EFFECT OF CADMIUM ON ACTIN CYTOSKELETAL STRUCTURE IN SKATE (RAJA ERINACEA) HEPATOCYTES

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Cadmium (Cd^{2+}) is a heavy metal pollutant of the environment which progressively accumulates in the liver, kidneys and reproductive tissues of mammals and marine animals. The mechanism by which Cd^{2+} damages tissues is unclear. One of the earliest detectable effects of cadmium is an increase in permeability to low molecular weight proteins across mammalian epithelia. Prior studies suggest that this is due to the disruption of tight junctions between epithelial cells. The purpose of this study was to examine the effects of Cd^{2+} on cytoskeletal organization and tight junction permeability in polarized skate hepatocytes.

Hepatocytes from the little skate (Raja erinacea) were isolated by the method described by Smith and Boyer (J. Exp. Zool. 241:291, 1987), resuspended in elasmobranch Ringer's solution and maintained at 15°C. Immunocytochemical studies were done to study the effects of Cd^{2+} on the structural arrangement of F-actin, α-actinin and zona occludens-one (ZO-1), all important in tight junction formation. For these studies, hepatocytes were treated with $CdCl_2$ (100μM, 300μM, 600μM and 1mM) for 30-90min, then allowed to adhere to poly-L-lysine coated coverslips. Cells were fixed in 100% acetone and treated with rhodamine phalloidin for the staining of F-actin, and rat anti-ZO-1 polyclonal antibodies or bovine anti-α-actinin polyclonal antibodies, followed by fluorescein conjugated secondary antibodies, for the staining of ZO-1 and α-actinin. The cells were viewed with a Nikon Microphot 5-A epifluorescent microscope (60X objective, N.A. 1.4). In control (untreated) hepatocytes, actin filaments were present in the pericanalicular and submembranous cortical regions of the cell as described previously by Henson et al. (J. Exp. Zool. 271:273, 1995). α-actinin was present predominantly in the pericanalicular region of hepatocytes. ZO-1 staining was faint and not found in all hepatocyte clusters studied. Different fixation methods did not improve ZO-1 staining. No changes in actin or α-actinin organization were detected in skate hepatocytes treated with $CdCl_2$ (100μM over 90min and up to 600μM over 30 min). Hepatocytes exposed to 1 mM $CdCl_2$ showed both an enlargement in canalicular lumen size and an irregular (ruffled) cortical staining pattern of actin when compared to control cells. This may have been due to cell damage, as confocal fluorescence microscopic studies showed that exposure of hepatocytes to 1mM $CdCl_2$ caused a rapid uptake of fluorescent dextrans into the hepatocyte cytoplasm.

Studies were started to examine the effects of Cd^{2+} on tight junction permeability by measuring the uptake of fluorescent dextrans into the canalicular lumen of hepatocyte clusters using confocal fluorescence microscopy (Meridian Insight Plus frame scanning confocal microscope). Skate hepatocyte clusters with lumens were difficult to identify because of the small lumen diameter. For this reason, hepatocytes were treated with PMA, a phorbol ester which activates PKC and has been shown to increase tight junction permeability in rat hepatocyte clusters (Nathanson et al., Am. J. Physiol., 262:G1079, 1992 and 269:G789, 1995). Treatment of hepatocytes with PMA (1μM) in the presence of fluorescein-dextran (3000 M.W.) resulted in the appearance of dye in the canalicular lumen. Further studies need to be done to determine whether this was due to dye movement through the paracellular space or by transcytosis.

In conclusion, preliminary studies suggest that acute exposure of skate hepatocytes to high concentrations of Cd^{2+} does not alter skate hepatocyte cytoskeletal structure. In contrast, cultured mammalian epithelial cell monolayers develop a decrease in transepithelial resistance (Janecki et al., Tox.

Appl. Pharm., 112:51, 1992) and alterations in F-actin organization (Prozialeck et al., Tox. Appl. Pharm., 107:81, 1991) when exposed to much lower concentrations of Cd^{2+}(10-50μM), albeit over a longer exposure time. Whether Cd^{2+} can alter tight junction permeability without altering cytoskeletal structure in skate hepatocytes, perhaps by interfering with Ca^{2+}-dependent intercellular bridge formation, has not been determined. These findings suggest that skate hepatocytes, unlike mammalian epithelia, are relatively resistant to the effects of Cd^{2+} on cytoskeletal structure. Skate hepatocytes have also been shown to be relatively resistant to the effects of Cd^{2+} on cellular metabolism (Ballatori et al., Tox. Appl. Pharm., 95:279, 1988). The effect of longer exposure times of Cd^{2+} on the cytoskeletal structure of skate hepatocytes needs to be evaluated.

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