Biphasic, GPER-dependent regulation of Bcrp by bisphenol A in killifish (*Fundulus heteroclitus*) renal proximal tubules

Anne Mahringer[1,2], S. Nickel[1], Florian Umstätter[3], Alexander Zaremba[1,2], David S. Miller[4] and Gert Fricker[1,2]
[1] Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, 69120 Heidelberg, Germany
[2] Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672
[3] Goethe University Frankfurt, 60438 Frankfurt am Main, Germany
[4] Laboratory of Signal Transduction, NIH/NIEHS, Research Triangle Park, NC 27709

One function of the kidney is to excrete potentially toxic foreign chemicals, a process that occurs in the proximal tubule. Breast Cancer Resistance Protein (Bcrp) is an ATP-driven transport protein, expressed in proximal tubule that does just that. Here we show that bisphenol a, an environmental estrogen, regulates Bcrp activity in killifish proximal tubules through a novel mechanism involving a non-classical estrogen receptor.

Breast Cancer Resistance Protein (Bcrp) is an ATP-driven drug efflux pump that is highly expressed in barrier and excretory tissues. Estrogen regulates Bcrp expression in human placenta, breast cancer cells in culture and in rat and mouse brain capillaries. However, available evidence suggests tissue-specific modes of regulation. In addition to the hormone (estradiol, E2), persistent environmental pollutants also act through estrogen receptors to elicit estrogen-like effects. Bisphenol A (BPA) is one such environmental estrogen. It has a lower binding affinity to estrogen receptors than E2 and it exerts only a weak, genomic down-regulation of Bcrp in *rat* brain capillaries compared to E2[1,3]. However, BPA also binds to G-protein-coupled estrogen receptor 1 (GPER) at pico- to nanomolar concentrations and elicits even stronger, immediate effects than E2[1,4]. Thus, BPA is able to alter estrogenic signaling by interacting with non-classical receptors. Here we show that in killifish renal tubules BPA has a biphasic effect on Bcrp transport activity, with nanomolar concentrations reducing activity and micromolar concentrations increasing activity.

We used confocal microscopy to measure steady-state accumulation of mitoxantrone, a fluorescent Bcrp substrate, in lumens of freshly isolated renal tubules as described previously[2]. Exposure to 10 or 100 nM BPA reduced Bcrp-mediated mitoxantrone secretion into the lumen. In contrast, micromolar concentrations of BPA and of estradiol increased Bcrp transport activity. BPA exposure did not affect transport activity of P-glycoprotein or Mrp4, but did reduce Mrp2 transport activity. G1, an agonist for GPR, had a similar biphasic dose response. The GPER antagonist, G15, abolished the responses of Bcrp to nanomolar and micromolar G1 and to nanomolar BPA, indicating action through GPER. Effects of micromolar BPA were only partially reduced by G15. However, the nonspecific ER antagonist, ICI182.780, together with G15 completely abolished the BPA-mediated stimulation of Bcrp transport, indicating involvement of classical estrogen receptors. The increase in Bcrp transport activity caused by exposure to micromolar concentrations of BPA involved intracellular signaling, since it was reduced by Brefeldin A, an inhibitor of vesicular trafficking, and by H89, an inhibitor of protein kinase A, but not by a PI-kinase inhibitor.

To date, regulation of Bcrp activity has been lined to the action of classical estrogen receptors. The present results for renal proximal tubule provide evidence for a novel mechanism of Bcrp regulation through GPER and intracellular signaling.

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