 0, 1, or 3 min. Values are means ±SE.

These findings suggest that RVD in skate hepatocytes is an energy and sulfhydryl-dependent process. Mercury appears to be interacting with intracellular components, and presumably sulfhydryl groups to elicit its inhibitory effects on RVD.

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