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PRESENCE AND MEASUREMENT OF tele-METHYLIMIDAZOLEACETIC ACID AND pros-METHYLIMIDAZOLEACETIC ACID IN RAT BRAIN AND IN HUMAN CSF, PLASMA AND URINE. J.K. Khandelwal*, L.B. Hough*, and J.P. Green. Mount Sinai School of Medicine, N.Y., N.Y. 10029.

Endogenous tele-methylimidazoleacetic acid (t-MIAA) and pros-methylimidazoleacetic acid (p-MIAA) were shown and measured by a new GC-MS method developed by us. The acids were separated by ion-exchange chromatography, esterified with BF_3-butanol, and extracted with chloroform; 3-pyridylacetic acid (PAA) was the internal standard. Retention time, mass-ion ratios and chemical ionization mass spectra established the identity of the MIAAs in all biological extracts; and of imidazoleacetic acid in urine but not in CSF, plasma or brain. The MIAAs were quantified by monitoring m/e 95, PAA by monitoring m/e 93. The levels of t-MIAA and p-MIAA, respectively, were (ng/g or ml±S.E.M.): brain, 52.23±1.82 and 15.44±1.74; CSF, 3.89±0.30 and 11.31±2.64; plasma, 11.84±1.91 and 10.31±2.03. In urine the respective levels, μg/mg creatinine, were: 2.90 ±0.18 and 10.69±5.27. t-MIAA is formed by oxidative deamination (MAO-B) of tele-methylhistamine which was also measured in brain and in these body fluids. As no pros-methylhistamine was found in these extracts, p-MIAA likely originates from a source other than histamine metabolism. (Supported by NIMH grant MH-31805 and the Pharmaceutical Manufacturers Association).

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ONTOGENY AND SUBCELLULAR DISTRIBUTION OF RAT BRAIN TELE-METHYL-HISTAMINE. Lindsay B. Hough*, J.K. Khandelwal* and Jack Peter Green. Dept. Pharmacology, Mount Sinai School of Medicine, New York, NY 10029

The ontogeny of histamine (HA) in brain exhibits distinct differences from that of other putative transmitters; these differences have been attributed to the increased presence of neonatal brain mast cells (J. Neurochem 32:587,1979). To characterize further this anomaly, we studied the whole brain content and subcellular distribution of HA and its metabolite, tele-methylhistamine (t-MH) during postnatal development of the rat. T-MH was measured by our recently-improved GCMS method. Neonatal brain t-MH levels were similar to or greater than HA levels, indicating that HA methylation is a major metabolic pathway in neonatal brain, as it is in adults. When calculated per brain, HA, t-MH and histamine methyltransferase were all maximal ten days after birth. In neonates, brain HA was found almost entirely in nuclear (P_1) fractions, whereas t-MH was found nearly exclusively in supernatant fractions. By day 20, however, a greater proportion of both amines was localized in subcellular fractions containing synaptosomes, a finding consistent with HA's suggested transmitter role. The ontogenic pattern of brain t-MH and the very low t-MH content of peritoneal mast cells (see R.C. Goldschmidt et al., this volume) question the mast cell origin of neonatal brain HA, but may be consistent with a role for HA in brain development. (Supported by MH-31805 and the Pharmaceutical Manufacturers Association).

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LYSERGIC ACID DIETHYLAMIDE (LSD) ATTENUATES 5-HYDROXYTRYPTOPHAN (5-HTP) INDUCED INCREASES IN 5-HYDROXYINDOLEACETIC ACID (5-HIAA) IN VENTRICULAR PERFUSATE COLLECTED DURING OPERANT BEHAVIOR. M. S. Kleven*, L. P. Dwoskin*, and S. B. Sparber. Dept. of Pharmacology, Univ. of Minn., Mpls., MN 55455.

Cannulas were implanted in the lateral ventricles of rats responding on a fixed ratio 15 operant for food reinforcement. During an 80 min session, ventricles were perfused with saline (0.9%, 2.3 mM $CaCl_2$; 15 μl/min). Perfusate, collected for 20 min into acid buffer, was analyzed for dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), or 5-HIAA by loading directly onto a high performance liquid chromatograph: (ODS C_{18} reverse phase column; 25°C); 90% 0.1 M KH_2PO_4 (pH 3.0), 0.1 M Na_2EDTA, 0.25 mM sodium octane sulfonate, and 10% MeOH; glassy carbon electrode (+.80 V vs Ag/AgCl). Administration of LSD (400 μg/kg, ip) suppressed behavior without affecting DOPAC, HVA, or 5-HIAA in perfusate. A behaviorally inactive dose of 5-HTP (5 mg/kg, ip) resulted in an immediate increase in 5-HIAA without affecting DOPAC or HVA. The increase in 5-HIAA was attenuated by LSD. Monitoring perfusate for drug effects in this manner may be insensitive to transient or local changes; however, injection of 5-HTP amplifies the appearance of 5-HIAA so that drug effects can be demonstrated. (Supported in part by DA00532 and GM07397).

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PHENCYCLIDINE (PCP), IN NANOMOLAR CONCENTRATIONS, BINDS TO SYNAPTOSOMES, BLOCKS POTASSIUM CHANNELS, AND ENHANCES NEUROTRANSMITTER RELEASE. M.P. Blaustein[1], R.K. Ickowicz*[1], J.E. Warnick[2], L.G. Aguayo*[2] and E.X. Albuquerque[2]. Depts. of Physiol.[1] and Pharmacol. & Exp. Ther.[2], Univ. of Maryland Sch. of Med., Balto., MD 21201.

We have compared, quantitatively, the binding of ^{3}H-PCP to resealed rat brain synaptosomes with PCP's block of synaptosome K channels and its enhancement of transmitter release at neuromuscular junctions (nmjs). PCP blocked K depolarization-induced Rb efflux from ^{86}Rb-loaded synaptosomes, a measure of K channel conductance. Two classes of K channels were identified, with different affinities for PCP (K_I ~ 0.01 μM and K'_I ~ 150 μM); 0.1-1 μM PCP blocked 1/3 to 1/4 of the ^{86}Rb efflux into 75 mM K media at 20 sec. ^{3}H-PCP binding studies indicate two classes of "specific" binding sites in synaptosomes, with dissociation constants comparable to the aforementioned K_I values: K_D ~ 0.05 μM and K'_D ~ 100 μM, respectively; the number of binding sites were ~ 1 pmole and ~ 200 pmole/mg protein, respectively. Block of the K channels associated with the high affinity PCP binding sites may enhance transmitter release: PCP (0.075 - 1 μM) caused a significant increase ($p < 0.01$) in quantal content of endplate potentials in amphibian and mammalian nmjs. This suggests that PCP reacts with peripheral synapses with a K_I similar to that observed in central synapses. We postulate that the schizophrenia-like behavioral effects of PCP may be due to its ability to enhance release of neurotransmitter as a result of K channel blockade. (Supported by USPHS Grant NS-16106, NS-12063 and DA-02804.)

PULMONARY-RESPIRATORY PHARMACOLOGY I (4545–4546)

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EFFECTS OF INDOMETHACIN (INDO) ON THE CONTRACTILE RESPONSE OF GUINEA PIG LUNG PARENCHYMAL STRIPS (GPLS) TO LEUKOTRIENES (LT) B_4, C_4, D_4, AND E_4. A.G. Leitch*, K.F. Austen, E.J. Corey* and J.M. Drazen. Dept. Physiol., Harvard Sch. Pub. Hlth., Depts. of Med., Brigham & Women's Hosp., Harvard Med. Sch., Dept. Chem., Harvard Univ., Cambridge, MA.

We defined cumulative log concentration-effect curves for LTB_4, LTC_4, LTD_4 and LTE_4 on the GPLS in the absence and in the presence of INDO (0.1-10μg/ml) to determine if part or all of the observed contractile responses could be attributed to the formation and action of cyclooxygenase pathway products of arachidonic acid metabolism. LTB_4 (10^{-15}-10^{-7}M) was the least effective LT agonist with an EC25 of 10^{-8}M and 90% of its activity was abolished in the presence of INDO-1 & 10μg/ml. INDO (1 or 10μg/ml) did not significantly alter the concentration effect relationships for LTC_4 and LTE_4. Similarly INDO 0.1 or 10μg/ml had no effect on the LTD_4 concentration-effect relationship. However, INDO 1μg/ml had a moderate effect on the LTD_4 response at the highest concentration of LTD_4 studied (10^{-10}-10^{-8}M). Our data suggest that the contractile effect of LTB_4 on GPLS is almost entirely due to the release of cyclooxygenase pathway dependent secondary mediators. In contrast, the contractile effects of LTC_4, LTD_4 and LTE_4 in GPLS are independent of the cyclooxygenase pathway except for a moderate component of the response at high concentrations of LTD_4. Supported by NHLBI Grants HL00549, HL17382 and BMR.

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RELATIVE POTENCY AND PHARMACOLOGY OF ISOMERS OF 5-HYDROXY-6 CYSTEINYLGLYCINYL-7,9-TRANS-11, 14-CIS-EICOSATETRAENOIC ACID (LEUKOTRIENE D_4, LTD_4) ON ISOLATED GUINEA-PIG TRACHEA. R. D. Krell, B. S. Tsai*, P. R. Bernstein*, R. A. Macia*, J. Conaty*, R. E. Giles and M. E. Goldberg. Biomedical Research Department, Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, DE 19897

The relative contractile activity of C_5 and C_6 diastereomers as well as 11-trans-stereoisomers of LTD_4 were compared on isolated guinea-pig tracheal smooth muscle. Natural, 5(S) 6(R) LTD_4, was 1000, 143 and 714 times more potent than histamine, 5(R)6(R) LTD_4 and 5(R)6(S) LTD_4, respectively. The 5(S) 6(S) isomer of LTD_4 displayed no contractile activity at concentrations of 10^{-6}M or lower. Conversion of the 11-ethylenic bond from cis to trans in two of the diastereoisomers resulted in 4 fold or less changes in potency. At concentrations which lacked contractile activity, none of these isomers were antagonists of 5(S)6(R) LTD_4. FPL 55712 (20 μM) antagonized the contractile response of the tracheal strip to all isomers. Indomethacin (5 μM) markedly enhanced the efficacy of 5(S)6(R) LTD_4 but had little to no apparent effect on the other isomers. These results demonstrate the critical nature of the configuration of the 5-hydroxy and 6-peptide adducts of LTD_4 for maintenance of high affinity for receptors and, as well, demonstrate qualitative pharmacologic similarities and differences in the isomers.