

- 335.17 CYTOCHROME b561 IS ASSOCIATED WITH ASCORBIC ACID-REQUIRING ENZYMES IN MANY NEUROENDOCRINE SECRETORY VESICLES. Rebecca M. Pruss* and Emily A. Shepard* (SPON: R. Cook-Deegan). Lab of Cell Biology, NIMH, Bethesda, MD 20892.

Cytochrome b561 (cyt b561) is a major chromaffin granule protein. It represents between 10-20% of the granule membrane protein and about 3% of the total protein of the adrenal medulla. It is the only heme- or iron-containing protein in the granule membrane, and thus the only candidate for an electron carrier in these granules. Dopamine β -hydroxylase (DBH) is a mixed function oxygenase which requires copper, molecular oxygen, and ascorbic acid for converting dopamine to norepinephrine within chromaffin granules and adrenergic or noradrenergic synaptic vesicles. Ascorbic acid donates an electron during this reaction and cyt b561 is proposed as the electron carrier for regenerating reduced ascorbate in chromaffin granules. We have a monoclonal antibody which is specific for cyt b561. This antibody was generated by immunizing mice with chromaffin granule membranes. Although it detects cyt b561 in homogenates of adrenal medulla, this monoclonal antibody has not detected cyt b561 in homogenates of other tissues. Immunocytochemistry allows one to localize an antigen which is not abundant if it is concentrated in discrete subcellular regions. We have used our monoclonal antibody to study the distribution of cyt b561 in bovine tissues fixed by immersion in 4% paraformaldehyde. Since the function of cyt b561 as an electron carrier has not been absolutely established, we compared its distribution with that of catecholamine synthetic enzymes, DBH and PNMT, chromogranin A (another major chromaffin granule protein), and a number of neuropeptides and peptide hormones (VIP, enkephalin, NPY, galanin, and insulin). Cyt b561 was found in both the neural and endocrine lobes of the pituitary, the inner nuclear layer of the retina, gastrointestinal nerve fibers and plexuses, intestinal crypt cells (including typical enteric endocrine cells), heart muscle fibers, and all adrenal medullary chromaffin cells. Its distribution was best correlated with that of DBH or amidated neuropeptides. Many neuropeptides share the feature that their C-terminal amino acid is amidated. This modification is the result of a mixed function oxygenase found in peptidergic secretory vesicles. This peptide α -amidating enzyme, like DBH, requires copper, molecular oxygen, and ascorbic acid. Since the peptide-containing vesicles of the gut, pituitary, and retina do not contain DBH, it appears that cyt b561 may be required for ascorbic acid regeneration in peptidergic ascorbic acid-containing vesicles as well as DBH-containing granules. Cyt b561 may, therefore, be a useful and interesting marker for studying the development or pathology of peptidergic and DBH-containing neural and endocrine tissues.

SEROTONIN III

- 336.1 A PHARMACOLOGIC PROFILE OF MDA AND MDMA ON BRAIN RECEPTORS AND UPTAKE SITES. Theresa Kopajtic*, George Battaglia, and Errol B. De Souza (SPON: W. Kanner). Neuroscience Branch, Addiction Research Center, National Institute on Drug Abuse, Baltimore, MD 21224.

MDA and MDMA, ring substituted derivatives of amphetamine and methamphetamine, respectively, retain the stimulant properties of their parent compounds but additionally exhibit psychotomimetic properties. Although these compounds are reported to exhibit similar pharmacologic profiles in various species including man, recent behavioral data from stimulus generalization studies suggest that the effects of MDA and MDMA might differ markedly in some respects. For example, (+)MDA exhibits complete stimulus generalization to (+)MDMA, amphetamine, cocaine and hallucinogens (eg. LSD, (+)DOM) but (+)MDMA does not stimulate generalize to the hallucinogens. Additionally, in man, R(-)MDA is considered the more active isomer while for MDMA it is the S(+) isomer which exhibits greater activity. These data suggest that while some of the effects of MDA and MDMA might be mediated through similar neuronal systems their effects might be quite different on others. These studies were designed to delineate an in vitro pharmacologic profile for MDA and MDMA at various brain recognition sites using radioligand binding techniques. Our data indicate that MDA and MDMA exhibit similar and preferential high affinities at 5HT₂ and NE uptake sites (1-2 μ M) as well 5HT₂ receptors (4-5 μ M) with significantly lower affinities at DA uptake sites (45-70 μ M) and α 1 adrenergic receptors (25-50 μ M). In contrast to their affinities at α 1 adrenergic receptors, MDA and MDMA exhibit substantially higher affinities for α 2 sites (2-6 μ M). Of interest, (+)MDMA has a high affinity (2.5 μ M) for sigma receptors which is nearly 5-fold greater than that of (+)MDA (12 μ M), suggesting that perhaps some of the psychotomimetic effects of MDMA may be sigma receptor-mediated as are those of PCP, SKF 10,047 and other sigma agonists. Affinities in excess of 100 μ M were observed at mu and delta opiate, benzodiazepine and corticotropin-releasing factor receptors and calcium channels. The effects of in vivo administration of MDA and MDMA on the regulation of the higher affinity sites is currently being investigated. In summary, we report here the rank order of affinities of (+)MDA at various brain receptors and uptake sites as being: NE uptake $\geq$ 5HT uptake $\geq$ α 2 adrenergic $>$ 5HT₂ $>$ 5HT_{1A} = Sigma = M₁/M₂ muscarinic $>$ beta adrenergic $>$ α 1 adrenergic $>$ DA uptake $>$ D₂ dopamine. With respect to (+)MDMA we observed a rank order of: 5HT uptake $\geq$ NE uptake = Sigma $>$ 5HT₂ = M₁/M₂ muscarinic $>$ α 2 adrenergic $\geq$ 5HT_{1A} $>$ beta adrenergic $>$ α 1 adrenergic $>$ DA uptake $\geq$ D₂ dopamine. These data suggest that similarities in the effects of MDA and MDMA may be mediated by serotonin receptors, serotonin uptake sites and/or α 2 adrenergic receptors, while differences in discriminative stimulus properties may relate to their differential affinities at sigma receptors.

- 336.2 SYSTEMIC MDA AND MDMA, PSYCHOTROPIC SUBSTITUTED AMPHETAMINES, PRODUCE SEROTONIN NEUROTOXICITY. F.O'Hearn*, G.Battaglia, E.B.DeSouza, M.J.Kuhar and M.E.Molliver. Dept. of Neuroscience, Johns Hopkins Univ.Sch.of Med.; and NIDA, Baltimore, MD 21205

Several amphetamine derivatives are reported to have long-term toxic effects upon monoamine neurons. A recent study reports that the psychotropic drug methylenedioxymphetamine (MDA) selectively reduces serotonin (5-HT) levels in the rat brain [Ricaurte et al, Science 229 (1985)]. We have investigated the cytopathologic effects of systemic MDA and of methylenedioxymethamphetamine (MDMA), another substituted phenethylamine reportedly used for its mood altering effects. Immunocytochemistry of monoaminergic axons was used to identify the specific neuronal elements affected and to characterize the extent, regional distribution, and transmitter specificity of the drug induced neurotoxicity. Rats were administered (±)MDA, (±)MDMA (20 mg/kg) or saline s.c. every 12 hours for 4 days. The rats were sacrificed two weeks later and brain sections were incubated with antibodies to tyrosine hydroxylase (TH) [courtesy of Dr. T. Joh] and to 5-HT. To study acute toxic effects additional rats were killed 1 and 3 days after 4 doses of MDA.

MDMA and, more markedly, MDA produce profound reductions in the density of 5-HT axon terminals, most evident in cerebral cortex, thalamus, olfactory bulb, and striatum but also in hippocampus, hypothalamus, and septum. Ablation of 5-HT axons is as extensive at 1 day as at 2 weeks after injections. In cortex, the fine 5-HT terminals with small varicosities are especially vulnerable, whereas the larger axon terminals with spherical varicosities appear to survive. Remaining 5-HT axons are greatly reduced in number but are increased in diameter and 5-HT staining intensity, probably due to accumulation of 5-HT and other axonal components in fibers after loss of the terminal branches. Similar changes may cause the increased 5-HT staining in the basal forebrain, where dilated, intensely stained 5-HT axons ascend in the medial forebrain bundle. At 1 day survival, the swollen, intensely staining intervaricose segments and giant abnormal 5-HT varicosities in cortex are evidence for acute axonal degeneration. The same drugs do not affect the 5-HT staining or cytology of raphe cell bodies; nor are there changes in the density or morphology of catecholamine (TH-positive) axons. We conclude that MDA and MDMA produce rapid, selective and extensive destruction of 5-HT axon terminals, which may result from the binding of these compounds to the 5-HT uptake sites. The sparing of 5-HT cell bodies and preterminal axons may provide the potential for regenerative sprouting. [Support: NIH NS15199, NS21011, & NS19920]