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BRAIN NEUROTRANSMITTER RECEPTORS AFTER LONG-TERM HALOPERIDOL: DOPAMINE, ACETYLCHOLINE, SEROTONIN, α-NORADRENERGIC AND NALOXONE RECEPTORS

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SUMMARY

Since long-term neuroleptic therapy is known to alter brain dopaminergic sensitivity, we tested the effects of chronic haloperidol administration (10 mg/kg/day for over 3 weeks) on the amount of the dopamine receptors (using ^{3}H-apomorphine and ^{3}H-haloperidol) in various regions of the rat brain. To test whether the changes in dopamine receptors were selectively produced, we also assayed acetylcholine receptors (with ^{3}H-quinuclidinyl benzilate or ^{3}H-QNB), alpha-noradrenergic receptors (with ^{3}H-WB-4101), ^{3}H-serotonin receptors and ^{3}H-naloxone receptors.

The specific binding of ^{3}H-haloperidol increased significantly by 34% in the striatum and by 45% in the mesolimbic region after long-term haloperidol. The specific binding of ^{3}H-apomorphine also increased significantly by 77% in the striatum and 55% in the mesolimbic area. Although there was a small significant increase of 20% in specific ^{3}H-serotonin binding in the striatum, no such increment occurred in the hippocampus or the cerebral cortex. No significantly different binding occurred for the other ^{3}H-ligands in these brain regions except for a 13% increase in alpha-noradrenergic binding in the cerebral cortex. These results indicate that long-term haloperidol treatment produces rather selective increases in dopamine/neuroleptic receptors, without much change in 4 other types of receptors. Such relatively selective increments in these receptors may be the basis of dopaminergic supersensitivity (e.g. tardive dyskinesia) after long-term haloperidol.

INTRODUCTION

Tardive Dyskinesia is a condition of abnormal involuntary movements which may occur after long-term administration of neuroleptic drugs (1,2). The symptoms are difficult to treat and may persist for months or years after discontinuing the drug (3).

Klawans and McKendall (4) suggested that tardive dyskinesia is a result of postsynaptic dopaminergic receptor supersensitivity brought about by the protracted pharmacological block of dopaminergic neurotransmission by the neuroleptics. The hypothesis is supported by the following clinical observations:

1. Decreasing or discontinuing the neuroleptic treatment aggravates the dyskinesia (5).

2. Readministration of neuroleptics (6,7) or using α-methyl-paratyrosine (8) counters the dyskinesia symptoms.

3. L-DOPA aggravates the tardive dyskinesia symptoms (8,9,4).

The apparent clinical dopaminergic supersensitivity has its parallel in animal behaviour. Thus, rats chronically treated with dopaminergic antagonists (neuroleptics) show increased behavioural sensitivity to dopaminergic agonists such as apomorphine (10-13). This increased behavioural responsiveness to the dopaminergic agonists is also thought to be due to postsynaptic dopamine-receptor supersensitivity.

Attempts at direct demonstration of dopamine-receptor supersensitivity have yielded conflicting results. Thus, Yarborough (14) demonstrated that proportionately more cells (compared to control) in the striatum are inhibited by dopamine or apomorphine following heloperidol pretreatment. On the other hand, chronic neuroleptic pretreatment did not increase dopamine-sensitive striatal adenylate cyclase (15,16).

In the present experiments, we sought direct evidence for dopamine-receptor supersensitivity in both the rat striatum and the limbic region, using two different types of radioligands to tag the dopamine receptor: ^{3}H-haloperidol and ^{3}H-apomorphine. A preliminary report of some of this work was reported earlier (1976) [see also (17-21)]. We also studied the effect of haloperidol pretreatment on other neurotransmitter binding sites in different brain areas and compared them with dopaminergic binding.

METHODS

Rats were intubated daily with 10 mg/kg haloperidol dissolved in 0.1 M tartaric acid. Controls were intubated with the tartaric acid only. Both solutions were given as 1 ml/100 g of rat weight. The chronic treatment lasted at least three weeks. The animals were sacrificed 48 hours after the last intubation.

The experimental protocol for the homogenate preparation has been described previously (22). Briefly, the rats were sacrificed by cervical dislocation. The brains were removed and rinsed with ice-cold saline. The mesolimbic structures, cortex, hippocampus and striatum were dissected on ice. The mesolimbic structures were dissected by isolating tissues rostrally to the anterior commissure, caudally to the olfactory bulb and laterally to the striata. It contained nucleus accumbens, septum, olfactory tubercle, medial piriform cortex and part of the amygdala including nucleus amygdaloid centralis. The homologous structures from about five rats given the same treatment were pooled and teflon-homogenized in the modified TRIS buffer. The tissues were then incubated at 37°C for one hour, centrifuged at 40,000 g for 20 minutes and resuspended to contain about 70 mg of the original wet weight per ml of buffer (the buffer contained 15 mM Tris-HCl, pH 7.4, 5 mM Na_2EDTA, 1.1 mM ascorbate and 12.5 μM nialamide). This yielded approximately 1 mg of protein/ml for the homogenate solution. The homogenates were then stored in 3 ml volumes at -20°C [which preserves the binding sites; (21)]. The protein concentration was then determined for each batch of homogenates by the Lowry method.

Radioreceptor assays: Each individual radioreceptor assay consisted of 10 test-tubes containing homogenates from normal rats and 10 tubes with homogenates from rats treated with haloperidol. The protein content was about 0.2 mg/tube, and the total volume in each tube was 0.6 ml.

^{3}H-Haloperidol radioreceptor assay (22,23): The stereospecific binding of ^{3}H-haloperidol was measured by minor modifications of previously described methods (22,23). In each incubation tube the final concentration was between 1 and 2 nM for ^{3}H-haloperidol (7.7 Ci/mmole; originally prepared for this laboratory in May 1974, I.R.E. Belgique, with specific activity of 9.6 Ci/mmole), and either

100 nM (+)-butaclamol or 100 mM (-)-butaclamol. After incubation at 20-22° for 30 min, 0.5 ml was filtered under vacuum through a Whatman GF/B glass fiber filter followed by a wash of 5 ml (ice-cold buffer). The filters were placed in liquid scintillation vials, 8 ml of Aquasol (New England Nuclear Corp., Boston) were added, and the vials monitored for ^3H at least 6 hrs later. Stereospecific binding of ^3H-haloperidol was defined as that amount bound in the presence of (-)-butaclamol minus that bound in the presence of (+)-butaclamol (22,23).
^3H-Apomorphine radioreceptor assay (24): The stereospecific binding of ^3H-apomorphine was measured by minor modifications of a method previously described (22). In each incubation tube the final concentration of ^3H-apomorphine (13.1 Ci/mmole; originally custom-prepared at 14 Ci/mmole for this laboratory by New England Nuclear Corp., Boston) was between 1.5 and 6 nM; each tube also contained either 1000 nM (-)-butaclamol or 1000 nM (+)-butaclamol. Incubation and filtration was as above, and each filter was washed with 5 mls of buffer. Stereospecific binding of ^3H-apomorphine was defined as that bound in the presence of (-)-butaclamol minus that bound in the presence of (+)-butaclamol (24).
^3H-Quinuclidinyl benzilate radioreceptor assay for cholinergic receptors: The specific binding of ^3H-quinuclidinyl benzilate was done with minor modifications by the method of Yamamura and Snyder (25). Each tube contained a final concentration of 1 nM ^3H-quinuclidinyl benzilate (28.2 Ci/mmole; New England Nuclear Corp.), and half the tubes contained 100 nM scopolamine. Incubation for 30 min at 20°C was sufficient to achieve equilibrium. Filtration was as above (5 mls buffer). Specific binding of ^3H-quinuclidinyl benzilate was defined as that bound in the absence of scopolamine minus that bound in its presence.
^3H-Serotonin radioreceptor assay (26): The specific binding of serotonin was done by the method of Whitaker and Seeman (26). Each tube contained a final concentration of 3 nM ^3H-serotonin creatinine sulphate (12.5 Ci/mmole; Amersham/Searle), and half the tubes also contained excess (100 nM) serotonin creatinine sulphate. After incubation at 20-22°C for 30 min, 0.5 ml was filtered under vacuum through Whatman GF/B filters followed by a wash with 5 ml of ice-cold buffer. Specific binding was defined as that bound in the absence of excess serotonin minus that bound in its presence.
^3H-Naloxone radioreceptor assay (27,28): Specific naloxone binding was done by the method of Pert and Snyder (27,28) with minor modifications. Each tube contained a final concentration of 1 nM ^3H-naloxone (16.4 Ci/mmole; New England Nuclear Corp.) and half the tubes contained excess (500 nM) morphine. Incubation and filtration was as above, and the filters were washed with a rinse of 5 ml of buffer. Specific binding was defined as that occurring in the absence of excess morphine minus that in its presence.
^3H-WB-4101 radioreceptor assay for alpha-adrenergic receptors (29): The specific WB-4101 binding was modified from Greenberg et al. (29). Each tube contained a final concentration of 1 nM ^3H-WB 4101 (13.0 Ci/mmole, New England Nuclear Corp.) and half the tubes contained excess (100 nM) phenoxybenzamine. Incubation and filtration was as above (5 ml rinse). Specific binding was defined as that occurring in the absence of phenoxybenzamine minus that in its presence.

The percentage increment (or decrement) between the haloperidol-treated tissue and the matched control were obtained for each membrane preparation. The geometrical average, standard deviation and standard error of the mean were determined for these percentage increments. The geometrical mean was used instead of arithmetical mean to compensate for the bias towards elevated values, which would occur with the arithmetic mean. The significance of the changes were calculated using the Wilcoxon's rank test.

RESULTS

The long-term haloperidol pretreatment resulted in a 34% increase in ^3H-haloperidol binding and a 77% increase in ^3H-apomorphine binding in the rat striatum (Fig. 1). These increases were significant at the 0.05 and 0.02 levels,

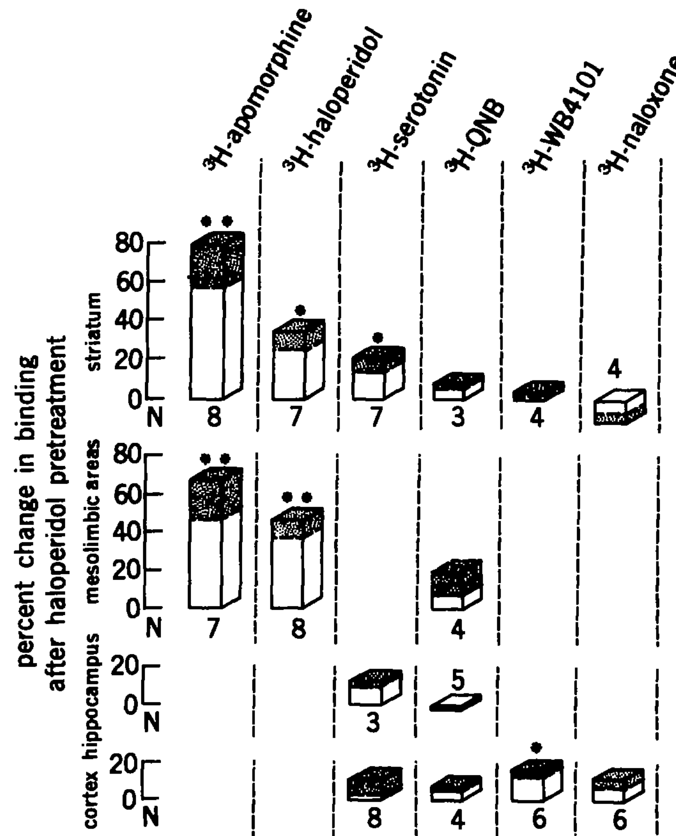

Fig. 1. Percentage changes in neurotransmitter receptors (rat brain regions) following long-term haloperidol treatment (10 mg/kg/day for at least three weeks). The height of the bars indicates the mean percentage change. The shaded portion indicates the standard error of the mean. The number of completely independently-assayed membrane preparations (from 2 to 15 rats per preparation) used in each experiment (N) is indicated below each bar (* = P < .05; ** = P < .02 using Wilcoxon's rank test). The long-term haloperidol treatment selectively increased the dopamine/neuroleptic binding without significant effects on acetylcholine receptors (^{3}H-QNB), alpha-adrenergic receptors (^{3}H-WB-4101) or opiate receptors (^{3}H-naloxone).

respectively, using the Wilcoxon's rank test.

In the mesolimbic brain areas ^{3}H-haloperidol binding increased by 45% and apomorphine binding by 55%. These increments were significant at the P < 0.02 level.

Striatal ^{3}H-serotonin binding increased by 20% (P < .05), although the hippocampal and cortical binding of ^{3}H-serotonin was not significantly affected by the chronic haloperidol treatment.

There was no significant change in ^{3}H-naloxone or ^{3}H-QNB binding in any of

the areas tested.

The chronic haloperidol administration induced a small, but significant, increase of ^{3}H-WB 4101 binding in the cortex, but not in the striatum. To test whether the increase in the striatal serotonin binding after chronic haloperidol was or was not due to a true increase in ^{3}H-serotonin binding sites, we also measured specific ^{3}H-serotonin binding in the presence of excess (500 nM) apomorphine. This ought to have eliminated any binding of ^{3}H-serotonin to dopamine binding sites. Under these conditions, the ^{3}H-serotonin binding was possibly somewhat reduced, but the increase in specific ^{3}H-serotonin binding was no less prominent than in the assays without the apomorphine present (Fig. 2). The assays were done using only two haloperidol-treated striatal preparations with their matched controls. The assays were done in duplicates. The same design was used for the cortex (Fig. 2).

DISCUSSION

These results indicate that long-term haloperidol treatment selectively increased the amount of specific binding of ^{3}H-haloperidol and ^{3}H-apomorphine in both the striatum and mesolimbic tissues without much change in other receptors. This increase may reflect a higher affinity of the receptor for these ligands, or there may have been an increase in the number of receptors (19).

These results are compatible with past behavioural observations made on animals undergoing long-term neuroleptic treatment. Such animals are apparently supersensitive to dopaminergic agonists (apomorphine; 10-13) as measured by stereotypy, for example. Some evidence has previously been presented indicating an increase in dopamine/neuroleptic receptors following chronic neuroleptic treatment in the striatum (17-21,30). Stereotypy, however, is not exclusively mediated by the striatal dopaminergic system. It is known that the mesolimbic system is also involved in eliciting stereotypy (31,32). It is known, furthermore, that locomotor activity also increases following chronic neuroleptic pretreatment (33-36). In locomotor behaviour, however, the striatal dopaminergic system seems to play a minor role, while the mesolimbic dopaminergic system is dominantly involved (36-40). It has been observed that rats display more locomotor behaviour (upon intra-accumbens injection of dopamine) following chronic penfluridol pretreatment than control rats. Our results showing increased haloperidol and apomorphine binding in the mesolimbic system are consistent with dopaminergic supersensitivity in the mesolimbic system.

Considerable supersensitivity (after chronic haloperidol) of both the mesolimbic system and the nigrostriatal system may arise from only rather small percentage changes in numbers of receptors. For example, Kelly and Moore (41) reported that the limbic dopaminergic system might be functioning as a modulator of the striatal dopaminergic system. Thus, if both systems are supersensitive as a result of chronic neuroleptic pretreatment, it is possible that the effect of these two regional supersensitivities is multiplied.

Behavioural supersensitivity might not necessarily be due to changes exclusively in the two dopaminergic systems. Other neurotransmitters and other receptors might be involved. Serotonin is an example of such possible influence of other neurotransmitters on dopaminergic behaviour (42). It appears that serotonin exercises a negative influence on dopaminergic activity; thus, 5-hydroxytryptophan inhibits apomorphine-induced stereotyped behaviour (43), while lesions of the raphe nuclei augment apomorphine-induced locomotor behaviour (44). In addition, serotoninergic antagonists potentiate dopaminergic action (43,45). In the present experiments we observed an increase in the specific ^{3}H-serotonin binding following chronic haloperidol pretreatment. This increase was not seen in the cortex or the hippocampus. The increase was not due to ^{3}H-serotonin binding to dopamine receptors, since excess of apomorphine in the incubation media did not eliminate the increase. It is possible, therefore, that the effect on the serotonin system we observed is either due to some direct effect of the dopaminergic tract on the serotoninergic pathway or synapse in the striatum.

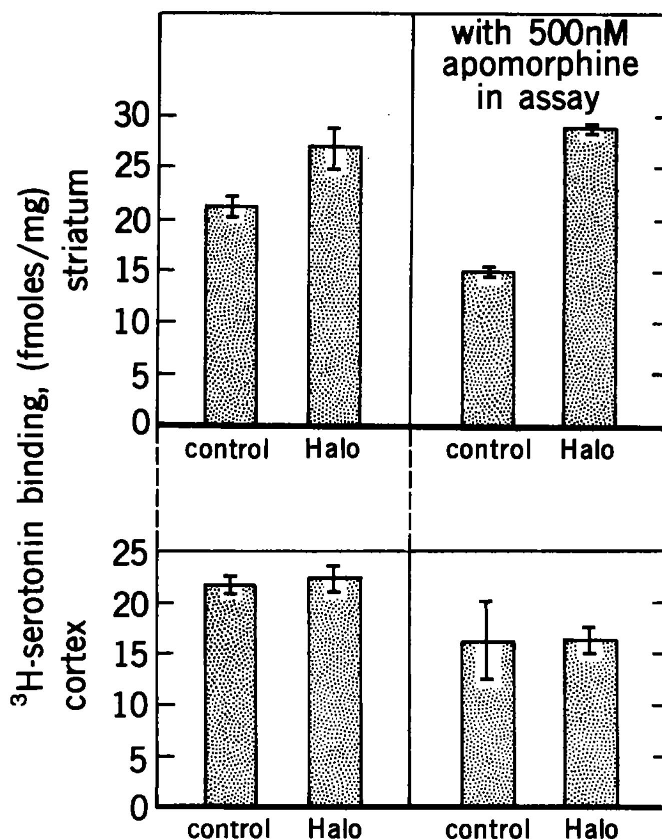

Fig. 2. Long-term haloperidol treatment increases the specific binding of ^{3}H-serotonin in the striatum. Left: Tissues from control rats and haloperidol-treated rats showing an increase in the striatum, but no change in the cortex. Right: Same membrane preparations, but the assays were done in the presence of an excess (500 nM) apomorphine to preclude any ^{3}H-serotonin from binding to dopamine sites.

This possibility is supported by reports showing that dopamine agonists affect serotonin turnover, even though the direction of the change is controversial (45-47). It appears more likely that serotonin receptors become supersensitive as a compensation for the changes in the dopaminergic system. This is further supported by the report that acute pretreatment with the neuroleptics has no effect on serotonin turnover (49-50), while chronic pretreatment with the same drugs induced an increase in serotonin turnover (49). These results suggest that the effect of the dopaminergic system on the serotoninergic system might not be direct and might develop over a period of time.

We have also measured the binding to the alpha-noradrenergic binding sites in the striatum. Alpha-noradrenergic binding in the striatum was reported previously (51), even though the functional significance is not clear, since

there is little noradrenaline found in the striatum (52). We would have not, therefore, expected any increase in the binding in the striatum following chronic haloperidol pretreatment.

We were unable to observe any significant change in opiate receptors following chronic neuroleptic pretreatment. Opiates are interesting drugs to study in relation to the striatal dopaminergic system. They share many properties with the neuroleptics. They increase the turnover of dopamine (53), induce catalepsy (53) and block apomorphine-induced stereotyped behaviour (54). Furthermore, cross-tolerance to some opiate-neuroleptic actions has been observed (55, 56). Because of this close relation between the opiates and the neuroleptics, one might have expected a change in the striatal ^{3}H-naloxone binding (57). Both cortex and striatum, however, showed no significant change in ^{3}H-naloxone binding.

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