REGULATION OF D-1 DOPAMINE RECEPTOR FUNCTION AND GENE TRANSCRIPTION.

Sidhu A, Olde B, Kimura K and Gardner N. Georgetown University Medical Center, 3900 Reservoir Rd, NW, Washington D.C.

SK-N-MC human neuroblastoma cells express functional D-1 dopamine (DA) receptors and has been used as a model system in desensitization studies to analyze D-1 receptor function and gene transcription. Stimulation of cells with DA or the D-1 selective agonist SKF R-38393 for 2 h, results in >95% attenuation of DA-mediated accumulation of cAMP, without any change in D-1 DA receptor levels. Prolonged (>4 h) exposure of cells to DA attenuates D-1 receptor levels to 45-50% of control and is accompanied by a loss of agonist high affinity binding sites. At the molecular level, the expression of D-1 receptor mRNA is bimodal: an initial increase (~60%) of receptor mRNA within 2 h of DA-treatment of cells, is followed by a decline to 50% below mRNA levels of untreated cells. Low concentrations (1-10 nM) of DA also enhances D-1 receptor transcription to 48% above control. SK-N-MC cells were transiently transfected with the D-1 receptor promoter region. Treatment of these transfected cells with DA results in activation of the D-1 promoter, indicating that the increased transcription of D-1 mRNA is a direct consequence of activation of receptor promoter by DA. The DA-mediated regulation of both D-1 receptor mRNA and promoter region is prevented by the D-1-selective antagonist, SCH 23390. Treatment of cells for 24 h with either forskolin or dibutyryl-cAMP fails to alter D-1 receptor mRNA levels; forskolin also fails to activate the D-1 receptor promoter. These studies indicate that D-1 receptor transcription is not regulated by the second messenger, cAMP.

THE EFFECT OF IBOGAINE ON SIGMA- AND NMDA- RECEPTOR-MEDIATED RELEASE OF [^{3}H]DOPAMINE

Sershen, H., Hashim, A., and Lajtha, A.
Nathan S. Kline Institute, Orangeburg, NY 10962

The indole alkaloid ibogaine has been suggested to have potential for inhibiting dependency on stimulant drugs. Radioligand binding studies have suggested possible multi-site actions of ibogaine: affinity at the kappa-opioid, NMDA, and sigma receptors, with effects on dopamine (DA) release. To further investigate the multiplicity of sites of action of ibogaine and the presynaptic regulation of DA release, its effect on NMDA- and sigma-receptor-mediated efflux of [^{3}H]DA was measured in striatal tissue. Striatal tissue was incubated in vitro with [^{3}H]DA and the effect of the sigma agonist pentazocine on NMDA (25 μM)-evoked DA release was measured. Pentazocine (20 μM) alone increased the efflux of DA. Pentazocine did not inhibit the NMDA-evoked release, as previously reported. MK-801 (1 and 5 μM) inhibited the NMDA-evoked release, and partially inhibited the pentazocine-evoked release. Ibogaine (10 μM) itself increased the efflux of DA; at 1 μM it was without effect. Ibogaine (1 μM) did not affect the NMDA-evoked release of DA, but partially inhibited the pentazocine-evoked release. In addition, the basal release of DA (release measured after the removal of NMDA or pentazocine) remained higher in the tissue exposed to ibogaine throughout. The results suggest that sigma receptors can regulate the release of DA, along with an action at the NMDA receptor. We have previously reported an action of ibogaine at the kappa-opioid site. The elevated basal release of DA in the presence of ibogaine after NMDA- or pentazocine-evoked release may reflect the ibogaine-induced removal of the tonically active kappa-opioid system that acts presynaptically to reduce dopamine release.

C

EFFECTS OF CHRONIC CLOZAPINE AND HALOPERIDOL TREATMENTS ON DOPAMINE D1, D2 AND SEROTONIN 5-HT2C RECEPTORS IN RAT BRAIN.

Huang, N., Radja, F., Hébert, C., van Gelder, N.M., Reader, T.A.
Centre de recherche en sciences neurologiques, Département de Physiologie, Faculté de médecine, Université de Montréal, Montréal (Québec) Canada H3C 3J7.

The antipsychotic effects of neuroleptics are believed to be related to dopamine D2 receptor blockade; however, the anatomical and pharmacological specificity of these drugs remain controversial, and interactions with serotonin receptors have also been proposed. The purpose of this study was to examine, using quantitative ligand binding autoradiography, the effects of chronic clozapine (CLZ) and haloperidol (HAL) treatments on the densities of dopamine D1 and D2, and on serotonin 5-HT2C receptors. After treating adult male Sprague-Dawley rats (300 g) with either HAL (1 mg/kg/day, i.p.), CLZ (20 mg/kg/day, i.p.) or saline for 21 days followed by 3 days of withdrawal, the brains were removed and sectioned for autoradiography. The D1, D2 and 5-HT2C receptors were labeled with [^{3}H]SCH23390, [^{3}H]raclopride and [^{3}H]mesulergine, respectively, and densitometric measurements were done with an MCID™ image analysis system. There were significant increases of D2 receptor densities in nucleus accumbens, in the dorso-lateral, ventro-medial and dorsal-medial quadrants of the rostral neostriatum, and in globus pallidus of both CLZ (17-73%) and HAL (27-52%) treated rats. In the latero-ventral rostral neostriatum, there was a significant increase induced by HAL (33%), but not by CLZ. Furthermore, HAL also induced a homogenous up-regulation of D2 receptors in rostral and caudal neostriatum, but CLZ produced a more heterogeneous increase; the highest densities were in the caudal neostriatum. For D1 receptors, CLZ but not HAL, produced significant increases (13-71%) in olfactory tubercles, nucleus accumbens, latero-dorsal rostral neostriatum, globus pallidus and substantia nigra. In addition, after CLZ there was a significant decrease of 5-HT2C binding sites (39-53%) in the dentate gyrus of dorsal hippocampus and caudal neostriatum. The changes in receptors observed after chronic treatment with the neuroleptics HAL and CLZ may underlie their therapeutic effects. The higher efficacy and lower extrapyramidal side-effects of CLZ might be due to interactions between the serotonin and dopamine systems.

D

TROPHIC ROLE OF THE NEURONAL SEROTONIN 1A RECEPTOR

J.K. Singh, T. Adayev, S. Vijayan, and P. Banerjee
Biol. Doc. Prog. of Grad. School & Dept. Chem., CUNY at Staten Island, NY.

A trophic role of the neural serotonin 1A receptor (5-HT_{1A}-R) was suggested in earlier studies, however, a direct demonstration of neuroprotective action of the 5-HT_{1A}-R has not been reported. Also, the possibility of stress-induction of a neuroprotective, heptahelical receptor protein has never been considered, even though protective proteins, such as heat shock proteins, have been shown to be induced during various types of stress. Our experiments demonstrate that the 5-HT_{1A}-R gene is transcriptionally activated during apoptosis in our engineered neuronal cell lines and that agonist activation of this receptor causes inhibition of apoptosis in the engineered neuronal cells as well as in hippocampal neurons. Apoptosis is initiated by conditions of stress, such as hypoxia (12 h) or heat shock at 42.5 °C (30 min), or a prolonged stress paradigm termed as nutrient deprivation, which creates in tissue culture, the steps, (i) proliferation, (ii) migration, (iii) differentiation and apoptosis, that pre-neuronal cells undergo during maturation in the brain. This paradigm also allows for the dissection of differentiation and apoptosis which occur simultaneously *in vivo* when the supply of trophic factors is limited. Upon continuous culture without feeding, which eventually causes nutrient deprivation, at an early, preconfluence stage, 8-OH-DPAT (1 μM) causes an increase in mitosis whereas at confluence, 8-OH-DPAT (1 μM) treatment causes inhibition of apoptosis, which is eliminated upon pertussis toxin (15 ng/ml) pretreatment. Under each of the three conditions of stress, the presence of the 5-HT_{1A} agonist, 8-OH-DPAT, causes a major decrease (~two-fold) in the proportion of apoptotic cells and this protection is eliminated in the presence of the 5-HT_{1A} antagonist, UH301 (1 μM), confirming the involvement of the 5-HT_{1A}-R. Similar 8-OH-DPAT treatment of an astrocyte-free and 16-DIV primary culture of hippocampal neurons from E16 mice also showed a dramatic decrease (~two-fold) in the proportion of apoptotic cells following hypoxia (40 min). Studies on possible mechanisms of this neuroprotection (e.g. via the MAP kinase or the PI-3 kinase pathway) will be presented.