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# THE USE OF THIOPROPERAZINE, A PHENOTHIAZINE DERIVATIVE, AS A LIGAND FOR NEUROLEPTIC RECEPTORS—II.

## *IN VIVO* STUDIES

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**Abstract**—Specific *in vitro* binding of [$^3$H]thioproperazine (a neuroleptic of the phenothiazine family) has previously been demonstrated in the rat brain. In this paper the *in vivo* binding of [$^3$H]thioproperazine to dopamine receptors is reported. After the administration of a tracer dose of [$^3$H]thioproperazine, this neuroleptic is preferentially accumulated in the dopaminergic areas of the brain. The accumulation of [$^3$H]thioproperazine is prevented by prior administration of various doses of unlabelled thioproperazine in the striatum but not in the cerebellum; thus accumulation in the cerebellum is considered to be nonspecific binding. Neuroleptics of different chemical families, phenothiazines (thioproperazine, pipotiazine, prochlorperazine and levomepromazine), butyrophenones (haloperidol, pipamperone) and a dibenzodiazepine (clozapine) are able to compete with [$^3$H]thioproperazine. Moreover, the pharmacologically inactive D-isomer (R.P. 7 185) of levomepromazine is devoid of such activity. These findings indicate that the *in vivo* binding of [$^3$H]thioproperazine is specific. It is concluded that thioproperazine is a suitable ligand for *in vivo* dopamine receptor studies as well as for *in vitro* studies.

In the previous paper [1], we reported the specific *in vitro* binding of [$^3$H]thioproperazine (a potent neuroleptic of the phenothiazine family) to membranes prepared from different dopaminergic regions of the rat brain. Apart from *in vitro* studies, one approach to providing further information on [$^3$H]thioproperazine binding is the labelling of binding sites after *in vivo* administration of this ligand. According to Laduron *et al.* [2], *in vivo* specific binding of a neuroleptic drug must primarily fulfil two criteria : (i) specific retention of the drug in the dopaminergic areas of the brain, and (ii) displacement of this binding by large doses of unlabelled neuroleptics. Moreover, this binding should be stereospecific. Such specific binding has been demonstrated using butyrophenone derivatives: [$^3$H]pimozide [3–5] and [$^3$H]spiperone [2, 4–7].

As far as we are aware, the only phenothiazine neuroleptic tested as a ligand for *in vivo* studies has been chlorpromazine [8]; however, with this drug, no specific binding has been observed.

In this paper, we report the *in vivo* binding of the phenothiazine neuroleptic [$^3$H]thioproperazine.

### MATERIALS AND METHODS

[$^3$H]Thioproperazine (sp. act. 18.5 Ci/mmole) was administered by intravenous injection (i.v.) to male rats (200 ± 10 g, $CD_1$, C.O.B.S., Charles River, France). The animals were decapitated 1 hr after the injection of a standard dose (5 $\mu g.kg^{-1}$) of this labelled neuroleptic. Brain regions were dissected and homogenized in 9 vol. (w/v) of 50 mM Tris buffer, pH 7.4. The radioactivity was counted in aliquots (220-$\mu$l) by liquid scintillation spectrometry. In the experiments with unlabelled compounds, neuroleptics and metopimazine (a phenothiazine antiemetic) were injected subcutaneously (s.c.) 1.5 hr before i.v. injection of [$^3$H]thioproperazine while apomorphine was administered by the intraperitoneal route (i.p.) at the same time as the i.v. injection of [$^3$H]thioproperazine. Results of accumulation were expressed in pmoles of labelled material per g wet wt. [$^3$H]Thioproperazine was prepared by Mr. Raballand (Rhône-Poulenc and C.E.A. Saclay, France). The other drugs were obtained from various pharmaceutical companies.

### RESULTS

Throughout this work, the total radioactivity was always considered as unchanged drug. Indeed, chromatographic studies using thin layer chromatography (t.l.c.) have shown that 1 hr after i.v. injection, 93 per cent of [$^3$H]thioproperazine was found in the rat brain as unchanged drug.*

*Regional distribution of* [$^3$H]*thioproperazine.* Table 1 shows the distribution of [$^3$H]thioproperazine in different areas of the brain. [$^3$H]Thioproperazine at a very low dose (5 $\mu g.kg^{-1}$) was found to be preferentially taken up in dopaminergic areas (striatum, nucleus accumbens, tuberculum olfactorium). In the cerebellum, the medulla oblongata + pons and the frontal cortex, we found comparable concentrations of [$^3$H]thioproperazine which were, however, lower than those found in the dopaminergic regions mentioned.

* D. Heusse, personal communication.

Table 1. Regional distribution of radioactivity in rat brain 1 hr after i.v. injection of [$^3$H]thioproperazine*

| Brain region | *In vivo* distribution of [$^3$H]thioproperazine (pmoles/g) |
|---|---|
| Striatum | 4.32 ± 0.17 |
| Nucleus accumbens | 3.61 ± 0.06 |
| Tuberculum olfactorium | 3.41 ± 0.19 |
| Frontal cortex | 1.94 ± 0.17 |
| Medulla oblongata + pons | 1.99 ± 0.14 |
| Cerebellum | 2.10 ± 0.10 |

* Results are the mean ± S.E.M. of at least four determinations.

*Effect of different doses of unlabelled thioproperazine on the accumulation of [$^3$H]thioproperazine in the striatum and the cerebellum.* This study was performed in the striatum, the main dopaminergic structure in the brain, and in the cerebellum, a well-known non-dopaminergic structure. Figure 1 shows that unlabelled thioproperazine in the dose range 0.005–0.1 mg.kg$^{-1}$ s.c. induces a dose-related reduction in striatum radioactivity; higher doses of thioproperazine had no further effect on the radioactivity accumulated in this structure. This reduction of radioactivity is not detectable in the cerebellum. Moreover, at high doses (0.1–2 mg.kg$^{-1}$ s.c.) of thioproperazine, the amount of radioactivity in the striatum was not significantly different from that found in the cerebellum. From these results it can be assumed that [$^3$H]thioproperazine accumulation in the cerebellum is equivalent to the non-specific binding of this drug in the striatum; thus the difference between the accumulation of [$^3$H]thioproperazine in the striatum and the cerebellum can be assimilated to a specific *in vivo* binding.

*Effect of different neuroleptics and apomorphine on the specific binding of [$^3$H]thioproperazine in the striatum.* In further experiments, different neuroleptics were tested for their ability to compete with [$^3$H]thioproperazine binding in the striatum. The efficacy of these drugs at preventing the binding of [$^3$H]thioproperazine is expressed as an $ED_{50}$, i.e. the dose for which the difference between striatum and cerebellum radioactivities is half of that found in animals receiving [$^3$H]thioproperazine alone. Figure 2 shows the potency of various neuroleptics (injected s.c.) in preventing specifically bound [$^3$H]thioproperazine. Neuroleptics of different chemical families (phenothiazines: thioproperazine, pipotiazine, prochlorperazine and levomepromazine; butyrophenones: haloperidol and pipamperone or a dibenzodiazepine: clozapine) are able to compete with specifically bound [$^3$H]thioproperazine. It

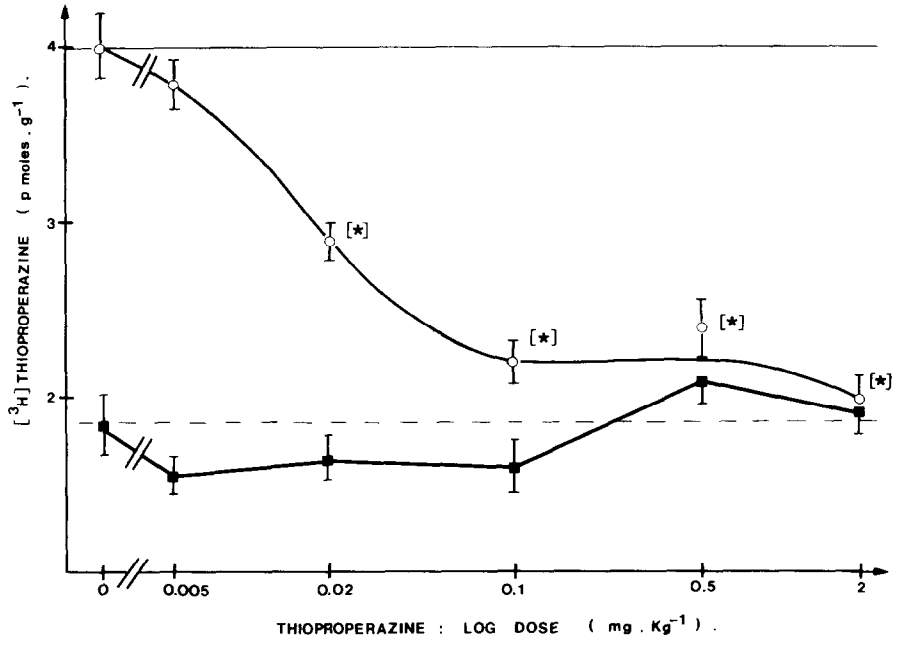


Fig. 1. Effect of different doses of unlabelled thioproperazine on [$^3$H]thioproperazine accumulation in the striatum (○——○) and the cerebellum (■——■). Each point is the mean of at least four determinations. [*] $P < 0.01$.

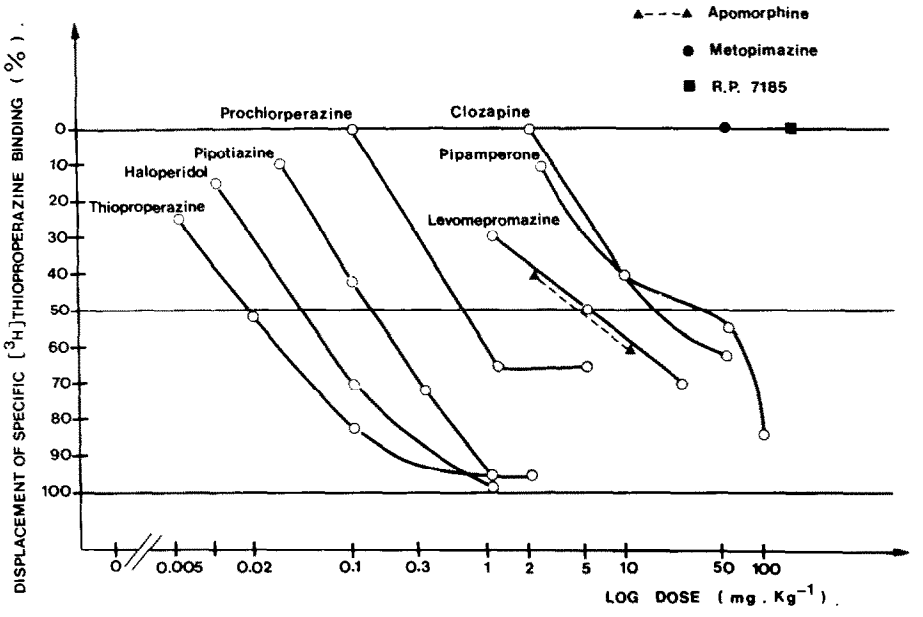


Fig. 2. Competition of specifically bound [$^3$H]thioproperazine by different compounds in the striatum. The neuroleptics and metopimazine were injected (s.c. route) 1.5 hr before the i.v. injection of [$^3$H]thioproperazine: apomorphine was injected (i.p. route) simultaneously with the labelled ligand.

is worth mentioning that the pharmacologically inactive D-isomer (R.P. 7 185) of levomepromazine is unable to compete with [$^3$H]thioproperazine at a dose 20 times higher than the $ED_{50}$ of levomepromazine.

Interestingly, metopimazine, a phenothiazine derivative with strong antiemetic activity, but devoid of neuroleptic activity, is not able to compete with [$^3$H]thioproperazine, even at high doses (50 mg. $kg^{-1}$ s.c.).

On the other hand, a potent dopamine agonist, apomorphine (injected i.p.) is able to prevent [$^3$H]thioproperazine binding (37 and 57 per cent inhibition at 2 and 10 mg.$kg^{-1}$ i.p., respectively).

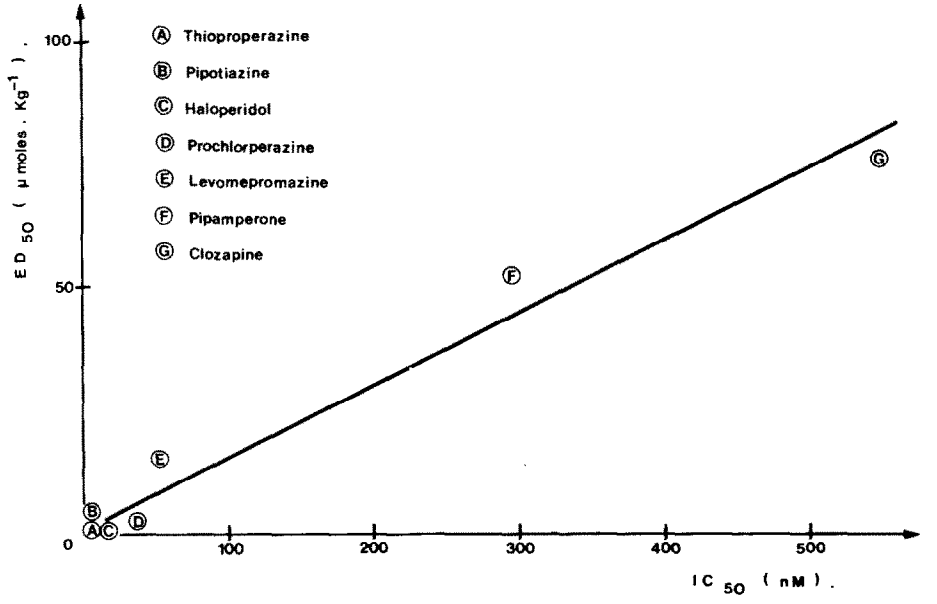


Fig. 3. Correlation between $ED_{50}$ ($\mu$moles . $kg^{-1}$) and $IC_{50}$ (nM) values from [$^3$H]thioproperazine binding studies. The $ED_{50}$ values (ordinate) are the doses of the different neuroleptics which reduce specific *in vivo* [$^3$H]thioproperazine binding by 50 per cent. The $IC_{50}$ values (abscissa) are the corresponding concentrations of the neuroleptics which reduce specific *in vitro* [$^3$H]thioproperazine binding by 50 per cent.

Figure 3 shows that there is a significant correlation ($r = 0.98$, $P < 0.01$, $N = 7$) between the *in vitro* $IC_{50}$ values [1] and the *in vivo* $ED_{50}$ (evaluated in $\mu$moles.kg$^{-1}$) of the different neuroleptics tested.

## DISCUSSION

In the previous paper [1], specific *in vitro* binding of [$^3$H]thioproperazine has been demonstrated in dopaminergic-rich regions of rat brain. In the striatum, kinetic data reveal that thioproperazine binds with a high affinity ($K_d = 0.3$ nM) and that the number of binding sites is 1.47 pmoles/mg protein. These values are similar to those found with other neuroleptics used as ligands [9]. Moreover, subcellular studies have indicated that the receptor sites of [$^3$H]thioproperazine are mainly associated with membrane-like structures.

In the present work, we investigated the possibility of specific *in vivo* binding of [$^3$H]thioproperazine in the rat brain.

Several lines of evidence are provided to demonstrate such specific *in vivo* binding.

Firstly, [$^3$H]thioproperazine is preferentially accumulated in the dopaminergic areas (striatum, tuberculum olfactorium, nucleus accumbens). We found that the ratio of radioactivity bound in the striatum compared to that bound in the cerebellum was smaller with [$^3$H]thioproperazine than that found by others using [$^3$H]spiperone, but relatively similar to that obtained with [$^3$H]pimozide (see Ref. 5). We did not find any difference between the level of accumulation of [$^3$H]thioproperazine in the frontal cortex and in the cerebellum. If we assume that the accumulation in the cerebellum corresponds to non-specific binding, no specific binding was observed in the frontal cortex. This *in vivo* result is in good agreement with the lack of specific binding observed in the frontal cortex under *in vitro* conditions using [$^3$H]thioproperazine as ligand [1]. On the contrary, using [$^3$H]spiperone as ligand, specific binding of this compound was observed under *in vitro* and *in vivo* conditions [2, 4–7, 9]; however, with this ligand, part of this binding could be linked to serotonergic sites [10–11].

Secondly, accumulation of [$^3$H]thioproperazine is reduced by unlabelled thioproperazine in the striatum but not in the cerebellum: low doses (0.02 and 0.1 mg . kg$^{-1}$ s.c.) of unlabelled thioproperazine can compete with [$^3$H]thioproperazine in the striatum, while, even at high doses, unlabelled drug is unable to compete with [$^3$H]thioproperazine in the cerebellum. The radioactivity levels observed in the striatum after the administration of high doses (0.5 and 2 mg . kg$^{-1}$ s.c.) of unlabelled thioproperazine were not different from those found in the cerebellum. In order to measure specific *in vivo* binding of [$^3$H]thioproperazine in the striatum, we have considered that [$^3$H]thioproperazine binding in the cerebellum is non-specific.

Thirdly, unlabelled neuroleptics of different chemical families compete with [$^3$H]thioproperazine in the striatum: the most potent compounds were major neuroleptics such as thioproperazine, haloperidol, pipotiazine and prochlorperazine whereas sedative neuroleptics such as levomepromazine, pipamperone and clozapine were less active. It should be stressed that [$^3$H]thioproperazine binding is stereospecific: the pharmacologically inactive D-isomer (R.P. 7 185) of levomepromazine is unable to compete with [$^3$H]thioproperazine.

On the other hand, when comparing the capacity of different neuroleptics to compete with [$^3$H]thioproperazine in the striatum, a good correlation was observed between *in vitro* and *in vivo* results. This could indicate that the *in vivo* antagonist effect of these neuroleptics is mainly due to the binding of these compounds to specific binding sites clearly demonstrated by *in vitro* studies.

The inability of metopimazine (a phenothiazine antiemetic drug devoid of neuroleptic activity) to compete with [$^3$H]thioproperazine *in vivo* binding (but with strong *in vitro* activity) could be the consequence of poor penetration of this drug into the brain.

Finally it is important to emphasize that apomorphine, a dopamine receptor agonist, is also able to compete with [$^3$H]thioproperazine *in vivo* in the striatum, as has already been shown *in vitro*.

In conclusion, we can consider that *in vivo* [$^3$H]thioproperazine binding in the rat brain occurs at the dopamine receptors, thus confirming the *in vitro* results. It is assumed that [$^3$H]thioproperazine can be used as a tool in the study of drugs acting on dopamine receptors in the brain.

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