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ARSENIC AS AN ENDOCRINE DISRUPTOR: LOW-DOSE AFFECTS ON STEROID HORMONE RECEPTOR SIGNALING EXHIBITING COMPLEX DOSE-RESPONSE RELATIONSHIPS

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Arsenic is an environmental agent of considerable human health concern in the U.S. and around the world. Exposure to elevated drinking water arsenic has been associated with increased risk of several cancers, vascular disease, diabetes, and reproductive and developmental problems. These risks have been shown to be elevated in populations with drinking water arsenic in the range near the current U.S. EPA drinking water standard found in NH and many other areas of the U.S. We previously reported that arsenite can act as an endocrine disruptor, almost completely blocking hormone-mediated activation of gene expression by the glucocorticoid receptor (GR) in cultured rat hepatoma H4IIE cells. At the cell physiological level, arsenic was shown to block GR hormone-mediated control of cell growth. We investigated these effects further in the current work. Using various GR mutants in a GR deficient H4IIE-derived cell line, we also observed that the effects of arsenic on GR signaling most closely mapped to the central DNA-binding domain of GR, which is highly conserved among steroid hormone receptors in the nuclear receptor superfamily. Previous work had shown that arsenic does not compete effectively for zinc binding or alter overall structure within the two zinc fingers of this region. Further mutational analysis within this domain indicated that the two cysteines outside the zinc fingers are also not direct targets for arsenic binding or effects. No other mutations within this region ablated the arsenic effect; however, many of these mutations qualitatively altered the overall hormone response in the presence of arsenic. Hyper-fine dose-response analysis revealed marked changes in response to modest alterations in dose, suggesting a titration of arsenic into multiple binding sites with different affinities, which combine for the overall affect on GR function. The lowest doses used – in the 0.1 – 1 μ M range, actually increased hormone-mediated activation of GR, whereas slightly higher doses led to suppression of hormone activation which was almost complete at 2-3 μ M in these cells. We then examined whether arsenic would disrupt other steroid receptors within this larger family of highly conserved proteins. Arsenic altered the hormone-mediated regulation of gene expression by the estrogen, progesterone, mineralocorticoid and androgen receptors in a manner very similar to that of the glucocorticoid receptor, although there were qualitative differences and complex dose-response patterns reminiscent of the mutational analysis of GR. Other experiments have shown that arsenic has profound effects on physiological processes dependent on these steroid receptors at equivalent doses in vivo, suggesting that these alterations in gene expression result in pathophysiological effects that may contribute to arsenic-induced human diseases including cancer, vascular and cardiovascular disease, diabetes, and developmental and reproductive problems. (supported by NIEHS SBRP grant ES07373 – Project 2).