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IN VIVO BLOCKADE OF DOPAMINERGIC RECEPTORS FROM DIFFERENT RAT BRAIN REGIONS BY CLASSICAL AND ATYPICAL NEUROLEPTICS

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Abstract—The effect of haloperidol, chlorpromazine, clozapine, benzamides (sulpiride and isosulpride), 6-chloropyrimidines (mezilamine, UK 177) on the *in vivo* binding of [^{3}H]spiroperidol to striatum, tuberculum olfactorium, frontal cortex and cerebellum in the rat brain was investigated. Since these neuroleptics of various chemical classes were able to prevent the selective retention of [^{3}H]spiroperidol in the striatum and tuberculum olfactorium without modifying the level in the cerebellum, it has been assumed that [^{3}H]spiroperidol is a suitable ligand to label dopaminergic receptors in the living animal. All the neuroleptics (except the benzamides) were able to displace [^{3}H]spiroperidol from its receptors in the frontal cortex, suggesting a serotoninergic component in neuroleptic binding sites. Classical neuroleptics (haloperidol, chlorpromazine, UK 177) or atypical neuroleptics (clozapine, sulpiride, isosulpride, mezilamine) did not induce a selective blockade of dopaminergic receptors in the striatum or in the limbic system, respectively. These results indicate that there is no correlation between the selective regional stimulation of dopamine turnover after neuroleptics and the *in vivo* blockade of postsynpatic dopaminergic receptors.

Since the initial observations that neuroleptics induce an accumulation of *O*-methylated [1] or acidic [2] dopamine (DA) metabolites in rodent brain, this increase has been related to a compensatory activation of dopaminergic neurons subsequent to postsynaptic receptor blockade [3–6]. More recently, neuroleptics have been shown to increase DA turnover within specific dopaminergic neuronal systems. Classical neuroleptics (haloperidol, chlorpromazine, etc.) with a high incidence of extrapyramidal side effects increase DA turnover in the nigrostriatal system more than in the mesolimbic and mesocortical systems, whereas atypical neuroleptics (clozapine, sulpiride, mezilamine) with a low incidence of extrapyramidal side effects are more effective on DA turnover in limbic areas than in the striatum [7–13].

The activation of DA neurons by neuroleptics is thought to be mediated by a neuronal feedback mechanism triggered by the blockade of postsynaptic DA receptors. If this is so, the blockade of DA receptors by atypical and classical neuroleptics should vary from one region of the brain to another. To test this hypothesis we have studied the *in vivo* displacement of the binding of [^{3}H]spiroperidol (an extremely potent neuroleptic which labels specific neuroleptic binding sites *in vitro* and *in vivo* [14, 15], by classical and atypical neuroleptics. No correlation between regional stimulation of DA turnover and the selective blockade of DA receptors has been found.

MATERIALS AND METHODS

We have used the method of Laduron *et al.* [15] with slight modifications. The neuroleptics were injected i.p. to male Sprague–Dawley rats (150±10g) 15 min before the i.v. injection of [^{3}H]spiroperidol (2 μg/kg, specific activity 26.4 Ci/mmole from N.E.N.). The animals were killed by decapitation 2 hr after the administration of [^{3}H]spiroperidol, the brains were removed, dissected and weighed. The radioactivity was determined in a liquid scintillation spectrometer (Packard Prias PLD Tricarb) after solubilization in Soluene (Packard). In control experiments, the radioactivity was extracted by heptane–isoamyl alcohol (8.5 : 1.5) and analyzed by TLC (benzene–ethanol–NH_4OH 90 : 10 : 1). Radioactivity was measured with a radiochromatogram reader (Chromelec 101) connected to a multichannel recorder (Interzoom).

RESULTS

Two hours after the intravenous injection of a low dose (2 μg/kg) of [^{3}H]spiroperidol, the radioactivity was found to be present preferentially in the dopaminergic areas (Table 1). The order of retention of radioactivity in different parts of the brain was similar to that found by Laduron *et al.* [15]: striatum > tuberculum olfactorium > frontal cortex > cerebellum. Chromatographic studies revealed that 2 hr after i.v. injection, at least 90% of the [3H]spiroperidol was found in the rat brain as the unchanged drug. All the neuroleptics used were able to prevent, in a dose dependent manner, the retention of [^{3}H]spiroperidol in the dopaminergic regions (striatum, tuberculum olfactorium, frontal cortex) without altering the level of [^{3}H]spiroperidol recovered in the cerebellum. For this reason and the fact that the radioactivity found in the cerebellum is exactly that found in the other brain regions after pretreatment with cold drug, the radioactivity in the

Table 1. Regional *in vivo* binding of [^{3}H]spiroperidol in the rat brain. [^{3}H]Spiroperidol (2 μg/kg) was injected i.v. and the animals were killed 2 hr later. N = 50 determinations

Brain regions	Total binding (pmoles/g tissue)	Specific binding (total binding–cerebellum binding) (pmoles/g tissue)
Striatum	3.59 ± 0.09	2.89 ± 0.08
Tuberculum olfactorium	2.64 ± 0.07	1.94 ± 0.06
Frontal cortex	1.50 ± 0.03	0.80 ± 0.03
Cerebellum	0.70 ± 0.02	—

cerebellum was chosen to represent nonspecific binding, thus allowing the results to be expressed in terms of specific binding as a percentage of the control (total binding–cerebellum binding) for the dose–response curves of the displacement of [^{3}H]spiroperidol from dopaminergic regions by the neuroleptics (Figs. 1 A, B, C). The ED_{50} for this prevention, i.e. the doses for which the difference between dopaminergic regions and cerebellum is half-maximal, are presented in Table 2. Haloperidol was the most potent in the tuberculum olfactorium and the striatum, whereas chlorpromazine was the most effective in the frontal cortex. Isosulpride [2-(1-ethyl-2-pyrrolidinyl)-*N*-(2-methoxy-5-sulfamoyl-phenyl) acetamide], a derivative similar to sulpiride, was the least active. Sulpiride in the tuberculum olfactorium and clozapine in the frontal cortex showed the greatest regional selectivity. All the neuroleptics including classical (haloperidol, chlorpromazine, UK 177) and atypical (clozapine, mezilamine, sulpiride, isosulpride) were more potent on the tuberculum olfactorium than in the striatum (ratio: ED_{50} in To/ED_{50} in St = <1). All the compounds except chlorpromazine and clozapine were less effective in the frontal cortex (ratios: ED_{50} in Fc/ED_{50} in To and ED_{50} in Fc/ED_{50} in St = >1).

DISCUSSION

As already stated in previous publications on [^{3}H]spiroperidol or [^{3}H]pimozide [15, 16], it can be assumed that tritiated potent neuroleptics are able to label *in vivo* specific binding site receptors and that the difference between levels in frontal cortex, tuberculum olfactorium or striatum and cerebellum represents specific binding to multiple neuroleptic receptors. This is supported by the fact that neuroleptics of various chemical classes such as butyrophenone (haloperidol), phenothiazine (chlorpromazine), dibenzodiazepine (clozapine), benzamides (sulpiride, isosulpride) and chloropyrimidines (mezilamine, UK 177) are able to prevent the selective retention of [^{3}H]spiroperidol in the tuberculum olfactorium or striatal area without modifying the level in cerebellum.

Leysen *et al.* [17] have shown that spiroperidol and LSD label the same receptors in the frontal cortex and that these receptors are different from those labelled by spiroperidol in the striatum. The receptors in the frontal cortex are predominantly serotoninergic in nature [17] indicating a serotoninergic component in neuroleptic binding sites. Similar results have been obtained in limbic brain areas [18]. Our results comply with this suggestion, since all the neuroleptics were able to displace [^{3}H]spiroperidol from its receptors in the frontal cortex or the tuberculum olfactorium.

Although it has been shown that increases in DA turnover occur preferentially in the mesolimbic region rather than the striatal one with atypical neuroleptics and the reverse with classical neuroleptics [7–13], we have been unable to demonstrate a selective blockade of dopaminergic receptors in the striatum by the classical neuroleptics or in the mesolimbic system by the atypical neuroleptics. Different observations have provided evidence that there is no correlation between a preferential activation of DA neurons and a selective blockade of postsynaptic DA receptors: (1) all the neuroleptics (classical and atypical) were more potent in the tuberculum olfactorium than in the striatum; even though the atypical neuroleptic clozapine was more

Table 2. ED_{50} of the *in vivo* displacement of [^{3}H]spiroperidol by different neuroleptics in the rat brain. Results were expressed in mg/kg i.p. ED_{50} were obtained from the dose–response curves of Fig. 1

Drugs	Tuberculum olfactorium (To)	Striatum (St)	Frontal cortex (Fc)	ED_{50} in To / ED_{50} in St	ED_{50} in Fc / ED_{50} in To	ED_{50} in Fc / ED_{50} in St
Haloperidol	0.08	0.2	1.1	0.4	14	5.5
UK 177	1.2	2	5	0.6	4	4.2
Chlorpromazine	1.4	3.5	0.6	0.4	0.4	0.17
Mezilamine	2	4	9	0.5	4.5	4.5
Clozapine	>50	>50	7	<1	<1	<1
Sulpiride	35	>100	>100	<1	>1	>1
Isosulpride	90	>100	>100	<1	>1	>1

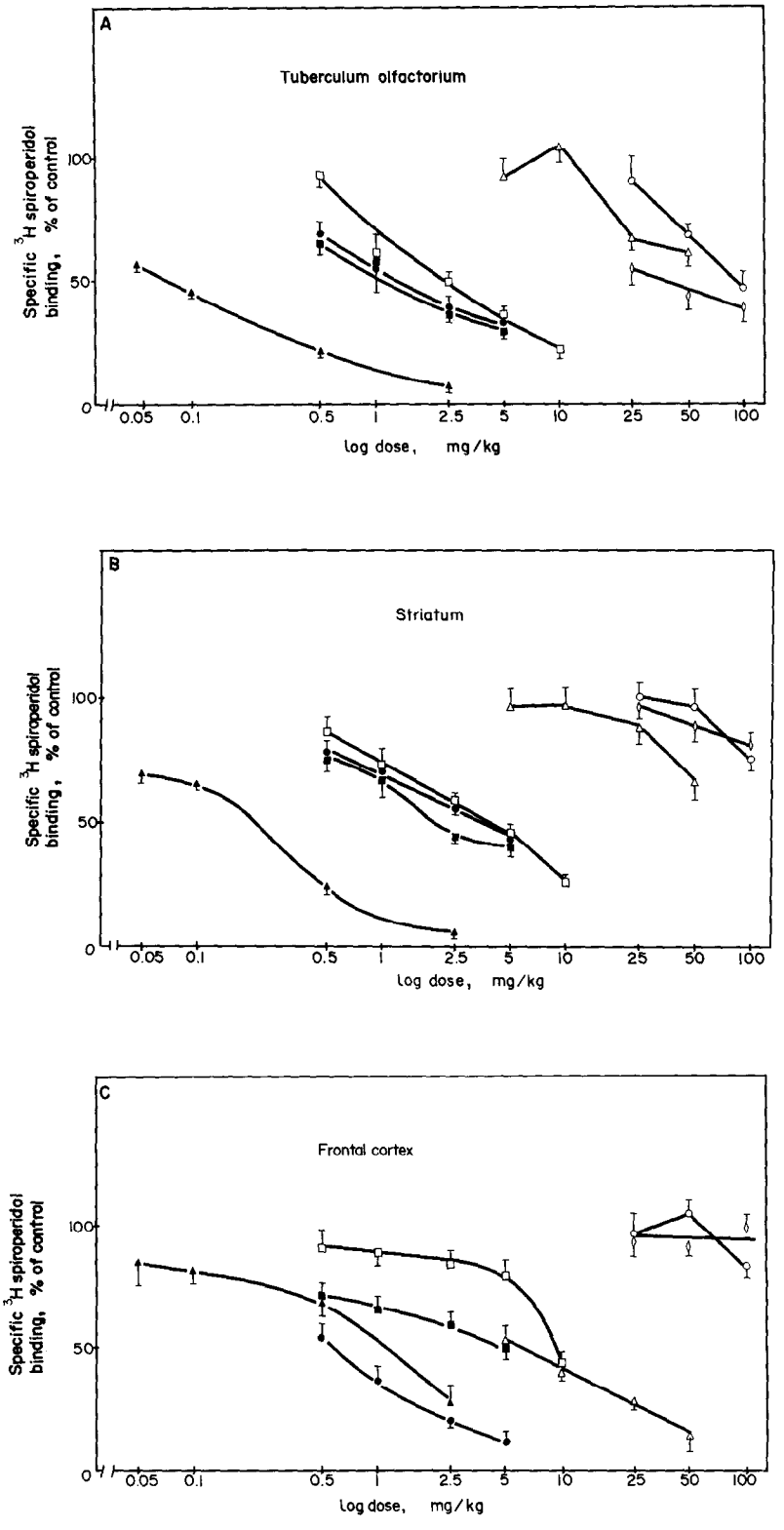

Fig. 1. Prevention of the binding of [^{3}H]spiroperidol by different neuroleptics in the rat brain. The drugs were injected i.p. 15 min before the i.v. injection of [^{3}H]spiroperidol (2 μg/kg). Rat brain was removed 2 hr after the administration of [^{3}H]spiroperidol and the radioactivity was measured in the tuberculum olfactorium (A), striatum (B), frontal cortex (C) and cerebellum. The results were expressed in terms of specific binding as a per cent of the control (total binding–cerebellum binding). Each value represents the mean of 5–15 determinations (±S.E.M.). ▲ Haloperidol; ■ UK 177; ● Chlorpromazine; □ Mezilamine; △ Clozapine; ◇ Sulpiride; ○ Isosulpride.

effective in the frontal cortex than in the other regions, the same result was obtained with the classical neuroleptic chlorpromazine relating such effects to the strong antiserotoninergic properties of these two compounds [17]; (2) in a new chemical class of potential anti-psychotics, mezilamine (2-methylamino-4-*N*-methylpiperazino-5-methylthio-6-chloropyrimidine) which has been related to the atypical neuroleptics [13] and UK 177 (2-benzylamino-4-*N*-methylpiperazino - 5 - methylthio - 6 - chloro - pyrimidine) which possesses a classical spectrum of neuroleptic with a high incidence of extrapyramidal side effects induce a similar regional blockade of DA receptors (To > St > Fc).

Such a lack of correlation between the differing effects of neuroleptics on DA turnover in the three DA areas and the effect *in vitro* of these drugs on [^{3}H]haloperidol binding [19] or DA sensitive adenylate cyclase has previously been suggested [20–23]. Moreover, we have obtained similar results *in vivo* which allow us to rule out a difference in drug kinetics and/or metabolism leading to a regional preferential accumulation of these compounds which could explain a different effect on DA turnover.

In conclusion, the selective regional stimulation of DA turnover after neuroleptics cannot be correlated with variations in the blockade of postsynaptic DA receptors. This suggests that the activation of DA neurons by neuroleptics is not exclusively mediated by a direct neuronal feedback mechanism triggered by the blockade of postsynaptic DA receptors. The possibility of a differential blockade of auto presynaptic (which could regulate DA synthesis) and postsynaptic DA receptors by neuroleptics could be put forward, but the existence of such autoreceptors must first be definitively demonstrated. Another possible explanation should be the different onset for the pharmacological activity of different neuroleptics.

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