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188: 135-153, 2003). To investigate molecular mechanisms, we used GC-mass spectroscopy to measure levels of the ACR-cysteine adduct, S-carboxyethylcysteine (SEC), in isolated brain synaptosomes exposed to ACR (1mM – 1 M x 60 mins incubation). Previous *in vitro* studies showed that this concentration range produced graded inhibition of synaptosomal neurotransmitter release that was correlated to a reduction in free sulfhydryl groups (Neurotoxicology, in press, 2003). Results of the present study show that *in vitro* ACR exposure produced concentration-dependent increases in SEC (range 0.10 ± 0.03 – 5.6 ± 1.2 ng CEC/μg synaptosomal protein), which suggests that ACR impaired transmission by adduction of functionally important cysteine groups on nerve terminal proteins. To identify putative targets, we exposed synaptosomes to ¹⁴C-ACR (7 μCi) and then separated proteins by 2-D gel electrophoresis. Labeled proteins were detected by phosphoimaging and subsequently identified by overlay immunoblot analysis. Results show that ACR adducted numerous presynaptic proteins including N-ethylmaleimide sensitive factor (NSF) and SNAP-25, but not synaptobrevin. Inhibition of NSF activity by sulfhydryl alkylation has been shown to impair neurotransmission by preventing disassembly of the 7S SNARE complex. In our studies, *in vitro* exposure of synaptosomes to ACR (175-700 mM) significantly increased 7S complex levels as determined by immunoblot analysis (mean fold increases = 1.8 ± 0.22 – 5.5 ± 0.81). These results are consistent with the possibility that ACR disrupts neurotransmission by adducting sulfhydryl groups on cysteine residues of presynaptic proteins such as NSF or SNAP-25 which inhibits corresponding function. Supported by NIEHS grant ESO3830-17.

427 DEGRANULATION OF DURAL MAST CELLS BY *IN VIVO* AND *EX VIVO* OPIATE EXPOSURE.

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Opiates produce degranulation of some, but not all populations of mast cells (e.g. cutaneous vs mucosal). Recent work showing that continuous intrathecal delivery of high concentration morphine sulfate (MS) leads to inflammatory mass (granuloma) formation localized at the catheter tip in humans and animals led us to focus on the effects of opiates on dural mast cells. We examined dural mast cell degranulation following MS exposure *in vivo* and *ex vivo*. For *in vivo* studies, beagle dogs were implanted with chronic intrathecal lumbar catheters and received MS infusions for 28 days at a granuloma inducing concentration (12.5 mg/ml, 0.96 ml/day). At necropsy dura was harvested from lumbar and cervical regions and stained for biogenic amines with acidic Alcian blue (pH 1.4). For *ex vivo* studies dura was harvested from opiate naive dogs. Dura was stripped of pia-arachnoid and dissected into 10 mg sections. Sections were incubated in Krebs-bicarbonate (95%:5% O₂:CO₂) for 30 minutes in duplicate. The ability of MS or hydromorphone to induce histamine release was examined using Compound 48/80 (0.1 mg/ml) as a positive control. Histamine release was quantified by EIA. Dura samples were then stained with acidic Alcian blue to examine degranulation. *In vivo*, intrathecal infusions of MS produced degranulation of mast cells in the lumbar, but not cervical regions in dogs receiving 12.5 mg/ml MS. *Ex vivo*, MS (0.1 mM, approximating concentrations in dog CSF following 12.5 mg/ml infusion) more than doubled histamine release over control treatment. This represented over half of the 48/80 sensitive histamine pool. Hydromorphone (0.01 and 0.1 mM) produced an increase of approximately 75% in histamine release. *Ex vivo* histochemistry displayed mast cell degranulation following MS and hydromorphone incubation. These data suggest opiates produce degranulation of dural mast cells both *in vivo* and *ex vivo*. Dural mast cells may be involved in formation of granulomas due to chronic intrathecal opiate infusions and may represent a target for therapeutic intervention to prevent granuloma formation. Supported by DA-15353

428 DYSREGULATION OF DOPAMINE HOMEOSTASIS AND OXIDATIVE STRESS IN PCB-EXPOSED NEURONAL CELLS.

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PCB exposure has been associated with neurological and cognitive impairments. These adverse health effects are thought to arise from disrupted neurotransmission. Previous studies have demonstrated that PCBs can impair dopaminergic (DAergic) systems by inhibiting DA uptake into synaptic vesicles and DA reuptake into presynaptic terminals. This study tests the hypothesis that subtoxic PCB concentrations alter DA homeostasis, increase oxidative stress, and render DAergic neurons vulnerable to subsequent insult. The direct toxicity of PCB treatment was evaluated in MN9D cells, a mesencephalic-derived DAergic cell line. Cultures were exposed to 0-20ppm Aroclor 1254 for 3-48h then assayed for cell *viability* by measuring LDH release and WST-1 response. Cell *viability* did not differ significantly between control and treated cells except in response to 20ppm, where approximately 60% cells survived. Additional experiments analyzed whether PCB treatment modulates DA

levels, which could lead to reactive oxygen species (ROS) production and subsequent activation of antioxidant defense systems. Neurochemical studies demonstrated that subtoxic PCB treatments produced a concentration-dependent reduction in intracellular DA levels, which were accompanied by an increase in DA turnover. Furthermore, PCB treatment induced a time- and concentration-dependent elevation in ROS production, as detected by increased dichlorofluorescein staining. Immunoblot analyses revealed that PCB-induced ROS production were accompanied by coincident alterations in antioxidant defense enzyme expression. While MnSOD protein levels exhibited a concentration-dependent decrease with PCB treatment, CuZnSOD protein levels remained unchanged. Additionally, the upregulation of heme oxygenase-1 levels provides supporting evidence that PCBs stimulate an oxidative stress response in dopamine neurons. Although the intracellular source of ROS remains unknown, these results suggest that PCBs activate an oxidative stress-related pathway potentially by disrupting DAergic neuron function. Supported by NIH ES00375 and ES01247.

429 OVEREXPRESSION OF BCL-XL ALTERS THE SUSCEPTIBILITY OF PRIMARY RAT ASTROCYTES TO 1, 3-DINITROBENZENE.

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Exposure to 1, 3-dinitrobenzene (DNB) produces an edematous, glio-vascular lesion that is initially confined to brainstem nuclei with high energy requirements. Selective vulnerability of brainstem astrocytes to DNB is mediated by a ten-fold lower threshold for opening of the mitochondrial permeability transition pore (mtPTP). Concentrations of 100 μM DNB induce complete loss of mitochondrial membrane potential by 10 min in brainstem astrocytes. Cortical astrocytes remain unaffected by 100 μM DNB for at least 1 hour. The BCL family of proteins is known to modulate the open probability state of the mtPTP. It is hypothesized that the balance in expression of BCL-2 agonist and antagonist proteins dictates regional astrocytic susceptibility to DNB. Western blot analyses demonstrate that *in vivo* exposure to 30 or 50 mg/kg 1, 3-DNB does not significantly alter the constitutive expression of BCL-XL, BAX, or BCL-2 in either the brainstem (sensitive region) or cortex (non-sensitive region) of F344 rats or C57/Bl6 mice relative to that observed in the vehicle control (DMSO) animals. *In vivo* exposure to DNB also fails to induce subcellular translocation of BAX or BCL-XL from the cytosol to the mitochondria, where the proteins are known to interact with one another and the mtPTP. However, overexpression of BCL-XL in primary rat astrocytes significantly reduces DNB-induced loss in cell *viability* when evaluated by an MTT assay following a 24-hour exposure up to 1 mM DNB. Therefore, while DNB exposure alone is not sufficient to alter the ratio of death promoting to death repressing members of the BCL-2 family, genetic manipulation of this ratio alters the susceptibility of sensitive astrocytes to DNB. Supported by R01 ES08846.

430 1, 3-DINITROBENZENE INHIBITS THE PYRUVATE DEHYDROGENASE COMPLEX.

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Prolonged or repeated exposure to 1, 3-Dinitrobenzene (DNB) produces topographically comparable lesions to those observed in various pathological conditions of energy deprivation including thiamine deficiency, exposure to misonidazole, metronidazole, and inborn errors of metabolism. A key enzyme complex involved in energy metabolism, pyruvate dehydrogenase complex (PDC), acts as the central gateway between glycolysis and the more efficient energy-producing pathway, TCA cycle, through production of acetyl-CoA. A disturbance in PDC activity compromises the brain's ability to maintain sufficient ATP levels needed for proper cerebral function. The present study investigated the potential inhibitory effects of DNB on PDC. Purified porcine heart PDC was used to determine the effect of DNB on activity. Kinetic analysis of NADH absorbance showed a rapid dose-dependent inhibition of PDC by DNB (31.25μM-1mM). Approximately 45% inhibition of activity occurred at the lowest dose and greater than 90% inhibition was observed at the highest dose. Inhibition of activity was isomer specific. The para isomer of DNB did not inhibit PDC activity significantly at 250, or 500μM. Consistent with the kinetic analysis, Acetyl-CoA production was also inhibited in a dose-dependent manner by DNB as revealed by HPLC analysis. C6 Gliomas were used to investigate DNB toxicity as a function of energy metabolite levels. Cell morphology, LDH release and lactate production were used as indices of cell damage. Treatment of C6 gliomas with 1mM DNB resulted in cytoplasmic swelling, extensive vacuolation, increased LDH release and nearly a 45% increase in lactate production, relative to controls. Co-treatment with 5mM acetoacetate, which is converted to acetyl-CoA, diminished lactate production by approx. 20% and resulted in complete inhibition of LDH release compared to DNB alone. Morphologically, no