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SPECIFIC *IN VITRO* AND *IN VIVO* BINDING OF ^{3}H-RACLOPRIDE

A POTENT SUBSTITUTED BENZAMIDE DRUG WITH HIGH AFFINITY FOR DOPAMINE D-2 RECEPTORS IN THE RAT BRAIN

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Abstract—The substituted benzamide drug raclopride, [((-)-(S)-3,5-dichloro-*N*-((1-ethyl-2-pyrrolidinyl) methyl)-6-methoxy-salicylamide tartrate; FLA 870(-); A40664] was shown to be a potent and selective antagonist of dopamine D-2 receptors by its high affinity for striatal ^{3}H-spiperone binding sites and low potency to block dopamine stimulated adenylate cyclase *in vitro*. *In vitro* studies showed that ^{3}H-raclopride binds with a high affinity (K_D = 1.2 nM) and a low proportion of non-specific binding to rat striatal homogenates. The binding of ^{3}H-raclopride is saturable (B_{max} = 23.5 pmoles/g wet wt) and reversible (dissociation half-time = 30 min) with a regional distribution of the specifically bound drug showing the following rank-order: striatum > nucleus accumbens > olfactory tubercle > septum > hypothalamus > hippocampus > frontal cortex.

After *in vivo* administration, ^{3}H-raclopride accumulates preferentially in dopamine rich brain areas with approximately 10 times higher levels in the striatum than in the cerebellum, when examined 30 min after injection. The *in vivo* binding of ^{3}H-raclopride was saturable, reversible and showed a low component of non-specific binding. More than 90% of the drug reached the brain in a non-metabolized form as judged by thin-layer chromatography. Pharmacological analysis of ^{3}H-raclopride binding showed that it could be displaced by dopamine agonists and antagonists but not by serotoninergic or noradrenergic drugs. Taken together, the results suggest that ^{3}H-raclopride labels dopamine D-2 receptors with high specificity in the rat brain both *in vitro* and *in vivo*, and thus, that it should be a useful tool in studies of central dopamine D-2 receptors.

Substituted benzamide drugs have become important tools to study dopamine receptors not linked to adenylate cyclase (the dopamine D-2 receptors, according to the nomenclature of Kebabian and co-workers [1, 2], and recently the interest has been focused on the benzamides as potential anti-psychotic agents [3–6]. Many pharmacological and biochemical studies of brain dopamine D-2 receptors have been performed using the benzamide drugs sulpiride and sultopride [7–10] as well as the butyrophenone domperidone [11, 12]. Although sulpiride and domperidone label the dopamine D-2 receptor relatively selectively *in vitro*, their poor penetration into the brain has partly limited their usefulness as pharmacological probes for studies of central dopamine D-2 receptors *in vivo*. In the search for novel substituted benzamide drugs, new classes of potent and selective D-2 receptor antagonists have been synthesized and tested [13–16] and some of these drugs have been radio-labelled to high specific activity [17]. In the present study we have analyzed the specific *in vitro* and *in vivo* binding of one of these substituted benzamide drugs, ^{3}H-raclopride [16], in the rat brain.

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Abbreviations used: ADTN, 2-amino-6,7-dihydroxy-tetrahydronaphthalene; ATP, adenosine 5′-triphosphate; BSA, bovine serum albumin; cAMP, adenosine 3′,5′-cyclic-monophosphate; DHA, dihydroalprenolol; EGTA, Ethylene glycos-*bis*-(β-aminoethyl ether)-*N,N,N′,N′*-tetra-acetic acid; IBMX, 3-isobutyl-l-methylxanthine; NPA, norpropylapomorphine; PAC, p-aminoclonidine; QNB, 3-quinuclidinyl benzilate; Tris, tris (hydroxymethyl)-aminomethane; WB4101, 2-(2′,6′-dimethoxy phenoxy-ethyl)amino methyl benzodioxan.

MATERIALS AND METHODS

Subjects. Male Sprague–Dawley rats (Anticimex, Sollentuna, Sweden) weighing 130–150 g at death were used in the *in vivo* binding experiments. The *in vitro* binding assays were conducted on brains from rats weighting 150–250 g.

Drugs. The compound, [(-)-(S)-3,5-dichloro-*N*-(1-ethyl-2-pyrrolidinyl)methyl)-6-methoxy-salicylamide tartrate, FLA 870(-),A40664], raclopride was synthesized at Astra Läkemedel AB (Södertälje, Sweden), and radio-labelled to high specific radioactivity by catalytic hydrogenation. The structure of raclopride and the position of the tritium atoms in the molecule is shown in Fig. 1. The specific activities of the two batches used in the present study were 40 and 21.4 Ci/mmole, respectively, ^{3}H-Spiperone was obtained from New England Nuclear (Boston, MA) (specific activity: 21 Ci/mmole). The non-radioactive drugs used included: raclopride (Astra Läkemedel AB, Sweden), eticlopride (Astra Läkemedel AB), remoxipride (Astra Läkemedel AB), (+)butaclamol (Research Biochemicals Inc., U.S.A.), spiperone

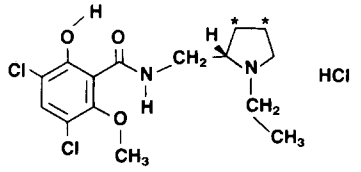

Fig. 1. Structural formula of ^{3}H-raclopride. The position of tritium atoms is indicated by asterisks.

(Janssen Pharmaceuticals, Belgium), D,L-sulpiride (DelaGrange, France), haloperidol (Janssen Pharmaceuticals), apomorphine(Ferak, FRG), metoclopramide (DelaGrange, France), *N-n*-propylnorapomorphine (Research Biochemicals Inc., U.S.A.), mianserin (Organon, Holland), phenoxybenzamine (Smith, Klein & French Laboratories, U.S.A.), propranolol (ICI, United Kingdom), morphine (May and Baker, U.K.), naloxone (Endo Laboratories, U.S.A.), domperidone (Janssen Pharmaceuticals), bromocriptine (Sandoz, Switzerland).

In vitro *receptor binding*. The binding assay for ^{3}H-raclopride was carried out using fresh rat striatal homogenates. Briefly, the rats were decapitated and their brains quickly removed and put on ice. The striatum was homogenized using a Branson B-30 sonifier and the homogenate was centrifuged at 48,000 *g* for 10 min. The pellet was homogenized and centrifuged a second time. The final pellet was resuspended in Tris–HCl buffer (0.05 M; pH 7.4) containing 0.01% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. Pargyline was included to inhibit breakdown of the monoamines used in the displacement studies. Non-specific binding was defined by adding 1 μM (+)butaclamol to the incubation medium. The incubation was carried out in microtitration plates for 60 min at room temperature and terminated by rapid filtration and washing on Whatman GF/B filter using a cell harvester. The radioactivity of the filters was determined in a liquid scintillation counter (Mark III, Nuclear Chicago Inc.). For details about the other binding assays used, see Table 1.

Adenylate cyclase assay. The determination of adenylate cyclase activity was made using a method modified from Kebabian *et al.* [2]. The striatum was put into ice-cold Tris–EGTA buffer (2 mM, pH 7.4). Subsequently, the tissue was blotted on filter paper, weighed and homogenized in a glass homogeniser. The homogenate was then filtered through a nylon net to get rid of cell debris. Aliquots of 1 ml homogenate were quickly frozen in plastic tubes in dry ice, and could then be stored at −70°. The homogenates was diluted 1:2 prior to use.

Tris–EGTA buffer (100 mM final concentration, pH 7.4, containing 1.25 mM IBMX, 5 mM $MgCl_2$, 0.5 mM EGTA and 0.3% BSA) was preincubated with 100 μl of the striatal homogenate (dilution 1:50), 50 μl dopamine (0.01 mM in 0.2% ascorbic acid) and 50 μl antagonist for 18.5 min at +4° and 1.5 min at +30°. ATP (2.5 mM) was then added and the incubation was continued for 5 min. The incubation was stopped by immersion of the tubes

Table 1. The inhibitory potencies (IC_{50}, μM) of raclopride, sulpiride, haloperidol and chlorpromazine on rat brain receptors using *in vitro* receptor binding assay. The binding experiments were carried out as is described in the text and in Hall and Ögren [35] and Leysen *et al.* [36]. The homogenates were prepared from rat striatum for the study of dopamine receptors (final concentration 5 mg/ml for D-2 and 10 mg for D-3 sites) and from rat cortex for the study of the other receptors (final concentration 20 mg/ml) for α_1, α_2 and histamine-H_1 receptors and 10 mg/ml for the other receptors). IC_{50} values were calculated using log-logit regression analysis of values falling in the range $5\% > X > 95\%$. Two or more experiments were used for each radioligand using five concentrations of the compound in each experiment. The data on inhibitory properties on dopamine stimulated adenylate sensitive cyclase are from Jerning and Hall (manuscript in preparation)

Receptor (radioligand)	Radioligand concentration (nM)	Raclopride	Sulpiride	Haloperidol	Chlorpromazine
Dopamine-D-1 (Adenylate cyclase)	–	>100	>100	0.34	0.39
Dopamine-D-2 (^{3}H-spiperone)	0.4	0.026	0.23	0.012	0.017
Dopamine-D-3 (^{3}H-ADTN)	8	0.90	45	0.009	0.035
α_1-adrenergic (^{3}H-WB4101)	0.2	32	54	0.035	0.014
α_2-adrenergic (^{3}H-PAC)	1	38	7.9	7.1	3.1
β-adrenergic (^{3}H-DHA)	1	>100	>100	>30	>30
5-HT_1 (^{3}H-5-HT)	7	49	>100	9.1	2.6
5-HT_2 (^{3}H-spiperone)	0.4	13	>100	0.036	0.031
Histamine-H_1 (^{3}H-mepyramine)	2	8.4	>100	5.0	0.026
Muscarinic (^{3}H-QNB)	0.5	>100	>100	15	0.38

Abbreviations: DHA, dihydroalprenolol; PAC, paraaminoclonidine; QNB, 3-quinoclidinylbenzilate; WB4101, (2-(2′,6′-dimethoxyphenoxyethylaminomethyl) benzodioxan.

into hot water (95°) for 4 min. The tubes were then stored in ice until determination of cAMP content. The tubes were centrifuged for 10 min at 10,000 rpm (5000 *g*) at +4°. The content of cAMP in the supernatant was then determined in duplicates (2 × 50 μl) using the cAMP-kit (Radiochemical Center, Amersham, U.K.). Triplicates were performed of each dilution of antagonist.

In vivo *receptor binding*. The procedure used for the *in vivo* binding was essentially the same as that previously reported for ^{3}H-spiperone [18, 19] and ^{3}H-*N*-*n*-propylnorapomorphine [20]. Non-anaesthetized, restrained rats were injected with different concentrations of ^{3}H-raclopride in a tail-vein and sacrificed at various times after the injections. The brain was removed and rapidly dissected on ice. The individual brain regions were weighed, dissolved in Soluene (Packard) and the radioactivity was counted in a Packard Scintillation counter (Packard Tricarb., eff. approximately 40%). The amount of binding was expressed in dpm/mg tissue. The total amount of radioactivity in a specific brain region minus the amount present in the cerebellum was used as a measure of specific binding. This way of estimating specific binding has been used in several previous studies of *in vivo* binding of ^{3}H-neuroleptic drugs [18, 19]. The amount of non-specific binding was found to correspond relatively well to that found with another measure of non-specific binding used: the blockade of ^{3}H-raclopride binding by the neuroleptic drug (+)butaclamol (5 mg/kg, i.p.) injected either before or directly after ^{3}H-raclopride. Thus, the amount of ^{3}H-raclopride that remained in a brain region after (+)butaclamol administration was approximately equal to or slightly lower than that present in the cerebellum at the same time.

In a separate series of experiments thin-layer chromatography (TLC) was performed on extracts from the striatum of rats injected with 15 μCi of ^{3}H-raclopride. The rats were sacrificed 45 min after the injection of ^{3}H-raclopride and the brains were then dissected and processed as described above. The radioactivity was extracted from the striatum with ethanol and TLC of the extract was performed on 0.25 mm silica gel plates (Polygram R Sil G/UV 254) using ^{3}H-raclopride as a reference. The solvent systems used were ethanol:acetic acid:water (6:3:1) or methanol:benzene:acetic acid:water (15:2:5:2).

Statistics. All comparisons between groups in the *in vivo* binding experiments were made using Student's two-tailed *t*-test.

RESULTS

Effects of raclopride on different receptors and on dopamine stimulated adenylate cyclase in vitro

The potency of raclopride to displace the binding of ^{3}H-spiperone from dopamine D-2 receptors in striatal membranes in comparison to sulpiride, haloperidol and chlorpromazine is shown in Table 1. Raclopride was found to be a potent displacer of the ^{3}H-spiperone binding with an IC_{50} of 0.026 μM, which is close to the potency of both haloperidol (0.012 μM) and chlorpromazine (0.017 μM) for this binding site, but is approximately 10 times lower than that found for D,L-sulpiride (0.23 μM). Although raclopride was more potent than D,L-sulpiride to displace the striatal ^{3}H-ADTN binding (IC_{50} value: 0.90 and 45 μM, respectively) it was less potent than both haloperidol (IC_{50}: 0.009 μM) and chlorpromazine (IC_{50}: 0.035 μM) (Table 1). Furthermore, raclopride had a very low affinity for central α-adrenergic, β-adrenergic, 5-HT_1, 5-HT_2, histamine, and muscarinic cholinergic receptors (Table 1).

Similar to what has been reported for other substituted benzamide drugs [23, 24], raclopride was inactive ($IC_{50} > 100$ μM) as an antagonist of the dopamine stimulated adenylate cyclase *in vitro*, which together with the findings of the *in vitro* binding, (see below), indicates that raclopride does not interact with the dopamine D-1 receptor.

In vitro *binding of ^{3}H-raclopride*. The association rate constant was determined by interrupting the incubation at various times before steady state. The rate constant was determined according to Weiland and Molinoff [25] and was found to be 0.084/min/nM. The steady state level was reached approximately 40 min after the start of the incubation (Fig. 2).

The dissociation rate constant was determined by the addition of (+)butaclamol (1 μM) 60 min after the start of the incubation followed by filtration and

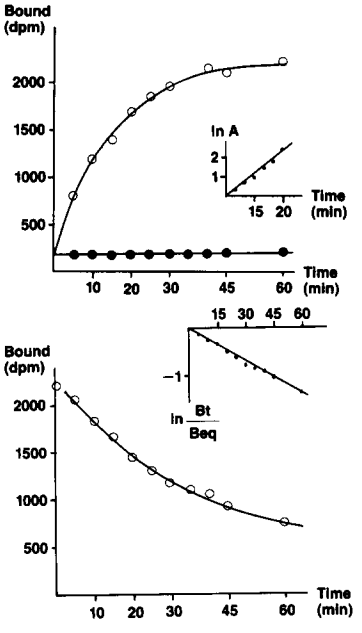

Fig. 2. Determination of rate constants. To determine the association constant ($k + 1$) the incubation (0.25 nM ^{3}H-raclopride) was stopped at various time intervals by immediate filtration and washing (upper graph). The specific binding was then determined and the constant was calculated using the integrated second order rate equation (see inset, where $\ln A = \ln \frac{B_{eq}(T\text{-}B_t \cdot B_{eq}/B_{max})}{B_{max}(B_{eq}\text{-}B_t)}$). The determination of the dissociation constant (k_{-1}) (lower graph) was performed by the addition of 1 μM (+)butaclamol at various time intervals after equilibrium was reached (60 min initial incubation) (see inset). The slope of the graph inset yielded the dissociation constant k_{-1}. The dissociation constant was then calculated using the formula $K_D = k - 1/k + 1$.

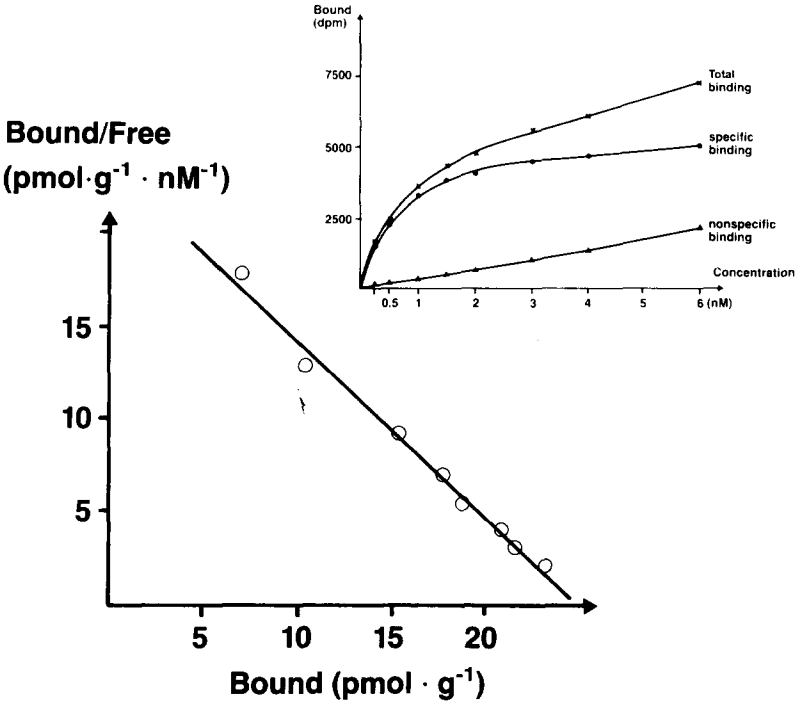

Fig. 3. Scatchard plot of the binding of ^{3}H-raclopride. Inset: Saturation curve showing total binding, specific binding and non-specific binding. The same data were used in both graphs. This Scatchard plot yielded B_{max} = 25.0 pmoles/g and K_D = 1.06 nM, which is close to the mean values of six experiments (B_{max} = 23.5 ± 2.0 pmoles/g, K_D = 1.15 ± 0.10 nM). Eight concentrations of ^{3}H-raclopride varying from 0.25 to 6 μM were used in the experiment.

washing at various time intervals thereafter. The rate of dissociation was very slow (T 1/2 = 30.5/min) and 60 min after the addition of (+)butaclamol, 25% was still bound. The dissociation rate constant was calculated to be 0.023/min/nM (Fig. 2).

Saturation studies showed that ^{3}H-raclopride binds to rat striatal membranes with a very low proportion of non-specific binding (see inset, Fig. 3). The specific binding of ^{3}H-raclopride was saturable with the B_{max} = 23.5 ± 2.2 pmoles/g (mean ± S.E.M., N = 6) as determined from the Scatchard plots. The Scatchard plots, furthermore, indicated that ^{3}H-raclopride binds to one single binding site. The K_D value for the ^{3}H-raclopride binding obtained from the Scatchard plots was 1.15 ± 0.10 nM (mean ± S.E.M.) (Fig. 3).

The binding capacity of ^{3}H-raclopride was studied in homogenates from different brain regions, which had been frozen prior to use. The regional distribution of ^{3}H-raclopride binding *in vitro* followed that of other neuroleptic drugs including other substituted benzamides, since the radioligand bound predominantly to dopamine rich areas (Fig. 4) with the following rank order: striatum > nucleus accumbens > olfactory tubercle > septum > hypothalamus > hippocampus > frontal cortex.

The specifically bound ^{3}H-raclopride was displaced at low concentrations only by dopamine D-2 antagonists (for example domperidone: IC_{50} < 0.025 μM). Drugs affecting other receptors were without activity at the ^{3}H-raclopride binding site at nanomolar concentrations. Of the monoamines tested dopamine was the most potent (IC_{50} = 0.33 nM). However, adrenaline also possessed some activity (IC_{50} = 0.69 nM) while serotonin was completely inactive.

In vivo *binding of* ^{3}H-*raclopride*. Intravenous administration of ^{3}H-raclopride resulted in a rapid accumulation of radioactivity in the various brain

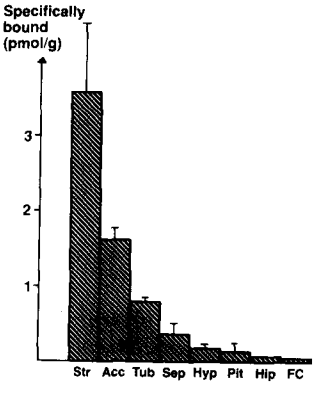

Fig. 4. Tissue distribution of the binding of ^{3}H-raclopride binding. Pieces of rat brain were rapidly dissected out and frozen on dry ice and stored at −70° until use. The columns and bars represent mean ± standard deviation of two separately run experiments except for hippocampus and frontal cortex which were run only in one single experiment. Abbreviations: Str, striatum; Acc, nucleus accumbens; Tub, olfactory tubercle; Sep, septum; Hyp, hypothalamus; Pit, pituitary; Hip, hippocampus; FC, frontal cortex.

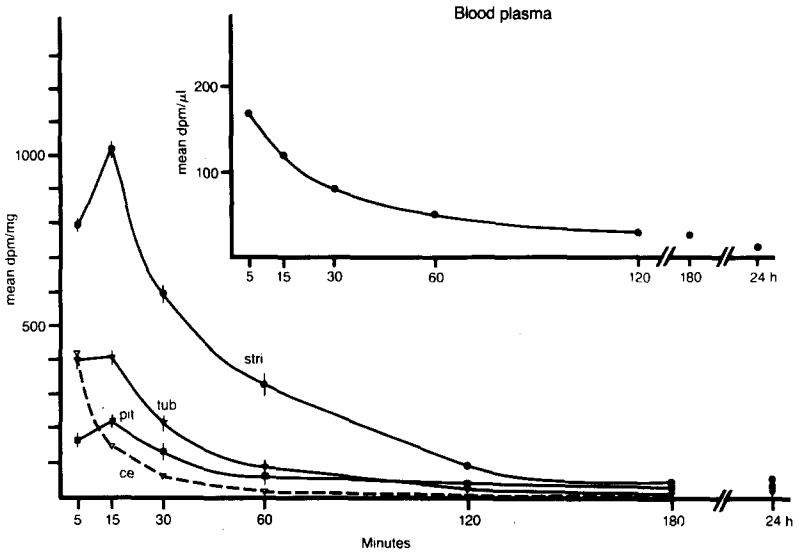

Fig. 5. Retention of radioactivity (in dpm) in different brain regions and blood plasma at various times after injection (15 μCi) of ^{3}H-raclopride. The S.E.M. values have been indicated. Abbreviations: ce, cerebellum; pit, pituitary gland; tub, olfactory tubercle; stri, striatum.

regions studied (Fig. 5). For at least 120 min after ^{3}H-raclopride injection the radioactivity showed a heterogeneous distribution in the brain: the highest levels were found in the striatum followed by the olfactory tubercle, the pituitary gland and the cerebellum. While the radioactivity in the cerebellum disappeared rapidly with only trace amounts present 1 hr after injection, relatively high levels of radioactivity were still present in all dopamine-rich areas up to 60 min after the administration of ^{3}H-raclopride (Fig. 5). Thus, between 20 and 60 min after ^{3}H-raclopride injections the ratio between the amounts of radioactivity present in the striatum and in the cerebellum was approximately 10:1. The levels of radioactivity were almost undetectable in the different brain regions after 3 hr, and at this time it did not exceed the levels found in plasma (see inset, Fig. 5). The relatively large difference between the radioactivity present in the striatum and the cerebellum after 30 min suggests that the highest degree of specific binding of the drug occurred at this time. In the subsequent pharmacological experiments, the rats were therefore sacrificed 45 min after injections of ^{3}H-raclopride.

Injections of increasing concentrations of ^{3}H-raclopride indicated that the binding in the striatum,

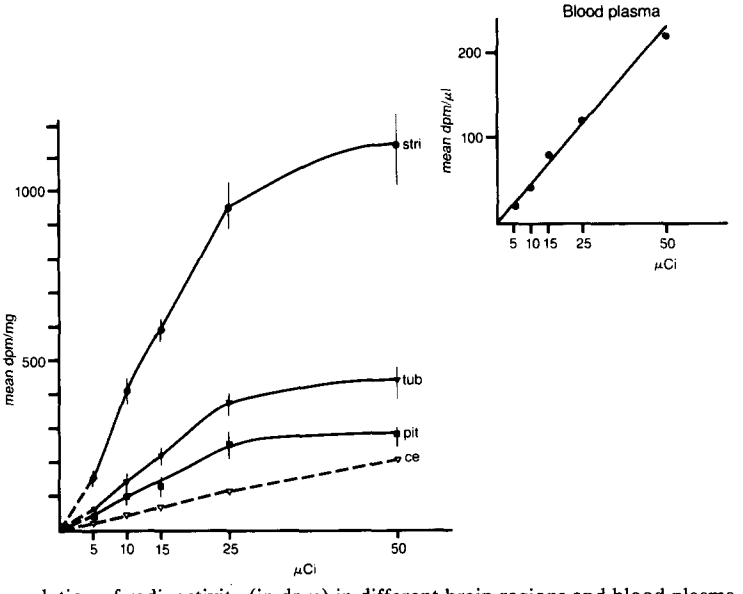

Fig. 6. Accumulation of radioactivity (in dpm) in different brain regions and blood plasma 45 min after injections of increasing doses (in μCi) of ^{3}H-raclopride. The S.E.M. values are indicated. Abbreviations as in Fig. 4.

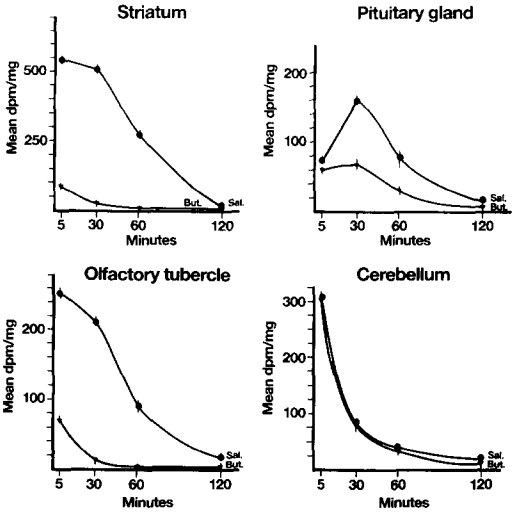

Fig. 7. Blockade by (+)-butaclamol (But) of the ^{3}H-raclopride (15 μCi/0.3 ml) binding at different times after injections. Saline treated rats (sal.) served as controls. Each point shows the mean ± S.E.M. of 5–6 rats. Specific binding is represented by the area between the two curves.

the olfactory tubercle and the pituitary gland was saturated at concentrations above 25 μCi (corresponding to 4.7 nmoles/kg of the drug). No saturation was observed for the radioactivity recovered from the cerebellum or from plasma (Fig. 6). The total amount of drug recovered from the brain at this point in the saturation-curve was approximately 12.5 pmoles/g in the striatum and approximately 6.0 pmoles/g in the olfactory tubercle as measured 45 min after injection of 4.7 nmoles/kg of ^{3}H-raclopride. The specific *in vivo* binding of increasing concentrations of ^{3}H-raclopride using (+)butaclamol (1 mg/kg, i.v.) as the specific displacer allowed the calculation of B_{max} and K_D with non-linear regression analysis. Using this technique the B_{max} value in the striatum was found to be 23.5 ± 4.0 pmoles/g (mean ± S.E.M., N = 5) with a K_D value of 1.0 ± 0.3 fmoles/kg body wt (mean ± S.E.M.). This B_{max} value is identical to that obtained with *in vitro* ^{3}H-raclopride binding (see above).

Analysis of the TLC performed on extracts of striata from rats injected with ^{3}H-raclopride showed only one major peak which run together with ^{3}H-raclopride. It could be estimated that this peak repre-

Table 2. Blockade (in% of saline treated control rats) by different drugs of the specific *in vivo* binding of ^{3}H-raclopride (15 μCi/0.3 μl) in the striatum, olfactory tubercle and pituitary gland. Number of rats in each group: 4–6, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; (Student's *t*-test). With exception of the pituitary gland the S.E.M. values did not exceed 7% in any of the experiments

Drug	Dose (μmoles kg^{-1})	Striatum	Olfactory tubercle	Pituitary gland
Dopamine-agonists				
Apomorphine[a]	0.37	3	13	11
	3.7	51 ***	58 ***	16
NPA[a]	0.5	78 ***	76 ***	41
	1.0	83 ***	83 ***	45
	10.2	96 ***	95 ***	59 *
	20.3	96 ***	92 ***	54 *
Bromocryptine[a]	0.63	0	0	41
	1.3	49 **	49 **	74 ***
Dopamine-antagonists				
Eticlopride[b]	0.003	1	4	2
	0.02	2	8	0
	0.2	73 ***	81 **	61 **
	1.3	94 ***	95 ***	70 ***
Metoclopramide[b]	2.8	37 **	29 *	60 ***
	7.9	70 ***	68 ***	66 ***
	18.4	85 ***	85 ***	71 ***
	56.6	88 ***	86 **	68
Domperidone[b]	11.7	4	7	72 **
Raclopride[b]	0.02	15	18	9
	0.12	13	9	8
	<2.4	90 ***	72 *	63
	20	95 ***	90 ***	67 **
Haloperidol[b]	0.27	52*	55*	30*
	1.3	91 ***	88 ***	64 ***
	2.7	89 ***	88 ***	56 **
	5.3	95 ***	93 ***	57 **
Miscellaneous drugs				
Mianserin[b]	18.9	0	0	0
Phenoxybenzamine[b]	32.9	0	0	5
Propranolol[b]	33.8	0	0	0
Morphine[b]	35	0	0	0
Naloxone[b]	30.5	0	0	3

[a] The drug given 5 minutes before ^{3}H-raclopride.
[b] The drug given 30 minutes before ^{3}H-raclopride.

sented 90% of the total radioactivity present in the extract.

In order to further determine the degree of specific *in vivo* binding of ^{3}H-raclopride, (+)butaclamol was given before ^{3}H-raclopride and the rats were sacrificed at various intervals after injection of the isotope. As can be seen in Fig. 7, the maximal displacement by (+)butaclamol occurred in the striatum and in the olfactory tubercle 30–60 min after the administration of ^{3}H-raclopride. From these studies as well as from the competition experiments described above, the amount of non-specific binding of ^{3}H-raclopride could be estimated to be in the range of 5–10% in all brain regions. This value corresponds well to the amount of radioactivity present in cerebellum 45 min after ^{3}H-raclopride injection. Taken together, these findings suggest that ^{3}H-raclopride penetrates readily into the brain, that its specific binding *in vivo* is saturable and that the binding in the dopamine rich brain areas is proportional to the relative density of neuroleptic or dopamine receptors in these regions as determined by the *in vitro* receptor assays (see above).

Pharmacological characterization of the in vivo ^{3}H-*raclopride binding*

The *in vivo* binding of ^{3}H-raclopride was found to be sensitive to several dopamine agonists and antagonists, but it was not blocked by the serotonin (mianserin), or the noradrenaline (propranolol; phenoxybenzamine) receptor antagonists tested. Opiate agonists and antagonists were not effective even when given in high doses (Table 2). As can be seen in Table 2, the dopamine D-2 receptor agonist *N-n*-propylnorapomorphine (NPA) potently blocked the ^{3}H-raclopride binding in a dose-dependent manner, as did the partial dopamine D-2 agonist bromocryptine, while the dopamine agonist apomorphine blocked ^{3}H-raclopride binding only when given in relatively high doses (3.7 μmoles/kg) (Table 2). Several drugs that are known [23, 24, 26–28] from *in vitro* binding and behavioural studies to be relatively selective antagonists of the dopamine D-2 receptor (e.g. spiperone, metoclopramide, sulpiride) blocked the *in vivo* ^{3}H-raclopride binding in a dose-dependent manner (Fig. 8 and Table 2). The following order of potency of the different dopamine D-2 antagonists to block the *in vivo* ^{3}H-raclopride binding in the striatum could be determined from the dose response curves and was found to be as follows: eticlopride > spiperone > remoxipride > metoclopramide > D,L-sulpiride. However, as can be seen in Fig. 8, both spiperone and D,L-sulpiride caused relatively modest reductions of the ^{3}H-raclopride binding when given at low doses. All dopamine antagonists tested prevented, in a dose-dependent fashion, the binding of ^{3}H-raclopride in the olfactory tubercle as well as in the pituitary gland (Table 2). The only exception in this regard is domperidone, which due to its poor penetration into the brain did not block the cerebral ^{3}H-raclopride binding. It did, however, produce a 72% reduction of the specific ^{3}H-raclopride binding in the pituitary gland.

The pattern of the regional blockade of the ^{3}H-raclopride binding found with D,L-sulpiride differs to some extent from that observed with the other substituted benzamides: D,L-sulpiride was a poor antagonist of ^{3}H-raclopride binding in the striatum at low dose-levels, while most of the specific binding was prevented by D,L-sulpride in the olfactory tubercle and the pituitary gland at these dose-levels.

Taken together, the present findings show that the specific *in vivo* binding of ^{3}H-raclopride is restricted to the dopamine-rich areas of the brain as well as to the pituitary gland. This binding is blocked by dopamine D-2 agonists and antagonists in a dose-dependent manner, which suggests that ^{3}H-raclopride labels those receptors under *in vivo* conditions.

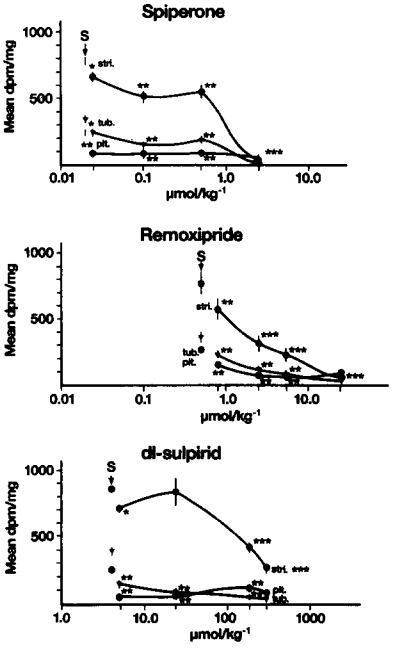

Fig. 8. Dose-response curves for the regional blockade by spiperone, remoxipride and D,L-sulpiride of specific *in vivo* ^{3}H-raclopride binding in the rat. The values for the saline treated control rats have been marked with arrows. Abbreviations as in Fig. 4. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$; Student's *t*-test.

DISCUSSION

In the present study we have shown that the substituted benzamide drug ^{3}H-raclopride binds to dopamine or neuroleptic receptors in dopamine rich areas of the rat brain under both *in vitro* and *in vivo* conditions. Similar to other substituted benzamide drugs (e.g. sulpiride) [8], raclopride appears to bind preferentially to the dopamine receptor not linked, or negatively coupled to an adenylate cyclase [1, 2, 29, 30], since it did not block dopamine stimulated adenylate cyclase and showed low affinity for the binding sites labelled by the D-1 antagonist ^{3}H-flupenthixol [24]. Raclopride did however have high affinity for the binding sites labelled by the dopamine D-2 antagonists ^{3}H-spiperone [22, 24] and ^{3}H-domperidone [11, 12, 24]. It appears that raclopride binds to these receptors with a lower component of non-specific binding than sulpiride since its affinity for these receptors is about 10 times higher. Like sulpi-

ride, raclopride showed little or no affinity for adrenergic, serotoninergic, histaminergic and muscarinic cholinergic receptors in homogenates from the rat striatum and neocortex.

Perhaps the most important finding of the present study is that ^{3}H-raclopride penetrates readily into the brain and that it binds with a high degree of specificity to dopamine receptors in the rat brain *in vivo* as well as *in vitro*. In fact, the *in vivo* binding of ^{3}H-raclopride showed several characteristics of the *in vitro* binding properties of the drug. Thus, the binding *in vivo* was of high specificity, the calculated B_{max} values for the *in vivo* and the *in vitro* experiments were similar, the regional distribution of the ^{3}H-raclopride binding sites were the same and the pharmacological properties of the ^{3}H-raclopride binding sites showed numerous similarities in the *in vivo* and the *in vitro* binding experiments.

However, it has not been possible at present to show *in vivo* binding of ^{3}H-raclopride to a D-2 receptor as conclusively as in the *in vitro* experiments, but, several observations indicate that a dopamine D-2 receptor is involved also in the *in vivo* binding of ^{3}H-raclopride. For example, the dopamine agonist NPA has been proposed as a relatively selective agonist for the D-2 receptor *in vitro* [31], and we [32] have reported previously that substituted benzamide drugs, including raclopride [16] are potent antagonists of the *in vivo* ^{3}H-NPA binding. The fact that NPA is a very potent blocker of the ^{3}H-raclopride binding *in vivo* provides further evidence that the substituted benzamide drugs interact with a dopamine D-2 receptor also under *in vivo* conditions.

Our demonstration that ^{3}H-raclopride readily penetrates into the brain and binds with high specificity to dopamine receptors *in vivo* is important in light of the very poor penetration by the selective dopamine D-2 antagonists sulpiride and domperidone. While these latter drugs have been successfully used to label dopamine D-2 receptors *in vitro*, their usefulness *in vivo* has been limited by their poor penetration into the brain.

Previous behavioural studies [16, 33] have shown that sulpiride has an atypical neuroleptic profile. Thus, unlike drugs of the butyrophenone class, e.g. haloperidol, sulpiride blocks apomorphine induced stereotypies at dose-levels far lower than that required to induce extra-pyramidal effects (e.g. catalepsy) [16, 33]. Since sulpiride penetrates poorly into the brain (Köhler, unpublished observations) it has been difficult to establish if its atypical behavioural profile is due to its interaction with a sub-population of dopamine receptors *in vivo* or if its pattern of distribution into the brain is responsible for these effects. While this question still remains unsolved, our present studies with ^{3}H-raclopride suggests that the atypical behavioural profile of the substituted benzamides cannot be explained only by a limited distribution of the drugs to the brain. The regional distribution of the ^{3}H-raclopride binding sites is similar *in vitro* and *in vivo*: the largest number of binding sites are present in dopamine rich brain areas such as the striatum and the olfactory tubercle and lowest in the cerebellum which lacks dopaminergic innervation. This distribution pattern resembles that seen for ^{3}H-spiperone [33] and ^{3}H-NPA [20] *in vivo* and for most dopamine agonists and antagonists *in vitro* (see [24]). Thus, *in vivo* ^{3}H-raclopride accumulated and bound to largest extent in the striatum in spite of the fact that this drug preferentially blocks apomorphine induced behaviours that are believed to be mediated through dopamine neuro-transmission in extra-striatal (e.g. mesolimbic or mesocortical) brain areas. Furthermore, it is of theoretical interest that ^{3}H-raclopride has a high affinity for dopamine D-2 receptors in the striatum *in vivo* since it does not cause extra-pyramidal side-effects, (e.g. catalepsy) in the rat [16], unless it is given in doses several orders of magnitude higher than those at which the striatal D-2 receptor have been saturated *in vivo*. These *in vivo* observations suggest that blockade of striatal dopamine D-2 receptors by neuroleptics is not in any simple way related to the induction of catalepsy in the rat (see [16]).

Drugs such as ^{3}H-spiperone, ^{3}H-domperidone and ^{3}H-sulpiride have been used as ligands for the dopamine D-2 receptor *in vitro* [24]. More recently ^{3}H-spiperone has also been introduced as a ligand for the dopamine receptor *in vivo* [18, 19, 33, 34]. However, ^{3}H-spiperone has been shown to bind to cortical 5-HT receptors *in vivo* [33] and to 5-HT and adrenergic receptors *in vitro* [21, 22, 37]. The selectivity of ^{3}H-raclopride for the dopamine D-2 receptor visavi other monoaminergic receptors *in vitro* and *in vivo* makes it a potentially very useful tool for studies of central D-2 receptors.

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