REGULATION OF SWELLING-ACTIVATED TAURINE EFFLUX IN SKATE (*RAJA ERINACEA*) HEPATOCYTES BY ATP AND PROTEIN PHOSPHATASE INHIBITORS, BUT NOT BY ARACHIDONIC ACID METABOLITES

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Cell volume regulation following hypoosmotic swelling is mediated in part by plasma membrane channels that allow taurine and perhaps other intracellular organic osmolytes to efflux from the cell. We demonstrated that skate hepatocytes also possess a swelling-activated taurine channel, and that the channel is regulated by intracellular ATP (Ballatori et al., Am. J. Physiol. 267:G285-G291, 1994; Ballatori and Boyer, Am. J. Physiol. 262:G451-G460, 1992; Ballatori et al., Mol. Pharmacol. 48:472-476, 1995). Channel opening is nearly completely blocked when ATP levels are lowered to approximately 50% of control in skate hepatocytes (Ballatori et al., Mol. Pharmacol. 48:472-476, 1995). Nonhydrolyzable analogues of ATP also sustain a swelling-activated anion conductance in skate hepatocytes (Jackson et al., Am. J. Physiol. 270:C57-C66, 1996), indicating that ATP binding rather than hydrolysis may be required for channel opening. Because the channels have not yet been isolated, it is not known whether this putative ATP activation site is on the channel itself or on an accessory regulatory protein.

Although intracellular ATP is required for proper function, the signaling pathways that mediate channel activation and inactivation have not been identified. A role for polyunsaturated fatty acids and their metabolic products has recently been suggested by studies in astrocytes and C_6 glioma cells (Sanchez-Olea et al., Am. J. Physiol. 269:C96-C102, 1995; Strange et al., Am. J. Physiol. 265:C244-C256, 1993). In order to test this hypothesis in our skate hepatocyte system, and to examine the effects of protein phosphatases in this process, we measured volume-activated [^{14}C]taurine efflux, and in some instances cell volume changes, in skate hepatocytes pretreated with modulators of arachidonic acid metabolism and protein phosphatase activity. Cellular ATP levels were measured to confirm that the effects of the various drugs were not due to changes in intracellular ATP levels, which would indirectly affect channel activation. Hepatocytes were isolated from male skates (*Raja erinacea*) and were preloaded with [^{14}C]taurine by incubating with 0.2 mM [^{14}C]taurine for 2 h at 15°C (Ballatori and Boyer, Am. J. Physiol. 262:G451-G460, 1992). Hepatocytes were then washed to remove extracellular radioactivity, and cells were pretreated with various drugs, as described below. Hypotonicity was induced by diluting the cell suspensions 40% with water. ATP was measured by the HPLC method of Hill et al. (Methods Enzymol. 148:132-141, 1987).

Swelling-activated taurine efflux from skate hepatocytes was inhibited in a concentration-dependent manner by the polyunsaturated fatty acids linoleic and linolenic acids (Table 1), confirming previous findings in astrocytes. However, inhibition of taurine efflux was observed only at concentrations of fatty acids that also decreased cellular ATP levels. Taurine efflux was also inhibited by 5,8,11,14-eicosatetraynoic acid (ETYA), an acetylenic analogue of arachidonic acid that inhibits cyclooxygenase, lipoxygenase and cytochrome P450, and by nordihydroguaretic acid, a lipoxygenase inhibitor, but once again the extent of inhibition was related to the extent of ATP depletion (Table 1). In contrast, arachidonic acid itself, as well as other inhibitors of cytochrome P450 activity (proadifen, metyrapone, and α-naphthoflavone) had no effect on either swelling-

activated taurine efflux or cellular ATP levels (Table 1), indicating that arachidonic acid metabolites do not regulate swelling-activated taurine efflux in skate hepatocytes.

TABLE 1. Inhibition of swelling-activated [^{14}C]taurine efflux by polyunsaturated fatty acids and modulators of arachidonic acid metabolism is related to the extent of ATP depletion.

	Concentration		[^{14}C]taurine efflux	ATP Content
	(μM)	n	*(% Control)*	
Arachidonic acid	100	3	95±3	103±4
	250	4	94±4	93±5
	500	5	84±6	92±3
ETYA	100	6	89±6	94±6
	200	3	80±3*	82±4*
	500	4	58±6*	74±3*
Linoleic acid	25	4	103±3	99±2
	100	4	94±7	89±3
	500	4	2±1*	38±7*
Linolenic acid	25	4	103±3	95±4
	100	4	101±3	95±2
	500	4	9±4*	43±6*
Nordihydroguaiaretic acid	50	5	95±4	93±2
	100	5	81±7	83±5
	200	4	63±16*	62±6*
Proadifen	100	4	108±2	103±5
	500	4	101±4	95±6
Metyrapone	100	4	105±4	103±5
	500	4	116±5	101±5
α-Naphthoflavone	100	3	104±5	97±7
	500	3	97±8	108±2

Values are means ± SEM. *Significantly different from controls, $P<0.05$.

The role of protein phosphatases in taurine efflux was examined in cells treated with microcystin-LR (10 μM), okadeic acid (2 μM), and calyculin A (2 μM). Each of these agents inhibited swelling-activated taurine efflux by ~30%, without affecting cellular ATP levels. These agents also inhibited the ability of skate hepatocytes to undergo the normal regulatory volume decrease as determined by video-microscopic analysis of individual skate hepatocytes perfused with the same concentration of inhibitors on the stage of an inverted Zeiss microscope (Fletcher et al., MDIBL Bull. 35:62, 1996). Cells swelled in response to hypotonic stress but did not volume regulate in the presence of these inhibitors when compared to control cells. Measurement of intracellular protein phosphatase activity demonstrated that most of the activity in skate hepatocytes is of type 1, and that this was nearly completely inhibited by a 30 min incubation with 10 μM microcystin-LR or 2 μM calyculin A. Okadeic acid, an inhibitor of phosphatase type 2A, only decreased hepatocyte phosphatase activity by approximately 20%. Because okadeic acid is a relatively selective inhibitor of phosphatase type 2A, the inhibition of taurine efflux may be mediated by this protein phosphatase activity.

The present study supports the previous finding that intracellular ATP is required to activate the volume-sensitive taurine channel of skate hepatocytes. The effects of lipoxygenase inhibitors and of unsaturated fatty acids were related to their ability to lower intracellular ATP, rather than their ability to alter arachidonic acid metabolism. In contrast, protein phosphatase inhibitors decreased swelling-stimulated taurine efflux without affecting cellular ATP levels, indicating an additional mode of regulation, although the mechanism involved has not yet been identified. (Supported by ES03828, ES01247, ES06484, DK34989, DK25636, and DK48823, and by the NSF Young Scholars Program, ESI9452682).