1361 5-HYDROXYTRYPTOPHAN TOXICOSIS IN DOGS.

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5-Hydroxytryptophan (5-HTP) is a nutritional supplement that has gained popularity within the alternative medical community because of its reported ability to treat conditions such as depression, chronic headaches and insomnia in humans. 5-HTP apparently exerts its effects by increasing the levels of serotonin in the body. Serotonin is a central nervous system neurotransmitter, has a stimulatory effect on smooth muscle of the gastrointestinal tract, and is a promoter of platelet aggregation. The objective of this study was to describe the clinical syndrome, toxic doses, and treatment methods associated with accidental 5-HTP ingestion in dogs. Searches of the database at the ASPCA National Animal Poison Control Center identified 15 cases of accidental ingestion of 5-HTP by dogs. Dosages of 5-HTP ingested ranged from 2.5 mg/kg to 557 mg/kg, with 27 mg/kg being the lowest dose at which clinical signs occurred. Clinical signs developed in 13 dogs (86.7%), with the onset of signs ranging from 10 minutes to 2 hours post-ingestion. The most common clinical signs included diarrhea (33%), tremors (33%), seizures (33%), hyperthermia (33%), mydriasis (27%), and ataxia (20%). In all cases where follow-up data were available (10/15), the dogs fully recovered within 24 hours with supportive treatment including anticonvulsants, IV fluids, and thermoregulation. The clinical syndrome seen in these dogs shares similarities with "serotonin syndrome" described in humans following ingestion of tryptophan or monoamine oxidase inhibitors.

1362 AN EXAMINATION OF THE BIOCHEMICAL AND BEHAVIORAL EFFECTS OF MPTP IN TWO SNAKE SPECIES.

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The synthetic neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces a Parkinsonian-like syndrome in humans and non-human primates, and also causes similar movement disorders in rodents, fish, amphibians and lizards. In this study, the behavioral and biochemical effects of MPTP were assessed in the black ratsnake *Elaphe o. obsoleta* and the banded watersnake *Nerodia f. fasciata*. We report that MPTP induces in *E. o. obsoleta* a depletion of norepinephrine, dopamine and serotonin in fore, mid and hindbrain regions and also impedes righting ability. In *N. f. fasciata*, norepinephrine and dopamine were also depleted by MPTP in all three brain regions, but serotonin was only significantly reduced in the forebrain and righting ability was not affected. This study demonstrates a behavioral and biochemical sensitivity to MPTP in *E. o. obsoleta* that differs from that in *N. f. fasciata*. This novel reptilian model is beneficial for assessing central dopaminergic and serotonergic involvement in motor control in the lower vertebrates.

1363 ACUTE AND LONG-TERM EFFECTS OF MPP+ IN CELL LINES COEXPRESSING PLASMA MEMBRANE AND VESICULAR DOPAMINE TRANSPORTERS.

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The plasma membrane dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT2) have been proposed to mediate the subcellular localization and ultimate toxicity of the active metabolite of MPTP, 1-methyl-4-phenylpyridinium (MPP^+). We have generated stable neuroblastoma cell lines (SK-N-MC) expressing either DAT or both DAT and VMAT2 to examine the effects of transporter expression on toxicity. Western blotting and immunofluorescence studies confirmed the expression of the transporter proteins. Basal levels of plasma membrane [^{3}H] dopamine uptake in either untransfected or VMAT2-expressing cell lines were 100 fmol/hr/10^6 cells and were unaffected by 10 μM nomifensine. Specific plasma membrane uptake was increased by 75-150 fold in cell lines expressing either DAT or DAT and VMAT2. Specific vesicular [^{3}H] dopamine uptake in digitonin-permeabilized VMAT2 cells was 500 fmol/hr/10^6 cells. Acute metabolic responses of these cell lines to MPP^+ were followed using a microphysiometer to measure proton excretion. Within 4 min. of application, MPP^+ (100 μM) caused a significant increase in proton excretion in DAT-expressing cells, which was attenuated by pretreatment DAT uptake inhibitors. The observed response was also attenuated in DAT/VMAT2 cells. Delayed toxicity was assessed by measuring the intercalation of the fluorescent probe, ethidium homodimer, into the DNA of dead cells. MPP^+ (10-500 μM) produced dose-dependent cell death in DAT-expressing cell lines during the 48 hr period after application. The rate of cell death (V_{max}) at an MPP^+ concentration of 10 μM was at least 40-fold higher than that in untransfected cells. In a cell line expressing DAT and VMAT2 (@ 500 μM) cell death was significantly attenuated compared to cells expressing DAT alone (V_{max} 110 and 240% of control). Together these data support the hypothesis that DAT acts as a gateway for MPP^+ entry into the cell and that intracellular compartmentalization of MPP^+ by VMAT2 can circumvent toxicity. (Supported by NIEHS 09248.)

1364 METHAMPHETAMINE NEUROTOXICITY : PROTECTIVE ROLE OF SELENIUM AND INVOLVEMENT OF S-ADENOSYLMETHIONINE.

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Methamphetamine (METH) is a drug of abuse known to cause dopaminergic neurotoxicity in rodents, non-human primates and humans. The present study was designed to evaluate if METH-induced dopaminergic toxicity can be protected by pre- and post-treatment with an antioxidant, selenium (Se) and how it correlates with changes in S-adenosylmethionine (SAM). Adult female C57BL/J6 mice were dosed with Se (0.5 mg/kg) in drinking water. One week later, animals were treated with 4 X 10 mg/kg METH, i.p. Se treatment was continued for the next week before mice were sacrificed, trunk blood was collected and the brains were quickly removed and dissected into caudate nucleus (CN), frontal cortex (FC) and hippocampus (HIP) for neurochemical analyses. Concentrations of dopamine (DA), serotonin (5-HT) and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) were measured using HPLC/EC. SAM levels were measured in these brain regions as well as in blood using HPLC/PD. METH treatment produced a significant depletion of DA and its metabolites (DOPAC & HVA) and a significant increase in SAM in CN. Pretreatment with Se prevented the depletion of DA and its metabolites and increase in the SAM level in CN resulting from the METH treatment. In contrast, SAM level was decreased in the blood of animals treated either with METH or Se but in the case of animals treated with METH and supplemented with Se, blood SAM levels were equivalent to control. These data suggest that SAM is involved in the manifestations of METH neurotoxicity and Se has a possible protective role against METH-induced neurotoxicity.

1365 INTRASTRIATAL, INTRACORTICAL, AND INTRAHIPPO-CAMPAL ADMINISTRATION OF GLUTATHIONE AND N-ACETYLCYSTEINE CONJUGATES OF α-MEHTYLDOPAMINE PRODUCES SEROTONERGIC NEUROTOXICITY.

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The serotonergic neurotoxicity of 3,4-(±)-methylenedioxyamphet-amine (MDA) and 3,4-(±)-methylenedioxymethamphetamine (MDMA) is dependent upon the systemic generation of a neurotoxic metabolite(s), the identity of which remains unclear. α-Methyldopamine (α-MeDA) is a major metabolite of both MDA and MDMA, and following oxidation to the *ortho*-quinone undergoes conjugation with glutathione (GSH). The thioether conjugates of α-MeDA maintain the ability to redox cycle and generate reactive oxygen species. We now report that direct intrastriatal or intracortical administration of 5-(glutathion-S-yl)-α-MeDA (4 X 200 nmol, 4 X 400 nmol), 2,5-*bis*-(glutathion-S-yl)-α-MeDA (4 X 150 nmol, 4 X 300 nmol), and 5-(N-acetylcystein-S-yl)-α-MeDA (4 X 7 nmol, 4 X 20 nmol) causes significant decreases in striatal and cortical serotonin (5-HT) concentrations, 7 days following the last injection. Interestingly, intrastriatal injection of 5-(glutathion-S-yl)-α-MeDA or 2,5-*bis*-(glutathion-S-yl)-α-MeDA, but not 5-(N-acetylcystein-S-yl)-α-MeDA, causes decreases in 5-HT concentrations in the ipsilateral cortex. A similar pattern of changes is seen in the ipsilateral striatum, when the thioether conjugates of α-MeDA are injected into the cortex. Intrahippocampal injection of the thioether conjugates of α-MeDA also pro-

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