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PRELIMINARY COMMUNICATIONS

DISTINCTION BETWEEN DOPAMINERGIC AND SEROTONERGIC COMPONENTS OF NEUROLEPTIC BINDING SITES IN LIMBIC BRAIN AREAS

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It has been well established that neuroleptic binding sites in rat striatum, labelled by ^{3}H-haloperidol or ^{3}H-spiperone are dopaminergic [1]. Recently we provided evidence that ^{3}H-spiperone binding sites in rat frontal cortex are serotonergic [2,3]. The limbic brain areas have been reported to contain a high density of stereospecific ^{3}H-haloperidol [4] and ^{3}H-spiperone [5] binding sites.

In the present investigations the rapid filtration technique [see: ref. 1] was used to measure in vitro binding, and experiments were performed using a total membrane preparation of rat nucleus accumbens and tuberculum olfactorium (10 mg tissue/ml Tris HCl salt buffer, pH 7.6) and 2 nM of ^{3}H-ligand in an incubation volume of 1.1 ml. Under these conditions the total binding and the percentage of stereospecifically displaceable binding at 10^{-6} M (+)-butaclamol, were for ^{3}H-spiperone: 46 pmoles/g tissue with 61 % stereospecific binding and for ^{3}H-haloperidol 25 pmoles/g tissue with 48 % stereospecific binding.

The relative affinities of drugs for serotonergic and dopaminergic receptors can be assessed using the in vitro assays of ^{3}H-spiperone binding in rat frontal cortex and ^{3}H-haloperidol binding in rat striatum. The ratio of these relative affinities (for serotonergic over dopaminergic binding sites) has been proposed as a means of drug classification. Amongst potent serotonin and dopamine antagonists and agonists, the most extreme ratios thus far reported are: for mianserine: 40, for bufotenin: 13, for haloperidol: 0.02 and for 2-(N,N-dipropyl)amino-5,6-dihydroxy tetralin: 0.0002 [3]. Recently we found a ratio of 200 for a new compound R 43 448, and a ratio of 0.0035 for domperidone. Compounds which show such high dissociation in their affinity for serotonergic and dopaminergic receptors can be used to distinguish between the two types of receptors in binding experiments. The tetralin derivative, domperidone, and R 43 448 inhibit high affinity, stereospecific ^{3}H-spiperone binding to membranes of limbic rat brain areas in a bi-phasic way. Inhibition curves of both the tetralin and R 43 448 show a pronounced intermediate plateau at concentrations between 5 x 10^{-8} M and 5 x 10^{-7} M; at which approximately 40 % of the stereospecific ^{3}H-spiperone binding is inhibited.
Hence, it seems that the limbic, stereospecific, spiperone binding comprises at least two different sites; and that a specific dopamine agonist or antagonist and a serotonin antagonist bind with high affinity to one of the sites, and with much lower affinity to the other site. In each case the high affinity binding sites are completely occupied at 10^{-7} M. When the specific dopamine antagonist, domperidone, is added to a fixed concentration of 10^{-7} M R 43 448, the residual stereospecific binding is inhibited, with an IC_{50} of 2 x 10^{-9} M; in addition, the specific serotonin antagonist R 43 448, added to a fixed concentration of 10^{-7} M domperidone, produces further inhibition of the stereospecific binding with an IC_{50} = 2 x 10^{-8} M. These IC_{50}-values are in good agreement with the IC_{50}-value of domperidone in the ^{3}H-haloperidol-striatum assay and with the IC_{50}-value of R 43 448 in the ^{3}H-spiperone-frontal cortex assay, respectively. The presently shown bi-phasic inhibition curves and the additive displacement at low concentrations of specific dopamine and serotonin antagonists indicate that limbic

rat brain areas contain both dopaminergic and serotonergic binding sites. A nearly equal number of both sites is labelled with ^{3}H-spiperone at 2 nM, and both sites are stereospecifically inhibited by (+)-butaclamol. In contrast, using ^{3}H-haloperidol at 2 nM, which is known as a specific dopamine antagonist, tetralin is much more active than R 43 448 and displaces nearly 62 % of the stereospecific binding at low concentrations ($< 10^{-7}$ M).

Evidence is thus provided that the limbic brain areas contain multiple neuroleptic binding sites which are related to both dopaminergic and serotonergic mechanisms. The importance of the blockage of both these sites for alleviating psychosis has yet to be considered.

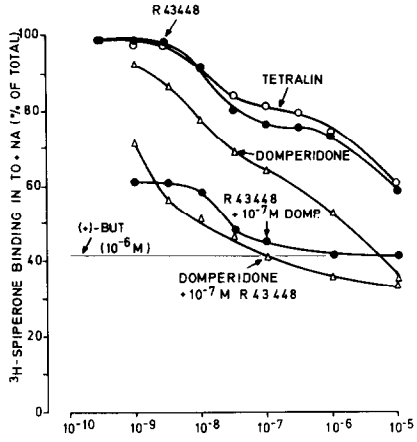

Fig. 1

In vitro ^{3}H-spiperone (2 nM) binding to membranes of rat nucleus accumbens and tuberculum olfactorium. Inhibition curves of 2-(N,N-dipropyl)amino-5,6-dihydroxy tetralin (tetralin), R 43 448 and domperidone, additive displacement curves of R 43 448 in the presence of 10^{-7} M domperidone; and of domperidone in the presence of 10^{-7} M R 43 448.

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