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# Comparison of the pharmacological characteristics of [$^3$H]raclopride and [$^3$H]SCH 23390 binding to dopamine receptors in vivo in mouse brain

Peter H. Andersen

*Department of Biochemical Pharmacology, NOVO Industri A/S, Pharmaceuticals R and D, NOVO Allé, DK-2880 Bagsvaerd, Denmark*

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In vivo binding of the benzamide derivative [$^3$H]raclopride was studied in mouse brain. The binding was saturable, reversible and stereospecific. Non-specific binding was 5-15% of the total binding. Pharmacological characterization of the binding indicated labelling of dopamine D2 receptors since the binding was potently inhibited by compounds with high affinity for this receptor in vitro. On the other hand, compounds with low affinity in vitro i.e., dopamine D1-selective compounds were weak or inactive as inhibitors of [$^3$H]raclopride binding. A comparison of the pharmacological characteristics of [$^3$H]raclopride and [$^3$H]SCH 23390 binding in vivo indicated that compounds with selectivity in vitro retained this selectivity in vivo. Thus, spiroperidol, haloperidol, l-sulpiride, clebopride, LY 171555 and (−)-NPA ((−)-N-propyl-norapomorphine) were D2 selective while SCH 23390, SKF 38393 and SKF 75670 were D1 selective. Clozapine, tilozepine, cis-flupentixol, chlorpromazine and butaclamol were non-selective both in vitro and in vivo. However, a few compounds changed profile in vivo compared to in vitro. Thus, fluperlapine and fluphenazine had a dual D1-D2 receptor profile in vitro but were D1- or D2-selective in vivo, respectively. Pergolide and molindone which were D2-selective in vitro both had a dual D1-D2 receptor profile in vivo. In conclusion, [$^3$H]raclopride, in vivo, selectively labels the dopamine D2 receptor. Comparison of the pharmacological characteristics of [$^3$H]raclopride and [$^3$H]SCH 23390 binding in vivo supported the previous observation that the dopamine D1 receptor is an important target for a variety of neuroleptics, especially of the clozapine type. This may indicate that blockade of the dopamine D1 receptor conveys antipsychotic action.

[$^3$H]Raclopride; [$^3$H]SCH 23390; Brain; Neuroleptics; Clozapine; Dopamine receptors; (Receptor profile, In vivo binding, Mouse)

## 1. Introduction

The antipsychotic efficacy of neuroleptics was thought until recently to be mediated by the blockade of dopamine D2 receptors (see e.g. Seeman (1980)). However, recent research has indicated that selective dopamine D1 receptor antagonists such as SCH 23390 ((R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-N-3-benzazepine-7-ol) are almost functionally equivalent to D2 receptor antagonists i.e. they inhibit dopamine-dependent stereotyped behavior and discriminative stimulus-effects, the conditioned-avoidance response, and they induce catalepsy (Iorio et al., 1983; 1986; Chistensen et al., 1984; Nielsen and Jepsen, 1985; Barnett, 1986). Further, recent experiments on in vivo [$^3$H]SCH 23390 binding showed the importance of the dopamine D1 receptor as target for atypical neuroleptics e.g. of the clozapine-type (Andersen et al., 1986). To analyze in more detail the importance of the dopamine D1 receptor as in vivo target for neuroleptics, we investigated the dopamine receptor profile/selectivity of a variety of neuroleptics (dopamine receptor antagonist) and dopamine agonists. For this purpose, in vivo binding of the D2 receptor-selective benzamide [$^3$H]raclopride (Köhler et al., 1985) and the D1 receptor selective benzazepine

[$^3$H]SCH 23390 (Andersen and Grønvald, 1986) was studied in mouse brain tissue.

## 2. Materials and methods

### 2.1. Materials

[$^3$H]Raclopride (60 Ci/mmol) was obtained from Astra Alab, Sweden, (N-[$^3$H]methyl)-SCH 23390 (85 Ci/mmol) was synthesized and purified by HPLC by Dr. F.C. Grønvald, Medicinal Chemistry, Lab. I, at NOVO. Male NMRI mice (20-25 g) bred at NOVO were used for in vivo binding studies, and male Wistar rats (125-200 g) from Møllegaards Lab. (Lille Skensved, Denmark) were used for in vitro binding studies.

### 2.2. Drugs

Clebopride was a gift from Almirall Laboratories, Barcelona, Spain; raclopride from Astra Alab (Södertälje, Sweden); sulpiride from Delagrange (Paris, France): loxapine from Lederle-Cyanamid (Copenhagen, Denmark); LY 171555, and pergolid from Lilly Research Laboratories (USA); cis- and trans-flupentixol form Lundbeck A/S (Copenhagen, Denmark); l- and d-sulpiride from Ravizza Research Laboratories (Milano, Italy); clozapine, fluperlapine and tilozepine from Sandoz (Basel, Switzerland); fluphenazine and SCH 23390 from Schering Corp. (Bloomfield, New Jersey, USA). All other drugs used were obtained from commercial sources or were synthesized at NOVO by Dr. F.C. Grønvald, Medicinal Chemistry Lab. I at NOVO.

### 2.3. Drug treatment

All compounds were dissolved in 1 M tartaric acid + propyleneglycol (1 : 1) and brought up to volume with distilled water. The drugs were injected i.p. 2 h before killing except when otherwise indicated. The injection volume was 300 $\mu$l (i.p. and s.c.) or 200 $\mu$l (i.v.).

### 2.4. In vivo binding

The mice received 4 $\mu$Ci of radioligand injected into the tail vein ([$^3$H]raclopride/[$^3$H]SCH 23390). The animals were decapitated 5/15 min later except when otherwise indicated, and the whole brain (240-260 mg) minus the cerebellum was homogenized in 14 ml (56 volumes, tissue wet weight) 50 mM Tris-citrate (pH 7.4 at 30 °C) containing 120 mM NaCl and 4 mM $MgCl_2$ ([$^3$H]raclopride binding) or 25 mM potassium phosphate buffer (pH 7.1) ([$^3$H]SCH 23390 binding). One ml of this homogenate was filtered through Whatman GF/C filters which were then rinsed with $2 \times 10$ ml of the same buffer. The entire procedure was completed within 30-50 s. Non-specific binding was defined as the radioactivity present after pretreatment of the mice with 20 mg/kg of cis-flupentixol i.p. 2 h before decapitation. The filters were counted in 4 ml of scintillation cocktail in a $\beta$ counter with an efficiency of 45%. Specifically bound radioligand was always corrected for the total amount of brain radioactivity relative to the controls i.e. mice not receiving any drug treatment.

### 2.5. In vitro binding

Binding in vitro of [$^3$H]SCH 23390 (0.2 nM) to dopamine D1 and [$^3$H]spiroperidol (0.05 nM) to dopamine D2 receptors was determined as described previously in detail (Andersen et al., 1985).

### 2.6. Localization of [$^3$H]raclopride binding

The mice were decapitated 5 min after the injection of 4 $\mu$Ci of [$^3$H]raclopride and the striatum, prefrontal cortex, residual cortex (minus the prefrontal cortex) and the residual brain tissue were homogenized in 56 volumes of 50 mM Tris-citrate (pH 7.4 at 30 °C) containing 120 mM NaCl and 4 mM $MgCl_2$. The homogenates were filtered through Whatman GF/C filters which were then rinsed with $2 \times 10$ ml 0.9% NaCl, and counted in a $\beta$ counter (efficiency 45%).

### 2.7. TLC analysis

The mice were decapitated 5 min after i.v. injection of 4 μCi of [$^3$H]raclopride and the brain was homogenized in 14 ml of 50 mM Tris-citrate (pH 7.4 at 30 °C) containing 120 mM NaCl and 4 mM $MgCl_2$. This homogenate was spun at 4 °C for 10 min at 25 000 × *g*. The following steps were then performed: (1) The pellet was homogenized with an ultra-turrax homogenizer in 2 ml ice-cold methanol and was centrifuged at 25 000 × *g* at 4 °C for 10 min. The supernatant from this centrifugation was analysed by TLC (below). (2) The supernatant (from the initial homogenization) was freeze-dried. The remnant was resuspended in 2 ml ice-cold methanol and centrifuged at 25 000 × *g* for 10 min at 4 °C. The resulting supernatant was analyzed by TLC (below).

Methanolic extracts (above), 5 μl, were applied on TLC plates (HPTLC-aluminium sheets, silica gel 60 F254, Merck). As reference, 5 μl methanol brain extract from mice not given [$^3$H]raclopride i.v., but with 0.01 μCi [$^3$H]raclopride added, was spotted separately. The TLC plates were run in ethanol : acetic acid : water (6 : 3 : 1). After drying, the plates were scraped in 0.5 $cm^2$ squares. Each square was counted in 4 ml scintillation liquid in a β counter.

## 3. Results

### 3.1. Characterization of [$^3$H]raclopride binding

High levels of radioactivity was found in the brain 5 min after i.v. injection of 4 μCi [$^3$H] raclopride (fig. 1). About 80% of this radioactivity was 'free', i.e. not bound to particles remaining on GF/C filters. (Compare fig. 1A and B). The half-life of bound [$^3$H]raclopride and of total [$^3$H]raclopride present in the brain was very short, being 15 and 8 min, respectively.

The level of non-specific binding i.e. radioactivity bound to particles remaining on the glass-fibre filters after pretreatment of the animals with 20 mg/kg (i.p. 2 h) of cis-flupentixol was low (5-15% of total binding) and did not change during the time interval examined. The amount of radioactivity hereafter termed 'specific binding' is the difference between total binding and non-specific binding and accounted for about 800 d.p.m./ml homogenate (see fig. 1A). The level of both bound and total radioactivity was considerably lower in the cerebellum than in the forebrain and no specific binding was detected in this brain area (fig. 1A).

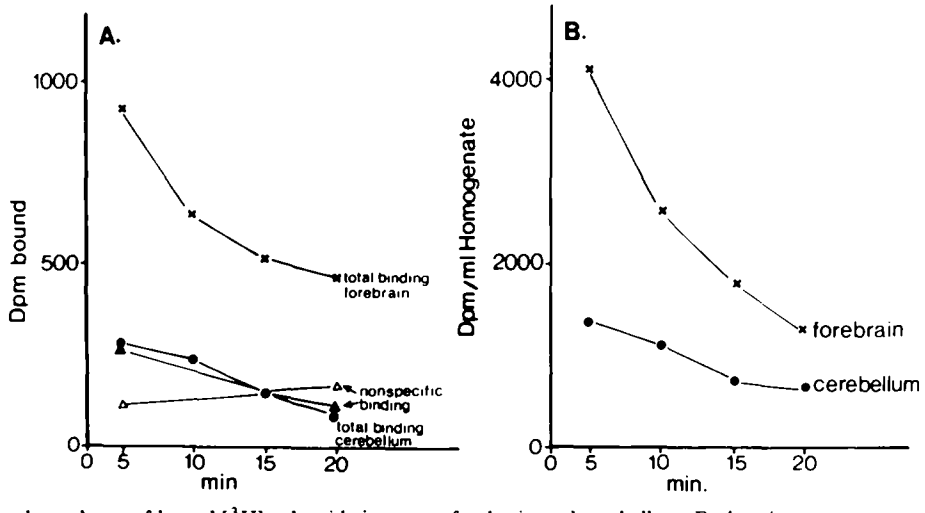


Fig. 1. (A) Time-dependence of bound [$^3$H]raclopride in mouse forebrain and cerebellum. Each point represents average data from 6 mice. △ and ▲ represent non-specific binding in the forebrain and cerebellum, respectively. (B) Time-dependence of total [$^3$H]raclopride in 1 ml mouse forebrain/cerebellum homogenates. See Materials and methods for experimental details.

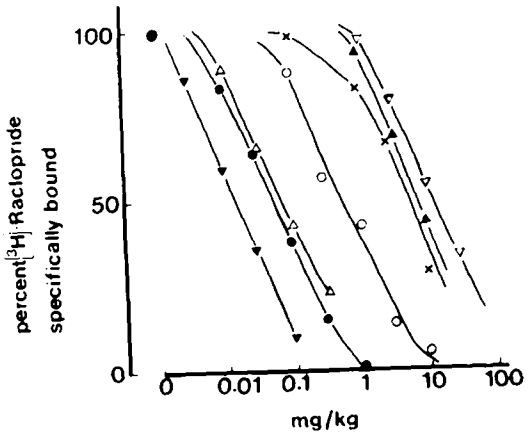


Fig. 2. Inhibition of specific binding of [$^3$H]raclopride by various compounds. Drugs were administered i.p. 2 h before killing except for (−)-NPA (s.c. 0.5 h). Each point represents average data from 2-4 separate experiments with at least 3 doses in each experiment, 2 mice on each dose. ▼ Spiperone; ● (−)-NPA; △ loxapine; ○ raclopride; × molindone; ▲ clozapine; ▽ fluperlapine.

TABLE 1

Localization of [$^3$H]raclopride binding in mouse brain. Data shown as means ± S.D. (N = 5-12) from mice injected i.v. with 5 μCi of [$^3$H]raclopride 5 min before killing.

| Brain area | Weight (mg) | Specific [$^3$H]raclopride binding (dpm/mg tissue) |
|---|---|---|
| Striatum | 30 ± 4 | 54 ± 11 |
| Prefrontal cortex | 45 ± 5 | ≈ 0 |
| Residual cortex | 52 ± 2 | ≈ 0 |
| Cerebellum | 84 ± 10 | ≈ 0 |
| Residual brain | 184 ± 18 | 4 ± 1 |

Specific [$^3$H]raclopride binding was localized mainly in the striatum, with only low levels in other brain areas (table 1). Binding of [$^3$H]raclopride was saturable since the binding was dose dependently inhibited by non-labelled raclopride to the level of the non-specific binding (see fig. 2 and table 2).

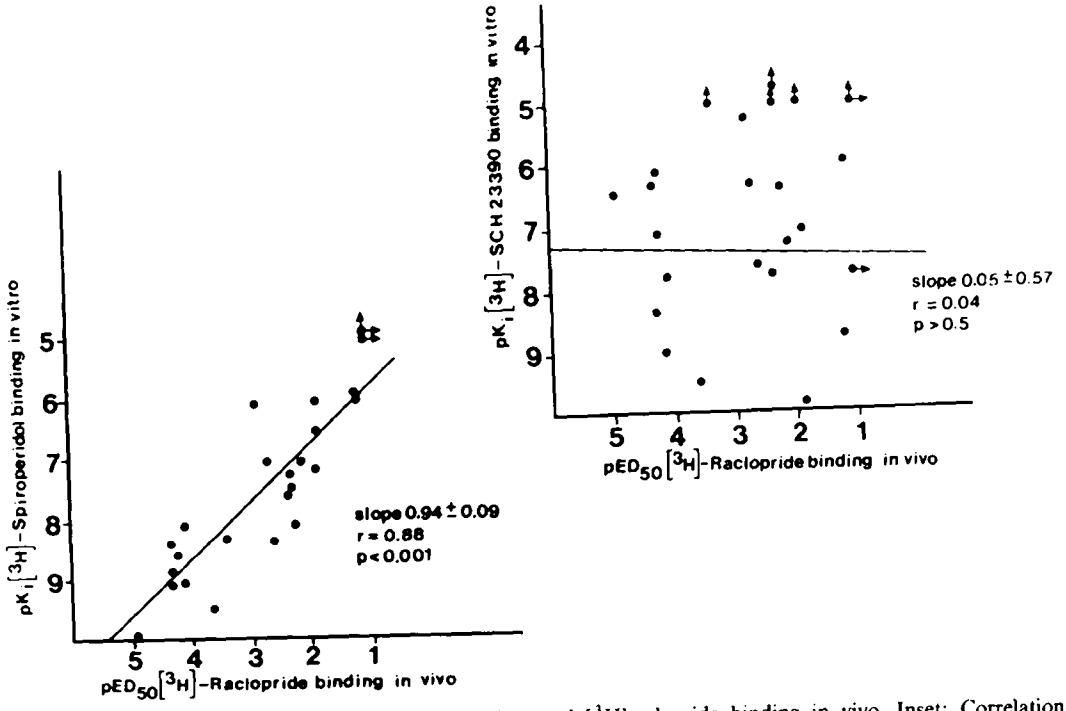


Fig. 3. Correlation between [$^3$H]spiroperidol binding in vitro and [$^3$H]raclopride binding in vivo. Inset: Correlation between [$^3$H]SCH 23390 binding in vitro and [$^3$H]raclopride binding in vivo. After points marked with an arrow were excluded, the data were correlated and the results were analyzed statistically using Spearman's $\rho$-test.

TABLE 2

Phamacological characteristics of dopamine D1 and D2 receptor binding in vivo and in vitro. Drugs were administered i.p. 2 h before killing, except when otherwise indicated. [$^3$H]SCH 23390 was used as radioligand for labelling of the dopamine D1 receptor both in vivo and in vitro, [$^3$H]raclopride for labelling the dopamine D2 receptor in vivo and [$^3$H]spiroperidol for labelling the dopamine D2 receptor in vitro.

| Compound | In vivo binding $ED_{50}$ (mg/kg) | | In vitro binding $K_i$ (nM) | | Selectivity (D1 : D2) [f] | |
|---|---|---|---|---|---|---|
| | D1 [b] | D2 [a] | D1 [c] | D2 [c] | In vivo | In vitro |
| *Benzazepines* | | | | | | |
| SCH 23390 s.c. 0.5 h | 0.017 | 13.5 | 0.14 | 895 | 1 : 1260 | 1 : 6400 |
| SKF 75670 s.c. 1 h | 0.4 | 66 | 1.9 | 840 | 1 : 165 | 1 : 443 |
| SKF 38393 s.c. 1 h | 52.4 | > 100 | 18 | 9300 | 1 : > 2 | 1 : 517 |
| *Thioxanthenes* | | | | | | |
| cis-Flupentixol (1) [e] | 0.4 | 0.24 | 0.32 | 0.34 | 1.7 : 1 | 1 : 1 |
| trans-Flupentixol | 192 | 2.0 | 474 | 100 | 96 : 1 | 4.7 : 1 |
| *Phenothiazines* | | | | | | |
| Chlorpromazine (2) | 7.3 | 2.5 | 25 | 4.6 | 2.9 : 1 | 5.4 : 1 |
| Fluphenazine (3) | 2.8 | 0.052 | 4.5 | 0.84 | 53.8 : 1 | 5.4 : 1 |
| *Butyrophenones* | | | | | | |
| Haloperidol (4) | 72.5 | 0.06 | 76 | 2.6 | 1200 : 1 | 29 : 1 |
| Spiroperidol (5) | 168 | 0.013 | 360 | 0.11 | 1300 : 1 | 3300 : 1 |
| *Benzamides* | | | | | | |
| Sulpiride (6) | 91 | 12.9 | > 10000 | 70 | 7.1 : 1 | 143 > : 1 |
| l-Sulpiride (7) | > 50 | 5.3 | > 15000 | 34 | 10 > : 1 | 441 > : 1 |
| d-Sulpiride | 61 | 73 | 10900 | 1300 | 1 : 1.2 | 8.4 : 1 |
| Raclopride (8) | > 100 | 0.44 (0.06) [d] | > 10000 | 4.8 | 23 > : 1 | 2083 > : 1 |
| Clebopride (9) | 126 | 0.06 | 820 | 1.3 | 4500 : 1 | 2631 : 1 |
| *Dibenzepines* | | | | | | |
| Clozapine (10) | 10 | 7.5 | 55 | 90 | 1.3 : 1 | 1 : 1.6 |
| Fluperlapine (11) | 1 | 14.7 | 85 | 245 | 1 : 14.7 | 1 : 2.9 |
| Tilozepine (12) | 9.3 | 4.4 | 17 | 27 | 2.8 : 1 | 1 : 1.6 |
| Loxapine (13) | 0.3 | 0.08 | 16 | 7.6 | 3.8 : 1 | 2.1 : 1 |
| *Miscellaneous* | | | | | | |
| (+)-Butaclamol (14) | 0.4 | 0.07 | 0.95 | 0.9 | 5.7 : 1 | 1 : 1 |
| (−)-Butaclamol | 194 | > 100 | > 10000 | > 10000 | – | – |
| Molindone (15) | 51 | 4.9 | > 10000 | 56 | 10.4 : 1 | 200 > : 1 |
| Pergolide | 11.5 | 6 | 400 | 7.8 | 1.9 : 1 | 51 : 1 |
| (−)-NPA s.c. 0.5 h | 35 | 0.05 | 480 | 4.1 | 700 : 1 | 117 : 1 |
| LY 171555 s.c. 1 h | 250 | 1.5 | > 5000 | 720 | 165 : 1 | 7 > : 1 |

[a] $ED_{50}$ values are means from 2-5 experiments; 2-6 doses in each experiment with two mice on each dose in each experiment. [b] Some of the data have previously been published in Andersen et al. (1986), Andersen and Grønvald (1986) and Andersen and Nielsen (1986a,b). [c] Parts of these data have previously been published in Andersen et al. (1985) and Andersen and Nielsen (1986a,b). [d] Raclopride administered s.c. 0.5 h. [e] Numbers in parentheses refers to fig. 5A and B. [f] D1 receptor-selective compounds are defined as having $ED_{50}$ (D2) $\geq 10 \times ED_{50}$ (D1) and D2 receptor-selective compounds are defined as having $ED_{50}$ (D1) $\geq 10 \times ED_{50}$ (D2). Compounds termed non-selective are those not fulfilling these criteria.

TLC of the brain extracts showed the presence of only a single peak of radioactivity. This peak of radioactivity was identified as [$^3$H]raclopride (not shown). It was estimated that this peak represented more than 90% of the radioactivity present in the brain.

Pharmacological characterization of [$^3$H]raclopride binding indicated that compounds with high

in vitro D2 receptor affinity were also potent inhibitors of in vivo binding (see fig. 2 and table 2), e.g. spiroperidol, haloperidol, cis-flupentixol, (+)-butaclamol and l-sulpiride. On the other hand, dopamine D1 receptor-selective compounds were inactive or were weak inhibitors of specific [$^3$H]raclopride binding (i.e. SKF 38393, SKF 75670 and SCH 23390). The close correlation between the affinities for [$^3$H]raclopride binding in vivo and [$^3$H]spiroperidol binding in vitro suggested D2 receptor labelling (fig. 3). This D2 receptor specificity was further supported by the complete lack of correlation between the affinities for [$^3$H]raclopride binding in vivo and [$^3$H[SCH 23390 in vitro (inset fig. 3).

[$^3$H]Raclopride binding appeared to be stereospecific since the pharmacologically inactive trans-, d- and (−)-isomers of flupentixol, sulpiride and butaclamol, respectively, were considerably less potent inhibitors of [$^3$H]raclopride binding than the respective cis-, l- and (+)-isomers.

### *3.2. Dopamine receptor profile*

To evaluate the receptor profile/selectivity of the reference compounds, the pharmacological characteristics of the in vivo binding of [$^3$H]raclopride were compared to those of in vivo [$^3$H]SCH 23390 binding. [$^3$H]SCH 23390 is a radioligand which selectively labels the dopamine D1 receptor both in vivo (Andersen et al., 1986; Andersen and Nielsen, 1986a,b; Andersen and Grønvald, 1986) and in vitro (Billard et al., 1984; Andersen et al., 1985). This comparison (see table 2) indicated that compounds with in vitro selectivity retained with only few exceptions this selectivity in vivo also i.e. haloperidol, spiroperidol, l-sulpiride, raclopride, clebopride, (−)-NPA ((−)-N-propyl-norapomorphine) and LY 171555 were D2-selective while SCH 23390, SKF 75670 and SKF 38393 were D1-selective. Further, most compounds exhibiting a dual D1-D2 receptor profile in vitro also exhibited this dual profile in vivo i.e. cis-flupentixol, chlorpromazine, d-sulpiride, clozapine, tilozepine, loxapine and (+)-butaclamol.

A few compounds did not follow the above general rules. Thus, molindone and pergolide, being D2 selective in vitro, appeared to be non-selective in vivo; fluphenazine and fluperlapine which exhibited a dual D1-D2 receptor profile in vitro appeared to be D2 and D1 receptor selective in vivo, respectively.

## 4. Discussion

The present results indicate that [$^3$H]raclopride binding to mouse brain tissue in vivo is stereospecific and saturable and further that [$^3$H]raclopride binds specifically to dopamine D2 receptors under the assay conditions used. Thus, [$^3$H]raclopride was accumulated in brain areas that receive a dopaminergic input i.e. striatum and residual brain (containing the substantia nigra). Binding of [$^3$H]raclopride was dose dependently inhibited by non-labelled raclopride to the level of non-specific binding (see fig. 2 and table 2) indicating a limited number of binding sites. Pharmacological characterization revealed that compounds with high affinity for the D2 receptor in vitro were potent inhibitors of [$^3$H]raclopride binding in vivo, whereas compounds with low or no D2 receptor affinity in vitro were weak or inactive inhibitors of in vivo binding. Further, the binding of [$^3$H]raclopride in vivo was only marginally affected by the pharmacologically inactive isomers, trans-flupentixol, d-sulpiride and (−)-butaclamol as compared to their corresponding cis-, l- and (+)-isomers, respectively. Finally, a close correlation was found between [$^3$H]raclopride binding in vivo and [$^3$H]spiroperidol binding in vitro. On the other hand, no correlation was observed between in vivo [$^3$H]raclopride binding and in vitro [$^3$H]SCH 23390 binding.

Thus, [$^3$H]raclopride labels in vivo a site in mouse brain which is closely related to the site labelled by [$^3$H]spiroperidol in rat brain tissue in vitro i.e. the dopamine D2 receptor. This specific and selective labelling of the dopamine D2 receptor by [$^3$H]raclopride is in agreement with previous reports (Köhler et al., 1985).

[$^3$H]Raclopride exhibits higher specificity and selectivity than [$^3$H]spiroperidol. Further, the very fast penetration of [$^3$H]raclopride into the brain, the reversibility and short half-life of the binding makes [$^3$H]raclopride the ligand of choice for in vivo D2 receptor labelling.

To examine the dopamine receptor selectivity of the reference compounds tested, the pharmacological characteristics of [$^3$H]raclopride binding in vivo were compared to those of [$^3$H]SCH 23390 binding in vivo. [$^3$H]SCH 23390 has previously been shown to label selectively the dopamine D1 receptor both in vitro (Billard et al., 1984; Andersen et al., 1985) and in vivo (Andersen et al., 1986; Andersen and Grønvald, 1986; Andersen and Nielsen, 1996a,b). It appeared from the comparison that most compounds being either D1/D2-selective or non-selective in vitro, retained this receptor profile in vivo also. A few compounds changed profile in vivo as compared to in vitro i.e. fluphenazine and fluperlapine, with a dual D1-D2 receptor profile in vitro were D2 and D1-selective in vivo, respectively. Further, molindone and pergolide that were D2-selective in vitro had a dual D1-D2 receptor profile in vivo. These pharmacological characteristics of dopamine D1 and D2 receptor binding in vivo support the view that the D1 receptor is an important target for a variety of neuroleptics e.g. of the clozapine-type, cis-flupentixol, chlorpromazine and (+)-butaclamol (Andersen et al., 1986).

It has been shown previously that a close correlation exists between the $IC_{50}$ values for D2 receptor binding in vitro and the 'average clinical daily doses' of a variety of neuroleptics (Seeman et al., 1976; Creese et al., 1976). A close and significant correlation was observed (fig. 4A) between the $pED_{50}$ values for [$^3$H]raclopride binding in vivo and the 'average clinical daily dose'. On the other hand, no correlation was observed between [$^3$H]SCH 23390 binding in vivo and the 'average clinical daily dose' except when D2-selective compounds were excluded from the correlation (fig. 4B and legend for details concerning the correlation). Thus, this could indicate blockade of either the D1 or the D2 receptor can mediate the antipsychotic efficacy of neuroleptics.

The involvement of the D1 receptor in the mediation of the antipsychotic action has been suggested lately, on the basis of both biochemical and behavioral data (Andersen and Braestrup, 1986; Barnett 1986).

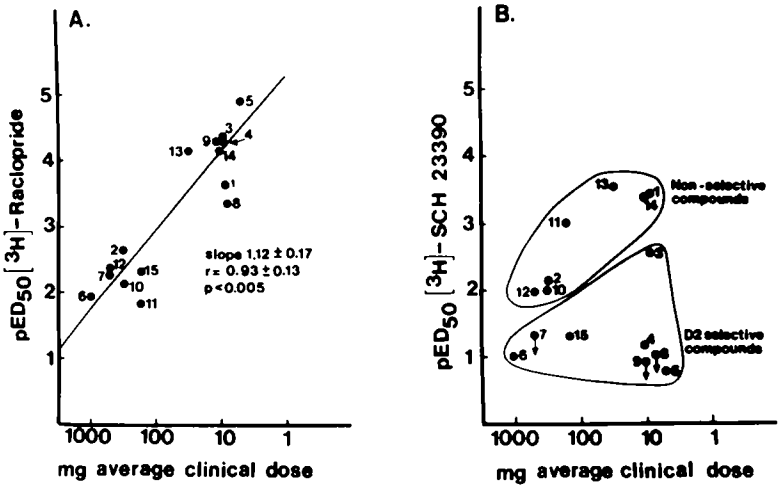


Fig. 4. (A) Correlation between 'average clinical daily doses' and $pED_{50}$ from [$^3$H]raclopride binding in vivo. (B) Correlation between 'average clinical daily dose' and $pED_{50}$ from [$^3$H]SCH 23390 binding in vivo. After exclusion of the points marked with an arrow, the data were correlated and the results were analyzed statistically with Spearman's $\rho$-test. No correlation was observed when all points were included (slope = $0.11 \pm 0.58$, $r = 0.09 \pm 0.43$, $P > 0.1$). A significant correlation was observed when D2-selective compounds (no 3, 4, 5, 6 and 15) were excluded (slope = $0.97 \pm 0.11$, $r = 0.98 \pm 0.09$, $P < 0.01$). Data concerning the clinical daily doses were obtained from the Danish Drug Compendium 1987 or Psychotropics '84 (Lundbeck, the Netherlands) or from Farde et al. (1986) (raclopride) and Dr. H. Remmer, Sandoz (tilozepine). In the case of cis-flupentixol and (+)-butaclamol the doses of the marketed racemic mixtures were halfed.

This postulate is supported by the present results. It is of special interest that fluperlapine, that has well documented antipsychotic efficacy with no extrapyramidal side-effects (Angst and Hippius, 1984), apparently has the D1 receptor as its primary target. However, the final conclusion as to whether D1 receptor blockade conveys antipsychotic efficacy must await clinical trials with a dopamine D1 receptor-selective compound.

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