Thesis Title RECEPTOR BINDING CHARACTERIZATION OF NOVEL D_1 AND D_2 DOPAMINERGIC DRUGS USING AUTORADIOGRAPHIC TECHNIQUES AND FUNCTIONAL MODEL

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ABSTRACT

The dopaminergic receptor binding in rat brain was characterized by using autoradiography and functional model. The major disadvantages of ^{3}H-spiperone, a widely used labeled ligand in receptor binding studies, were demonstrated. The most important drawback was that it bound non-specifically not only to "spirodecanone" binding site and also bound to 5-HT_2 receptors. The novel ligands ^{3}H-SKF 38393 (a selective D_1 agonist) and ^{3}H-CV-205-502 (a selective D_2 agonist) did not exhibit these characteristics of binding. The use of these two ligands in receptor autoradiography has revealed the presence of dopamine receptors in significant densities in areas where other dopaminergic ligands including spiperone did not exhibit relevant binding as well as in other well-characterized brain areas for which agreement already exists about the presence of dopamine receptors.

The receptor autoradiography was shown to have a certain disadvantage in that the tissue itself may quench some of the signals from the (^{3}H) ligands, altering grain densities in the autoradiography image. The quenching problem was successfully corrected in this study by determining correction factor. This factor was obtained by using the chloroform extraction method.

The non-catechol, ^{3}H-CV-205-502, was used to localize dopamine D_2 receptors by autoradiography after *in vitro* labeling of rat brain sections. The characteristics of the binding of this ligand to tissue sections were those expected from the labeling of dopamine D_2 receptors. The binding of ^{3}H-CV-205-502 was inhibited selectively and stereospecifically by dopamine D_2 agents but not by dopamine D_1 compounds. The autoradiographic localization of ^{3}H-CV-205-502 binding sites showed high densities of dopamine D_2 receptors in areas such as the glomerular layer of the olfactory bulb, the nucleus accumbens, caudate putamen, the lateral septum, and the islands of Calleja. The distribution pattern showed similarities and differences

with previously reported distributions of dopamine D_2 receptors visualized autoradiographically using ^{3}H-labeled agonists and antagonists.

^{3}H-SKF 38393 was used to study the regional distribution of dopamine D_1 receptor in the rat brain. The binding of ^{3}H-SKF 38393 to striatal sections was saturable and stereospecific. The highest levels of ^{3}H-SKF 38393 binding sites were found in cerebral structures such as caudate putamen, nucleus accumbens, olfactory tubercle, septum lateralis, central amygdaloid nucleus and substantia nigra.

A remarkable observation in this autoradiographic study was that the distribution of receptors did not precisely parallel or match with the distribution of related neurotransmitters. The lack of matching may be either due to the fact that a neurotransmitter is present where there is no receptor or a receptor exists without the presence of neurotransmitters.

The studies of the effects of guanine nucleotide and N-ethylmaleimide (NEM) on dopamine receptor binding characteristics in whole calf brains confirmed the finding that the effect of Gpp(NH)p, a stable GTP analog, was to lower agonist receptor affinity without affecting antagonist receptor affinity. The results also suggested that alkylation of sulfhydryl groups by NEM did not appear to significantly affect antagonist binding, while sulfhydryl groups appeared to be functionally important in the regulation of high affinity binding sites.

Stimulation of dopamine receptors can also lead to changes in the levels of second messengers. Significant changes in phosphatidylinositol (PI) turnover were not demonstrated when rat striatum slices were being stimulated by LY 141865, a D_2 agonist, despite the fact that activation of D_2 receptors has been previously shown to inhibit PI turnover in rat anterior pituitary gland. It is possible that the changes of PI turnover by activation of D_2 receptor in rat striatum are relatively small and rapid.

The main results of this thesis demonstrated the superior advantages of two novel ligands, SKF 38393 and CV 205-502, over other currently available radiolabeled compounds. They can be used to localize specific brain regions known to be innervated by dopaminergic neurons and also in areas that the innervation seemed to be absent or not well established.Dopaminergic receptors have been shown to be present in significant amount in mesolimbic brain areas as being labeled by ^{3}H-SKF 38393 and ^{3}H-CV-205-502 in the autoradiographic studies. The mesolimbic brain regions are also widely known to be associated with emotion and behaviours. This is the first study to suggest that D_2 receptor plays an important role in stressful situation at least in noise-induced stress model. Although quite simple to perform, this model was shown to be useful in differentiating the physiological role of D_1 and D_2 receptors

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