Vol. 105, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1982 Pages 117-120

EARLY SUPERSENSITIVE CHANGES IN [^{3}H]SPIROPERIDOL BINDING IN THE ISOLATED STRIATUM

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Received October 24, 1981

Adult mouse striatum was dissected and incubated as an organ culture in chemical medium for up to 24 hours to measure alterations in [^{3}H]spiroperidol binding. High-affinity [^{3}H]spiroperidol binding (ligand concentration 2 nM) was constant throughout the incubation. However, there was a 33% increase in lower-affinity binding (ligand concentration 20 nM) during 24 hours of incubation. Inhibition of dopamine synthesis with alpha-methyl-p-tyrosine methyl ester enhanced the increase in 20 nM [^{3}H]spiroperidol binding by 65%.

Introduction

Dopaminergic synapses in the corpus striatum undergo supersensitive changes in adaptive response to receptor blockade. Friedhoff (1) proposed that clinical phenomena such as schizophrenia and tardive dyskinesia could be manifestations of post-synaptic dopamine receptor supersensitivity. Animal models of dopaminergic supersensitivity such as apomorphine-induced stereotypy and tolerance to cataplexy have been developed (2). Direct measurement of dopamine receptor binding by radiolabelled ligands such as [^{3}H]spiroperidol or [^{3}H]apomorphine demonstrated that supersensitive behavior in the rat is accompanied by increases in the number of radioligand binding sites (3). Supersensitive increases in binding are approximately 25-80%, varying on whether [^{3}H]spiroperidol or [^{3}H]apomorphine is used as the radiolabelled ligand. Increases in [^{3}H]apomorphine binding are generally greater that increases in [^{3}H]spiroperidol binding (3). Supersensitive increases are generally detectable after 2-3 weeks of neuroleptic treatment, yet the process of supersensitive change may well begin as soon as the neuroleptic blockade occurs. To see whether a supersensitive increase in [^{3}H]spiroperidol

0006-291X/82/050117-04$01.00/0

binding is detectable early after denervation of the tissue, we incubated the isolated mouse striatum as an organ culture for up to 24 hours.

Methods

The technique of Klein and Rowe (4), developed for the organ culture of pineal gland, was modified for the incubation of mouse striatum. C57 black male mice were sacrificed by decapitation and the striata were dissected using aseptic technique under a stream of 95% O_2, 5% CO_2 gas. Each striatum was placed in incubation whole. Incubation flasks (Falcon Tissue Culture flasks) were prepared aseptically by placing a lens paper raft supported by a glass fiber filter (Whatman GF-B) in each flask with 3-4 ml of BGJ (original formula) medium to the level of the lens paper raft, and then gassing the flask with 95% O_2, 5% CO_2 gas. Striata were incubated at 37° C; drugs were added directly to the medium when indicated.

After incubation, striata were homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.7) using a motor-driven Teflon homogenizer. The homogenate was centrifuged at 800 x g for 10 minutes. The resultant supernatant was centrifuged x 2 at 50000 x g for 10 minutes. The final pellet was rehomogenized in ice-cold Tris-HCl buffer (pH 7.6), containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. For a standard [^{3}H]spiroperidol binding assay an incubation mixture contained 1.0 ml tissue (0.1-0.2 mg protein), 2 or 20 nM [^{3}H]spiroperidol (25.6 Ci/mmole, New England Nuclear) and 20 uM (+)-butaclamol in those samples used to determine non-specific binding. The incubation mixture was incubated for 10 minutes at 37° C, and then rapidly filtered through Whatman GF-B filters with 2 rinses of 5 ml ice-cold Tris-HCl buffer. The filters were counted using 10 ml of Aquasol scintillation cocktail at an efficiency of 40% after 24 hours. Samples were assayed in triplicate. Protein was assayed using the Bio-Rad method (5).

Results

We checked tissue viability by microscopic examination of representative tissue samples and by the uptake of [^{14}C]lysine (1.0×10^{-6} M) into incubated striata. There was no change in the uptake of [^{14}C]lysine for up to 24 hours of incubation and visible deterioration on microscopic examination did not occur until after 48 hours. The amount of [^{3}H]spiroperidol binding using 2 nM as the ligand concentration remained constant throughout the 24 hour incubation. Interestingly, [^{3}H]spiroperidol binding using 20 nM as the ligand concentration decreased during the first 4 hours of incubation and then increased by 33% over the next 20 hours ($p < .005$). (See Figure 1)

Alpha-methyl-p-tyrosine methyl ester (AMPT), a tyrosine hydroxylase inhibitor, was added to incubating striatal cultures (5.35×10^{-3} M).

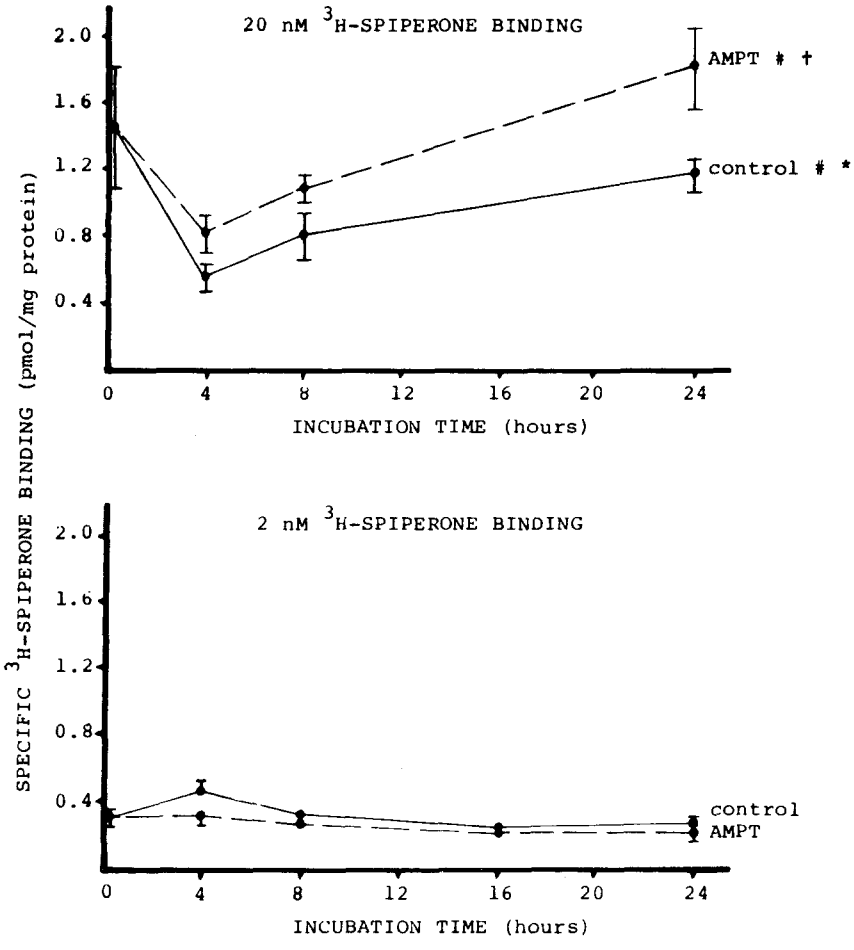

Figure 1. [^{3}H]Spiroperidol (2 or 20 nM) binding was performed in triplicate at the indicated time points for up to 24 hours after isolation and incubation of whole mouse striata. Straita were incubated in the presence and absence of alpha-methyl-p-tyrosine methyl ester (AMPT) (5.35×10^{-3} M). Protein determinations were made in duplicate using the Bio-Rad protein assay. Each point represents the mean of 3-5 separate experiments +/- SEM. *20 nM control culture, 4 vs. 24 hours, $p < .005$. †20 nM AMPT culture, 4 vs. 24 hours, $p < .025$. #20 nM 24 culture, control vs. AMPT, $p < .05$.

AMPT significantly enhanced the increase in lower affinity (20 nM) [^{3}H]spiroperidol binding at 24 hours by 65% ($p < .05$). However, AMPT had no effect on 2 nM [^{3}H]spiroperidol binding. (See Figure 1)

Discussion

Although we observed no change in high-affinity (2 nM) [^{3}H]spiroperidol binding during the incubation, an increase in [^{3}H]spiroperidol binding was observed when higher ligand concentrations (20 nM) were

used. This data suggests that there may be an increase in a [^{3}H]spiroperidol binding site with lower affinity. Such lower-affinity binding sites have not been described previously (3), perhaps because [^{3}H]spiroperidol concentrations too low to detect them were used. However, the relatively low affinity of such a binding site makes identification and characterization by Scatchard analysis difficult.

The incubating striata were treated with AMPT in the hope that such treatment would further reduce the amount of dopamine in the denervated synapse. Therefore, an enhancement of the increase of the lower-affinity [^{3}H]spiroperidol binding by AMPT treatment suggests the increase in lower-affinity binding is a response to denervation, rather than an artifact of high ligand concentrations or incubation. The early decrease in 20 nM [^{3}H]spiroperidol binding may represent an artifact of dissection and tissue destruction.

An increase in lower-affinity [^{3}H]spiroperidol binding may represent an early event in the development of dopaminergic supersensitivity. Increases in lower-affinity binding observed after 24 hours may precede the development of increases in higher-affinity (2 nM) binding, usually observed after several days. If the process of dopamine receptor supersensitivity involves the synthesis of new receptors, a possible locus for lower-affinity binding may be a dopamine pro-receptor, not yet completed or membrane-bound. This possibility requires further investigation.

Acknowledgements

This work was supported by grant DA 01113 and grant MH 08618 from USPHS.

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