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Short communication

SELECTIVE LABELING OF APOMORPHINE RECEPTORS BY ^{3}H-LSD

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There are at least two types of dopamine receptors: the ^{3}H-dopamine or ^{3}H-apomorphine receptor (with high or nM affinity for dopamine), and the ^{3}H-neuroleptic receptor (with low or μM affinity for dopamine). While ^{3}H-LSD can label the ^{3}H-neuroleptic receptor, this study was done in order to label the ^{3}H-apomorphine/dopamine receptor site. In the presence of excess phentolamine, serotonin and spiperone (to preclude binding to α-adrenergic, serotonergic and neuroleptic receptors, respectively) similar concentrations of dopaminergic drugs inhibited the binding (to calf caudate) of ^{3}H-LSD and ^{3}H-apomorphine. This is compatible with the concept that the ^{3}H-apomorphine/dopamine receptor and the ^{3}H-neuroleptic/dopamine receptor are separate.

Dopamine receptors Neuroleptic receptors Aminotetralin Bromocryptine Haloperidol

1. Introduction

There is considerable indirect evidence that the effects of LSD (d-lysergic acid diethylamide) on the brain are not only mediated via serotoninergic neurones, but also through dopaminergic, α-adrenergic and possibly histaminergic neurones (see Whitaker and Seeman, 1978, for complete Refs.). It is known that there are at least two types of dopamine receptors (Titeler and Seeman, 1978; Seeman et al., 1978). The ^{3}H-neuroleptic/dopamine receptor has a low affinity for dopamine (μM concentrations of dopamine are required to inhibit ^{3}H-neuroleptic binding), and this receptor is presumably post-synaptic. The receptor for ^{3}H-dopamine or ^{3}H-apomorphine, on the other hand, has a very high affinity for dopamine or apomorphine (in the nM range) and a low affinity for neuroleptics (μM range); these receptors may be pre-synaptic (Nagy et al., 1978).

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There is direct evidence that ^{3}H-LSD labels serotonin receptors (Bennett and Snyder, 1976; Leysen et al., 1978; further Refs. in Whitaker and Seeman, 1978). It has also been reported that ^{3}H-LSD labels postsynaptic neuroleptic receptors (with a low affinity of 4 μM for dopamine) in the snail esophageal ganglion (Drummond et al., 1978); such postsynaptic receptors (with low affinity for dopamine) are also labeled by ^{3}H-LSD in the calf caudate and hippocampus (Burt et al., 1976).

In order to obtain direct evidence that ^{3}H-LSD can also attach to high-affinity dopamine receptors, therefore, the present study was done using excess concentrations of other drugs to preclude the binding of ^{3}H-LSD to other receptors. Phentolamine (50 nM) and serotonin (5000 nM) were present in the ^{3}H-LSD-containing test-tubes in this study and served to preclude the binding of ^{3}H-LSD to α-adrenergic and serotonin receptors, respectively.

Furthermore, in order to preclude ^{3}H-LSD binding to the ^{3}H-neuroleptic/dopamine recep-

tor, an excess of spiperone (100 nM) was present in all the test tubes. The prediction was that if this strategy of precluding sites for adrenoceptors, serotonin and neuroleptic/dopamine receptors was appropriate, then the pattern of ^{3}H-LSD binding should be similar to that for ^{3}H-dopamine or ^{3}H-apomorphine binding. The results support this prediction.

2. Materials and methods

The binding of ^{3}H-LSD (11.5 Ci/mmol), ^{3}H-apomorphine (11.7 Ci/mmol) and ^{3}H-spiperone (23-26 Ci/mmol) were done on calf caudate homogenates (see Whitaker and Seeman, 1978, for details). The incubation tubes contained (final concentrations): 4 nM ^{3}H-LSD in the presence of 50 nM phentolamine, 5000 nM serotonin and 100 nM spiperone; 3—3.5 nM ^{3}H-apomorphine; 0.2 nM ^{3}H-spiperone. The tubes were incubated at 22°C for 30 min and the contents then filtered through Whatman GF/B filters. The filters were washed with either 5 ml (for ^{3}H-LSD) or 10 ml of buffer (for ^{3}H-apomorphine and for ^{3}H-spiperone; see Whitaker and Seeman, 1978). Specific binding of ^{3}H-LSD was defined as that displaceable by 200 nM LSD (in the presence of phentolamine, serotonin and spiperone) and this amounted to 125 ± 10 cpm (mean ± S.E.M.) per test tube (25% of total binding). Specific binding of ^{3}H-apomorphine was defined as that displaceable by 200 nM apomorphine, while that for ^{3}H-spiperone was defined as that displaceable by 100 nM spiperone. The ^{3}H was monitored as before (Whitaker and Seeman, 1978).

3. Results

The IC_{50} values (concentrations inhibiting specific binding by 50%) for a variety of drugs are shown in fig. 1. It can be seen (fig. 1, lower part) that the IC_{50} values against ^{3}H-LSD binding were approximately of the same magnitude as those against ^{3}H-apomorphine binding.

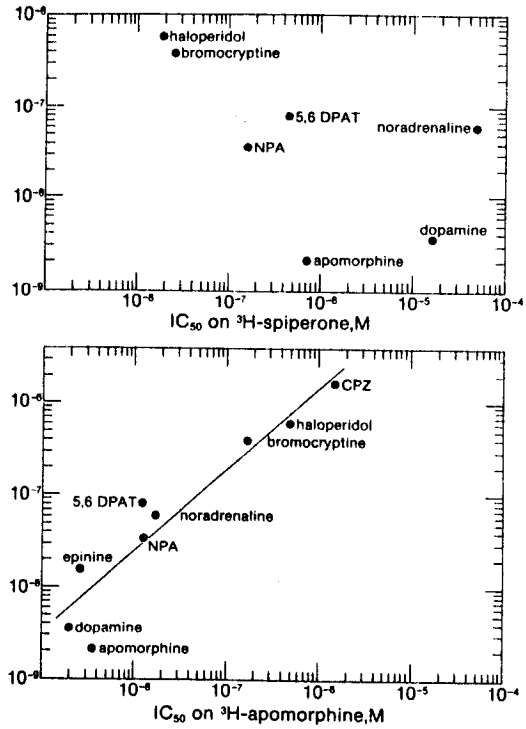

Fig. 1. The IC_{50} values for ^{3}H-LSD binding correlated with those for ^{3}H-apomorphine binding (bottom) in calf caudate homogenate. The binding of ^{3}H-LSD was done in the presence of excess concentrations of spiperone (100 nM), phentolamine (50 nM) and serotonin (5 000 nM) in order to preclude ^{3}H-LSD from binding to neuroleptic, α-adrenergic and serotonin receptors, respectively (the triple drug combination was abbreviated as SP5). Specific binding of ^{3}H-LSD (SP5) was defined as that displaceable by 200 nM LSD in the presence of SP5. Abbreviations: NPA, N-propylnorapomorphine; 5,6-DPAT, 5,6-dihydroxy-N,N-dipropyl-2-aminotetralin; CPZ, chlorpromazine. *Ordinates:* moles/liter IC_{50} on ^{3}H-LSD (SP5).

4. Discussion

Fig. 1 (bottom) illustrates that the IC_{50} values against ^{3}H-LSD binding correlated with those against ^{3}H-apomorphine binding. This finding suggests that ^{3}H-LSD attaches to sites similar to those labeled by ^{3}H-apomorphine,

providing that the α-adrenergic receptors are simultaneously occluded by phentolamine, while the serotonin and neuroleptic receptor sites are also occluded by excess concentrations of serotonin and spiperone, respectively.

Fig. 1 (top) reveals that there may have been an inverse correlation between the IC_{50} values against ^{3}H-LSD with those against ^{3}H-spiperone. The regression coefficient (R = −0.1) is low, however, for this relation. Omitting the values for noradrenaline (in fig. 1) did somewhat enhance the regression coefficient to −0.5, but this was still considerably lower than the regression coefficient of +0.9 for the ^{3}H-LSD-^{3}H-apomorphine correlation (fig. 1 bottom). Since an excess concentration of 100 nM spiperone was always present in these experiments measuring the binding of ^{3}H-LSD, the neuroleptic receptor should have been occluded by spiperone and inaccessible to ^{3}H-LSD. Should there have been unoccluded neuroleptic receptors available for the binding of ^{3}H-LSD, one might have expected a *direct* correlation between the IC_{50} values of the various drugs against the binding of ^{3}H-LSD and ^{3}H-spiperone. This is because both agonists and antagonists attach to the ^{3}H-neuroleptic/dopamine receptor in direct relation to their pharmacological potencies (Titeler and Seeman, 1978; Seeman et al., 1978).

Thus, the observation that the ^{3}H-LSD data better correlated with the ^{3}H-apomorphine data provides additional support for the idea that the ^{3}H-apomorphine/dopamine receptor and the ^{3}H-neuroleptic/dopamine receptor may be separate entities (Titeler and Seeman, 1978; Seeman et al., 1978).

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