Identification and characterization of dopamine transporter in bovine pineal gland

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The mammalian pineal gland contains several neurotransmitters and receptors for amino acids, biogenic amines and peptides. Some of these, such as dopamine and D_1 and D_2 dopamine receptors, have been identified and characterized in bovine pineal gland. This leads us to hypothesize that this organ possesses a high-affinity dopamine transporter mechanism for dopamine substrate. This study is an attempt to investigate the existence of dopamine transporter in the bovine pineal gland. The radioligand used was [^{3}H] GBR 12935 and the drug to define non-specific binding was GBR 12909. Tissue dependence of specific [^{3}H] GBR 12935 binding, [^{3}H] GBR 12935 (0.1 nM) binding to bovine pineal membranes was studied, and resulted in a linear increase of the specific binding as a function of tissue protein concentration between 0.14 – 0.3 mg/ml. The association rate of [^{3}H] GBR 12935 binding to pineal gland membrane was examined as a function of time. The binding reached equilibrium within 45 min of incubation at 25°C. The dissociation time course of specific [3H] GBR 12935 binding from bovine pineal membrane was also studied, allowing the binding of [^{3}H] GBR 12935 0.1 nM to reach equilibrium by incubation for 60 min at 25°C. Thereafter, 10 μM GBR 12909 was added, the incubation was continued for various times, and the reactions were terminated by filtration. A dissociation rate constant (k_d) of 1.23 nM was obtained from the association rate as ln(Be/Be-B) (Be; bound at equilibrium, B; bound at various times. Using the relationship $k_{ob} - (k-1) = k_{+1} \cdot L_T$, where L_T equals total ligand concentration and k_{ob} ; observed association rate constant of 0.075 per min). A half-life ($T_{1/2}$) of 14 min was obtained from analysis of the slope plotted between ln (B/Bo) (B; bound at various time, Bo; bound at 0 min) in relation of time. In the inhibition experiment, the concentration of drugs required to inhibit 50% of the binding (IC_{50}) was determined in descending order of potency in competition with radioactive ligand binding: GBR 12909 > nomifensine > amitriptyline > imipramine > desipramine > fluoxetine > fluvoxamine > nisoxetine. This result showed that drugs that block dopamine transporters1 were effective in displacing [^{3}H] GBR 12935, whereas norepinephrine and serotonin transporter inhibitors were ineffective. Drugs that block the uptake of dopamine were effective in displacing [^{3}H] GBR 12935 from bovine pineal gland, whereas drugs that block the uptake of serotonin, norepinephrine and mixed reuptake inhibitors affecting serotonin and norepinephrine were ineffective. The results indicate that dopamine transporters are present in the bovine pineal gland. Overall interpretation of the results indicates that dopamine transporter exists in bovine pineal. This unique transporter in the bovine pineal gland may have functional and pharmacological significance.

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