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Short communication

# THE [$^3$H]5-HT AND [$^3$H]SPIROPERIDOL BINDING SITES IN THE RAT FRONTAL CORTEX ARE THERMALLY INACTIVATED AT DIFFERENT RATES

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The rate of thermal inactivation of [$^3$H]5-HT, [$^3$H]spiroperidol and [$^3$H]LSD binding sites in a membrane preparation from the rat frontal cortex has been studied. The binding sites were inactivated as different rates and the results are interpreted as supporting the claim that [$^3$H]5-HT and [$^3$H]spiroperidol bind to different sites within the frontal cortex of the rat.

Serotonin Receptor Frontal cortex Spiperone LSD

## 1. Introduction

[$^3$H]spiroperidol was originally introduced as a ligand for the investigation of the dopamine receptor in the central nervous system. It has however subsequently been claimed to bind to both a dopaminergic site in the striatum and a mainly serotoninergic site in the frontal cortex (Leysen et al., 1978; Peroutka and Snyder, 1979; Seeman et al., 1980).

A comparison of the characteristics of the [$^3$H]5-HT and [$^3$H]spiroperidol binding sites in the frontal cortex of the rat has led Peroutka and Snyder (1979) to suggest that these ligands may label distinct non-interconverting sites, possibly representing two 5-HT receptor sub-types. [$^3$H]LSD was claimed to label both the [$^3$H]5-HT and [$^3$H]spiroperidol binding sites in this brain region.

We have attempted to differentiate further between these three binding sites by following the rate of thermal inactivation of specific [$^3$H]5-HT, [$^3$H]spiroperidol and [$^3$H]LSD binding in a membrane preparation of the rat frontal cortex. The results show that the rates of inactivation of [$^3$H]5-HT and [$^3$H]spiroperidol binding at 45°C are different and that [$^3$H]LSD binding is inactivated at a rate which is intermediate between that for the two other ligands. The results support the claim that [$^3$H]5-HT and [$^3$H]spiroperidol bind to different sites within the frontal cortex.

## 2. Materials and methods

The preparation of frontal cortex homogenates was carried out using an adaptation of the method of Bennett and Snyder (1976). The frontal cortices of 10 male Alderley Park rats (250—350 g) were dissected out and a crude homogenate was made using a Polytron homogeniser (setting 5, 20 sec) in 24 ml of Tris-HCl buffer (pH 7.4). After centrifugation at 48 000 × *g* for 20 min, the pellet was resuspended by gentle glass-teflon homogenisation in the same volume of Tris-HCl buffer and stored in liquid $N_2$ until needed.

Binding studies were performed as previously described (Middlemiss et al., 1977) after exposing the membranes to elevated temperatures (45°C) for the specific time

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periods in Tris-HCl buffer (pH 7.4 at 20°C). Subsequently the solutions were rapidly cooled to 37°C and binding studies initiated by the addition of [$^3$H]ligand ± cold drug to define non-specific binding. Binding incubations were for 15 min and were terminated by filtration through GF/C filters. After liquid scintillation in PCS (Amersham), specific binding was calculated as the difference between total and non-specific binding. Binding studies were carried out using 5 nM [$^3$H]5-HT (30 Ci/mM — New England Nuclear), 0.6 nM [$^3$H]spiroperidol (20 Ci/mM Amersham) and 2 nM [$^3$H]LSD (13 Ci/mmol — Amersham). Non-specific binding was defined in the presence of 1 μM 5-HT ([$^3$H]-5-HT) or 1 μM LSD ([$^3$H]spiroperidol and [$^3$H]LSD).

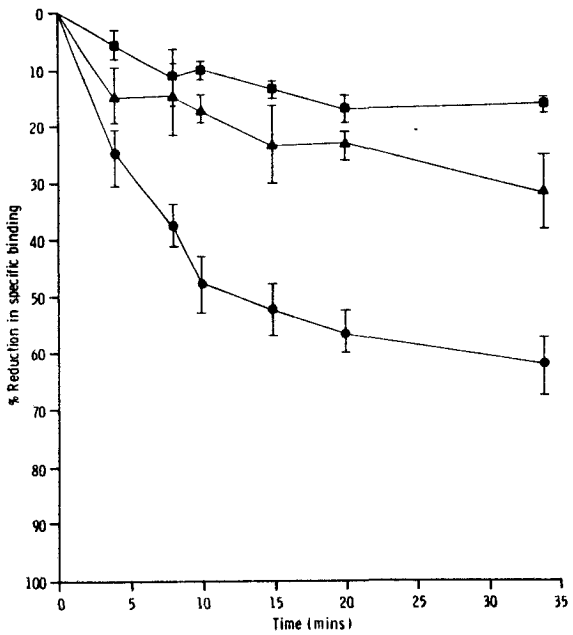


Fig. 1. Percentage reduction of specific binding for the ligands [$^3$H]5-HT (○), [$^3$H]spiroperidol (□), [$^3$H]-LSD (△): after incubation for various times at 45°C. Results are expressed as % reduction relative to control specific binding at 0 time (no exposure to 45°C inactivation). Control specific binding at 0 time was 6.0 ± 0.5 pmol/g wet weight for [$^3$H]5-HT binding, 9.4 ± 0.9 pmol/g wet weight for [$^3$H]spiroperidol binding and 12.3 ± 0.7 pmol/g wet weight for [$^3$H]-LSD binding. Non specific binding did not change over the time period studied at 45°C and represented 41 ± 4% ([$^3$H]5-HT), 35 ± 2% ([$^3$H]spiroperidol) and 18 ± 2% ([$^3$H]LSD) of total binding at 0 time. Results are mean ± S.E.M. of three separate experiments each performed in triplicate.

## 3. Results

Specific binding of the three [$^3$H]ligands showed a progressive decline over a 35 min incubation period at 45°C (fig. 1). There was however a marked difference between the rates of inactivation of [$^3$H]5-HT and [$^3$H]-spiroperidol binding with a 55% loss of specific [$^3$H]5-HT binding after 15 min incubation at 45°C as compared with only a 15% decrease in the specific binding of [$^3$H]spiroperidol over the same time period. The rate of loss of specific [$^3$H]LSD binding was intermediate to that of [$^3$H]5-HT and [$^3$H]spiroperidol binding (fig. 1).

## 4. Discussion

Peroutka and Snyder (1979) have suggested on the basis of the potency of a number of drugs in displacing [$^3$H]5-HT and [$^3$H]spiroperidol binding in the frontal cortex of the rat that these two ligands label distinct non-interconverting receptors for the neurotransmitter 5-hydroxytryptamine. In addition they have claimed that [$^3$H]LSD labels both of these receptor sites. The present study provides further evidence that these ligands label separate binding sites in the frontal cortex since the rates of thermal inactivation of these sites have been shown to be different, suggesting that they may represent separate physical entities. Furthermore, the intermediate rate of inactivation of specific [$^3$H]-LSD binding is consistent with this ligand labelling both the [$^3$H]5-HT and [$^3$H]spiroperidol binding sites.

The present study does not provide any information as to the possible physiological roles of these three binding sites.

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