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In vivo labelling of pituitary dopamine D-2 receptors in the male rat using [^{3}H]-raclopride

Ch. Köhler and **G. Karlsson-Boethius**

Department of Neuropharmacology, Astra Alab AB, Södertälje, Sweden

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Summary. The substituted benzamide drug [^{3}H]-raclopride (Köhler et al., 1985) was used to label dopamine D-2 receptors within the individual lobes of the pituitary gland as well as in the brain of male rats in vivo. The in vivo [^{3}H]-raclopride binding was found to be saturable, reversible and of high specificity. Between 5–30% of the binding was non-specific at saturating concentrations dependent upon the lobe of the pituitary gland as well as of the brain region (e.g., caudate nucleus and olfactory tubercle) studied. Saturation analyses revealed B_{max}-values of 12.9 ± 1.6 and 2.2 ± 0.9 pmol $\cdot$ g^{-1} wet weight in the intermediate and anterior lobes, respectively with respective K_D values of 6.5 ± 4.6 and 7.3 ± 2.4 nmol $\cdot$ kg^{-1}. Quantitative autoradiographic studies using a single concentration of [^{3}H]-raclopride showed a similar relationship with regard to binding densities in the different lobes, and showed, in addition, that the posterior lobe contained the lowest number of specific [^{3}H]-raclopride binding sites. The binding capacities and affinities of binding were 12.9 ± 1.7 and 9.2 ± 2.8 respectively in the caudate nucleus and 6.1 ± 0.7 and 9.3 ± 2.7 respectively in the olfactory tubercle.

The pharmacological analysis revealed that (S)sulpiride, remoxipride and raclopride were 10 to 125 times more potent than their corresponding isomers [(R)sulpridie, FLA 731(—), and FLB 472, respectively] in blocking the in vivo [^{3}H]raclopride binding in the pituitary gland as well as in brain. The in vivo potency of different D-2 antagonists in preventing the [^{3}H]-raclopride binding in the anterior and intermediate lobes was: spiperone > domperidone > raclopride > (S)sulpiride > remoxipride. The D-1 selective antagonist SCH 23390 did not block the in vivo binding of [^{3}H]-raclopride neither in the pituitary lobes nor in the brain. In agreement with these findings the D-2 agonists N,N-propylnorapomorphine and quinpirole (LY 171555) but not the D-1 agonist SKF 38393-A blocked the specific in vivo [^{3}H]-raclopride binding in the pituitary gland as well as in the brain. Comparisons between the relative potencies of different drugs in blocking pituitary and brain D-2 receptors in vivo showed that some drugs, including sulpiride and domperidone, were more potent in the

pituitary gland than in the brain, while remoxipride and raclopride were equipotent in the two areas. The D-2 agonists tested appeared to be slightly more potent in the brain than in the pituitary gland.

Keywords: Dopamine, D-2 receptors, in vivo binding, ^{3}H-raclopride, rat.

Introduction

Dopamine (DA) plays an important role in the regulation of prolactin and of pro-opiomelanocortin derived peptides from the anterior and intermediate lobes, respectively, of the pituitary gland (Apud et al., 1985; Beaulieu et al., 1986; Ben-Jonathan, 1985; Borgundvaag and George, 1985; Caron et al., 1978; George and Kertez, 1985; Horowski and Gräf, 1976; Meites and Sonntag, 1981; Yeo et al., 1979). Pharmacological studies indicate that these functions of DA are mediated through a D-2 receptor (Beaulieau et al., 1986; DeLean et al., 1982; Kebabian and Calne, 1979; Munenura et al., 1980; Stoof and Kebabian, 1984). Indeed, in vitro radioligand binding studies have demonstrated the existence of DA D-2 receptors in the anterior and the intermediate lobes of the pituitary gland (Brown et al., 1979; Cole et al., 1981; Cronin et al., 1978; Cronin and Weiner, 1979; Creese et al., 1977; George et al., 1985; Munemura et al., 1980; Seeman, 1980; Stefanini et al., 1980), while the posterior lobe is relatively poor in such receptors (Pazos et al., 1985).

Although the pituitary D-2 receptor has been extensively characterized in vitro, relatively little is still known regarding its pharmacological characteristics and regulation in vivo. In recent years, in vivo receptor binding has become a tool increasingly used to study dopamine receptors in experimental animals and in man (Bischoff et al., 1980; Farde et al., 1985; Hall et al., 1983; Köhler et al., 1979, 1981, 1984a, b; Kuhar et al., 1978; Hall et al., 1983; Laduron and Leysen, 1977; van der Werf et al., 1986; Wagner et al., 1983). The usefulness of the in vivo binding method depends partly upon the availability of suitable ligands with high selectivity for the receptor under study. Most of the in vitro pharmacological characterization for D-2 receptors has been performed using ligands such as [^{3}H]-spiperone (Leysen et al., 1978), [^{3}H]-domperidone (Baudry et al., 1979), and [^{3}H]-sulpiride (Theodorou et al., 1979). While these ligands have provided important information regarding the D-2 receptor in vitro, their usefulness for in vivo binding studies has not always been optimal due to factors such as irreversible binding, non-selectivity vis-a-vis other receptors and poor penetration across the blood-brain barrier. Recently, a novel benzamide compound has been introduced (Köhler et al., 1985; Ögren et al., 1986). This compound, [^{3}H]-raclopride, shows a high selectivity for D-2 receptors both in vivo and in vitro, it penetrates readily the blood-brain barrier and binds reversibly to D-2 receptors in vivo (Köhler et al., 1985; Köhler and Karlsson-Boethius, 1988). In the present study, [^{3}H]-raclopride was used to label DA D-2 receptors in the pituitary gland and in DA rich brain regions (e.g., caudate nucleus and olfactory tubercle) of the male rat in vivo.

Materials and methods

Male Sprague-Dawley rats (Laboratorietjänst AB, Sollentuna, Sweden) weighing 150 g were used. The rats were housed four in each cage and fed food and water ad lib. The light of the housing quarters went on 7.00 a.m. and off 7.00 p.m.. All experiments were performed between 9.00 a.m. and 4.00 p.m.

The procedure for in vivo binding used here was the same as that previously reported (Köhler et al., 1979, 1986; Köhler and Fahlberg, 1985). Briefly, the rats were restrained and received intravenous injections (0.3 ml) of [^{3}H]-raclopride. (Astra Alab AB, Södertälje, Sweden: specific activity: 46 and 74 Ci · mmol^{-1}, in the two batches used). Previous studies have shown that raclopride enters the bain in an unmetabolized form (Köhler et al., 1985). The animals were killed by decapitation at different times after injection of [^{3}H]-raclopride. The brains and the pituitary glands were removed and dissected out on ice. The anterior (mean weight in mg ± SEM: 4.3 ± 0.1), intermediate (0.3 ± 0.04) and posterior (0.5 ± 0.02) lobes as well as the caudate nucleus (23.4 ± 0.7), olfactory tubercle (14.1 ± 0.6) and cerebellum (14.1 ± 0.4) were dissected out and weighed individually. The pituitary lobes and the brain regions were dissolved in 0.5 ml Soluene® (New England Nuclear, Mass., USA) without previous homogenization or filtration. After adding 5 ml of Econofluor® (NEN), the radioactivity was determined in a scintillation counter (Packard, Tri-Carb efficiency: approx. 50%). In the pharamcological analysis of the in vivo [^{3}H]-raclopride binding, each drug was injected (i.v. in 300 µl) at different times, either before or after [^{3}H]-raclopride (see Table 1; see also Köhler and Fahlberg, 1985). The times chosen depended upon the known kinetics and half-life of the particular drug under study. In previous studies of in vivo DA receptor binding (see Köhler et al., 1984; Kuhar et al., 1978; Laduron and Leysen, 1977), specific binding was determined through the subtraction of the radioactivity present in the cerebellum of each rat from that found in the particular brain region under study in the same animal. In the present study, non-specific binding of [^{3}H]-raclopride was defined by measuring residual binding after intravenous injections of (+) butaclamol (0.6 µmol · kg^{-1}, i.v.). In each rat the amount of radioactivity present in the cerebellum was subtracted from the total binding since cerebellar values are likely to represent both bound and free (e.g., blood) [^{3}H]-raclopride not associated with dopamine receptors. However, this procedure may not be equally valid in the brain and pituitary gland (see Köhler and Fahlberg, 1985).

Table 1. The capacity (B_{max}) and affinity (K_D) of [^{3}H]-raclopride binding in the pituitary gland and the brain in vivo

Region	B_{max} (pmol · g^{-1} tissue)	K_D (nmol · kg^{-1})
Pituitary gland		
Anterior lobe	2.2 ± 0.9	7.3 ± 2.4
Intermediate lobe	12.9 ± 1.6	6.5 ± 4.6
Brain		
Caudate nucleus	12.9 ± 1.7	9.2 ± 2.8
Olfactory tubercle	6.1 ± 0.7	9.3 ± 2.7

The values were calculated using non-linear regression analysis and are based upon six different concentrations of [^{3}H]-raclopride ranging from 1.0 to 17.0 nmol · kg^{-1} i.v. (n = 4–6 rats at each conc.)

Four rats injected with 70 μCi (7.8 nmol · kg^{-1}, i.v.) [^{3}H]-raclopride alone or together with (+)butaclamol (0.6 μmol · kg^{-1} i.v., 30 min before [^{3}H]-raclopride) were taken for autoradiographic analysis of the pituitary [^{3}H]-raclopride binding. The procedure was similar to that previously reported for in vivo autoradiography (Unnerstall et al., 1982; Köhler et al., 1986). Briefly, sections (30 μm thick) were collected onto gelatine coated glass-slides, dried and put in contact with [^{3}H]-sensitive film (Ultrofilm®, LKB, Sweden) and exposed for two months. The binding was quantified using an IBAS 2000 (Zeiss Kontron, Federal Republic of Germany) image analyzer equipped with a program which converted optical densites into molar quantities of bound drug using commercially available calibrated plastic standards (Amersham). Specific binding was obtained through the subtraction of residual binding in pituitary glands remaining after (+)butaclamol treatment.

The following drugs were used to characterize the in vivo [^{3}H]-raclopride binding: (—)N,N-propylnorapomorphine · HCl (NPA; dissolved in Saline with 0.1% ascorbic acid), (+)-butaclammol · HCl (Research Biochemicals Inc., Boston, USA; ethanol and distilled water); spiperone (Janssen Pharmaceutical, Beerse, Belgium); R- and S-sulpiride (Delagrange, France); domperidone · HCl (Janssen Pharmaceutical, Beerse, Belgium). All compound dissolved in hydrochloric acid and distilled water, remoxipride · HCl [FLA 731(—) · HCl], FLA 731(+) · HCl, raclopride tartrate, FLB 472-tartrate (Astra Alab, Södertälje, Sweden) all dissolved in Saline; SCH 23390; maleate (Schering Corporation, Bloomfield, USA; propylene glycol and distilled water); SKF 38393-A · HCl (Smith Kline & French Laboratories, Philadelphia, USA; distilled water); Quinpirole · HCl (LY 171555, Lilly Research Laboratories, Indianapolis, USA; distilled water).

Statistical analysis

The statistical comparisons between drug treated and control rats were made using Student's t-test.

Results

Injections of [^{3}H]-raclopride (2.6 nmol · kg^{-1}, i.v.) resulted in a rapid accumulation of radioactivity in the anterior, intermediate and posterior lobes of the pituitary gland (Fig. 1) as well as in the caudate nucleus and the olfactory tubercle. The highest levels of radioactivity was detected within 5 min after [^{3}H]-raclopride injections with a rapid decline of the total radioactivity over a period of 2 hrs. Throughout this period the following rank-order was noted amoung pituitary and brain structures with regard to the amount of radioactivity recovered: striatum > olfactory tubercle > intermediate lobe > posterior lobe > anterior lobe > cerebellum (see Fig. 1 for the pituitary values). The relatively high values found in the posterior as compared to the anterior lobe most probably represent contamination of the posterior lobe samples with radioactivity present in cells of the intermediate lobe that remained attached to the posterior lobe during the dissection procedure, since in vitro binding studies (Pazos et al., 1985) have indicated lower densities of D-2 receptors (labelled by [^{3}H]-spiperone) in the posterior compared to the anterior lobe. To address this question, in vivo autoradiography of [^{3}H]-raclopride binding in the pituitary gland was performed after injection of a high dose (7.8 nmol · kg^{-1}, i.v.) of the [^{3}H]-raclopride. Using computer assisted microdensitometry, the specifically bound [^{3}H]-raclopride was found to be (means ± s.e.m. pmol · g^{-1} tissue)

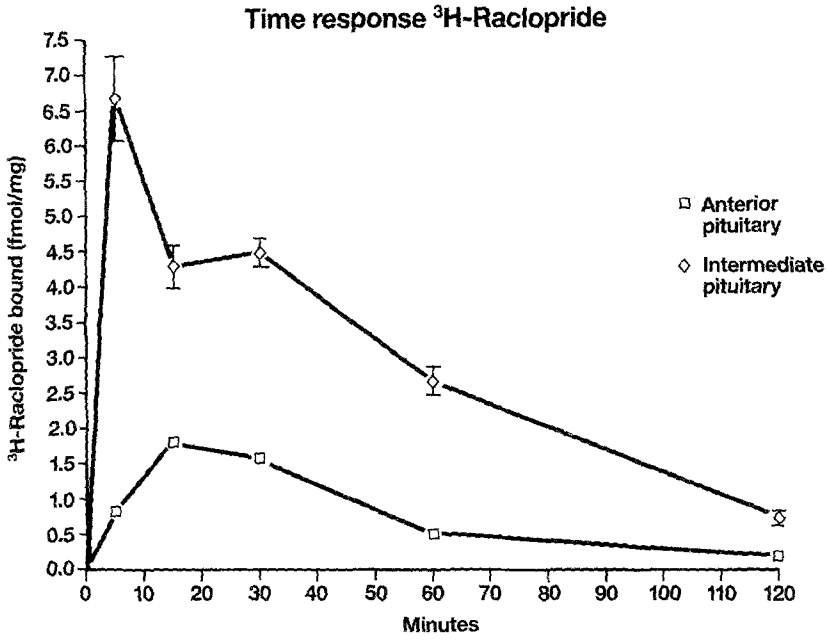

Fig. 1. Time-curve for the in vivo binding of [^{3}H]-raclopride in the anterior and intermediate lobes of pituitary gland. Values are expressed as mean ± S.E.M. fmol · mg^{-1} tissue wet weight

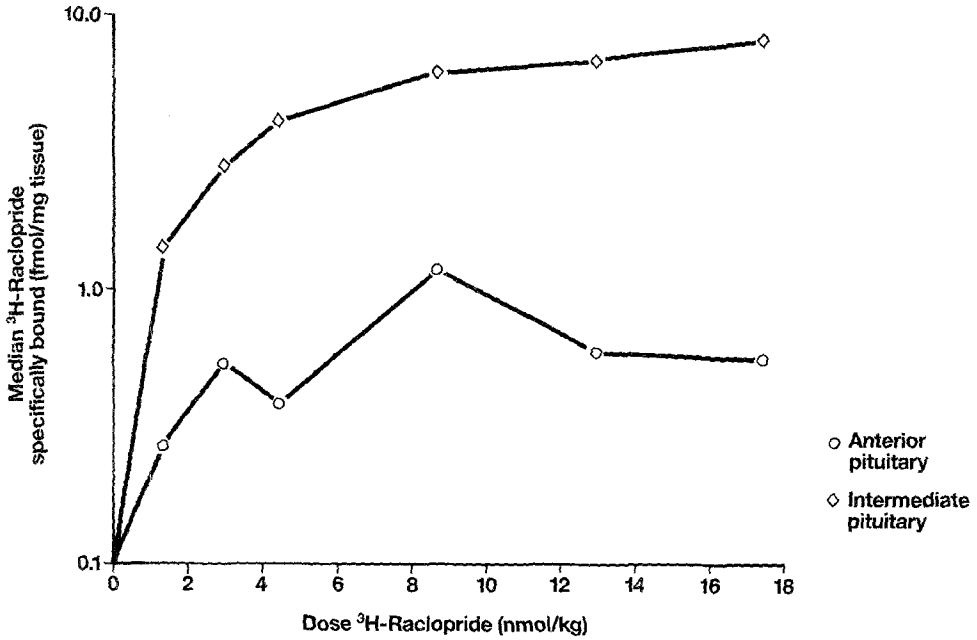

Fig. 2. Dose-response curve showing the saturation of specifically bound [^{3}H]-raclopride in the anterior and intermediate lobe of the pituitary gland log scale. Non-specific binding was defined as the binding remaining after treatment with (+)butaclamol (0.6 µmol · kg^{-1}, i.v.) before [^{3}H]-raclopride (see Methods). The variance among individual values did not exceed 5 percent

1.0 ± 0.2, 18.2 ± 0.5 and 0.3 ± 0.1 in the anterior, intermediate and posterior lobes, respectively. The autoradiographic analysis revealed that the intermediate lobe contained a central core of high density of [^{3}H]-raclopride binding which was found to be as high as 26.0 ± 0.8 (mean ± S.E.M.) pmol · g^{-1} tissue surrounded by a zone of less dense binding. In light of the fact that most of the binding in the posterior lobe may represent binding to cells from the intermediate lobe, remaining on the posterior lobe samples, the present pharmacological analysis will not include this part of the gland. In the brain (e.g., caudate nucleus and the olfactory tubercle) as well as in the pituitary gland an optimal ratio between specific and non-specific binding was obtained between 30 to 60 min after [^{3}H]-raclopride injections (Köhler et al., 1985; present study). As a result of this finding and together with the observation that the plasma radioactivity reached very low levels (Köhler et al., 1985) at 60 min, all subsequent pharmacological analysis of the binding was performed 45 min after ^{3}H-raclopride injections.

Kinetic analysis of the in vivo [^{3}H]-raclopride binding showed that binding to all lobes of the pituitary gland was saturable (Fig. 2) with a relatively low component of non-specific binding. The low degree of non-specific binding was particularly prominent in the intermediate lobe. The specific [^{3}H]-raclopride

Table 2. Estimated ED_{50} values (μmol · kg^{-1}, i.v.) for blockade of the in vivo [^{3}H]-raclopride binding in the anterior and intermediate lobes of the pituitary gland and in the caudate nucleus and the olfactory tubercle

	Pituitary gland		Brain	
Drug	Anterior	Intermediate	Caudate nucleus	Olfactory tubercle
S-sulpiride	0.50	0.08	> 20.0	5.0
R-sulpiride	20.0	10.0	> 20.0	> 20.0
Remoxipride	0.90	0.70	0.90	0.90
FLA 731(+)	> 10.0	> 10.0	> 10.0	> 10.0
Raclopride	0.90	0.05	0.09	0.10
FLB 472	> 5.0	> 5.0	> 5.0	> 5.0
Domperidone	0.06	0.05	1.0	2.0
Spiperone	≤ 0.007	0.009	0.02	0.02
NPA	1.0	0.50	0.25	0.20
Quinpirole*	8.0	3.0	2.0	2.0

The ED_{50} values were calculated from dose response curves made up of 4–6 doses with 4–6 rats for each dose. The drugs were given (i.v.) 30 minutes before the ligand
Remoxipride is equal to FLA 731(—), FLB 472 is the (R)enantiomer of raclopride
* Drug given 5 min before [^{3}H]-raclopride

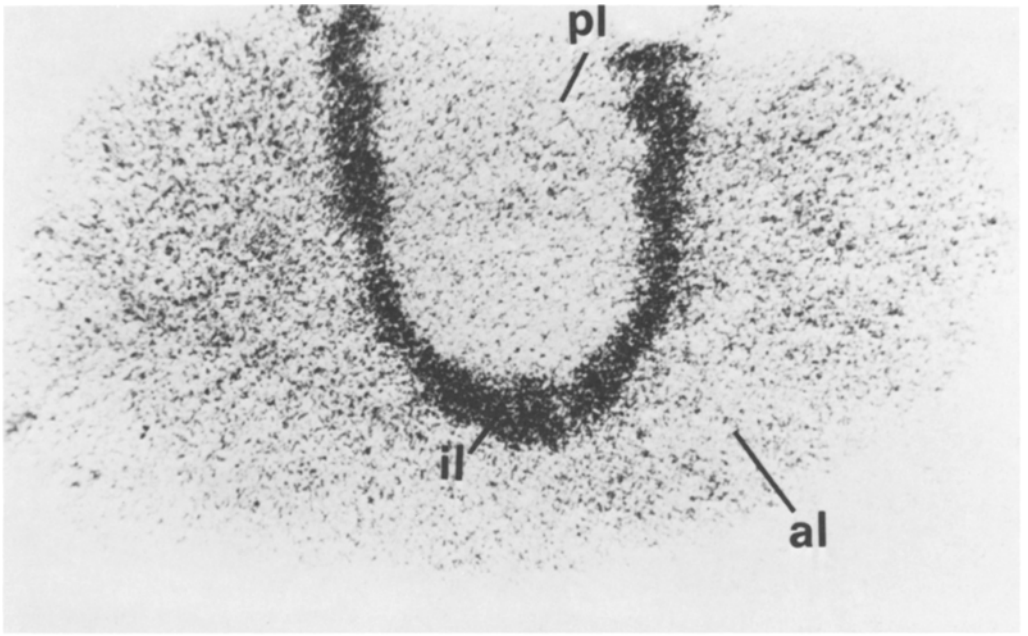

Fig. 3. Photomicrograph showing an autoradiogram generated from the pituitary gland of a rat injected with 70 μCi; 7.8 nmol · kg^{-1} i.v. of [^{3}H]-raclopride. *al* Anterior lobe, *il* intermediate lobe, *pl* posterior lobe

Table 3. Ratios between the estimated ED_{50} values for blockade by different drugs of the in vivo [^{3}H]-raclopride binding the anterior and intermediate lobes, respectively and the blockade of binding in the caudate nucleus

	Lobe of pituitary gland	
Compound	Anterior	Intermediate
S-sulpiride	40.00	250.00
R-sulpiride	1.00	2.00
Remoxipride	1.00	1.20
FLA 731(+)	1.00	1.00
Raclopride	0.10	1.80
FLB 472	1.00	1.00
Domperidone	16.00	20.00
Spiperone	2.80	2.20
NPA	0.25	0.50
Quinpirole	0.50	0.60

A value of 1 means equipotency in the pituitary gland and the caudate. The higher the ratio the more potent the drug is in the pituitary gland compared to the brain. For compounds where the ED_{50} was larger than the highest dose tested, the largest dose was used as a fictive ED_{50} value

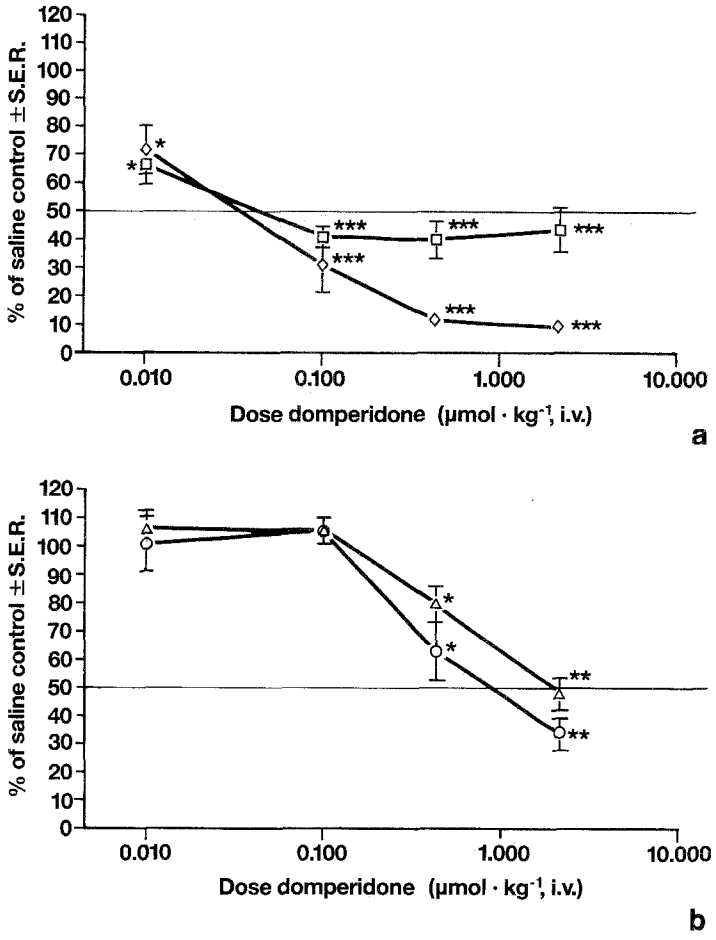

Fig. 4. Dose-response curves showing the blockade by domperidone of specific in vivo binding of [^{3}H]-raclopride in the anterior (□) and intermediate (◇) lobes of the pituitary gland (**a**) and in the caudate nucleus (△) and olfactory tubercle (○) (**b**). The values are expressed as the mean percent of saline treated control rats. Bars indicate S.E.R. * $p<0.05$; ** $p<0.01$; *** $p<0.001$, Student's t-test

binding in different lobes of the pituitary gland showed a rank-order that was similar along the entire dose-response curve (Fig. 2): intermediate > anterior. From the saturation analyses, the B_{max} values were estimated to be 12.9 ± 1.6 and 2.2 ± 0.9 pmol · g^{-1} wet weight and the K_D values were found to be 6.5 ± 4.6 and 7.3 ± 2.4 nmol · kg^{-1}, in the intermediate and anterior lobes, respectively. In the caudate nucleus and olfactory tubercle of the same animals a similar analysis revealed B_{max} values of 12.9 ± 1.7 and 6.1 ± 0.7 pmol · g^{-1} and K_D values of 9.2 ± 2.8 and 9.3 ± 2.4 nmol · kg^{-1}.

The specific in vivo binding of [^{3}H]-raclopride was dose-dependently prevented by several dopamine D-2 antagonists and agonists, while the D-1 receptor antagonist SCH 23390 and the D-1 agonist SKF 38393-A failed to block the

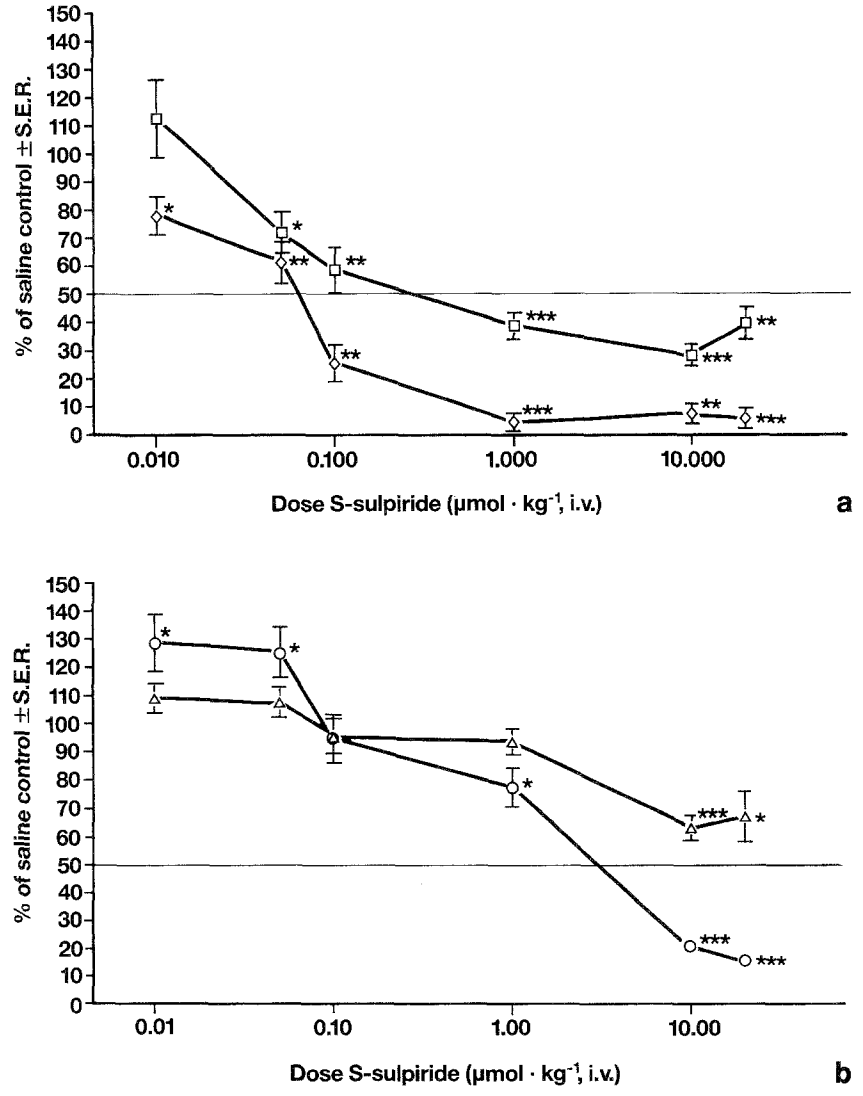

Fig. 5. Dose-response curves showing the blockade by S-sulpiride of specific in vivo binding of [^{3}H]-raclopride in the anterior (□) and intermediate (◇) lobes of the pituitary gland (**a**) and in the caudate nucleus (△) and olfactory tubercle (○) (**b**). The values are expressed as the mean percent of saline treated control rats. Bars indicate S.E.R. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, Student's t-test

binding of [^{3}H]-raclopride at behaviourally relevant doses (0.1 and 1.70 µmol · kg^{-1}, i.v., respectively). Interestingly, at 0.5 µmol · kg^{-1} (i.v.) of SCH 23390 a slight reduction of the specific binding was observed in the anterior and intermediate lobes but not in the caudate nucleus and olfactory tubercle. High doses of the serotonin S-2 antagonist ketanserin and the α adrenergic antagonist (phenoxybenzamine) failed to prevent specific [^{3}H]-raclopride binding in all lobes of the pituitary gland and in the brain (data not shown, see also Köhler et al., 1985).

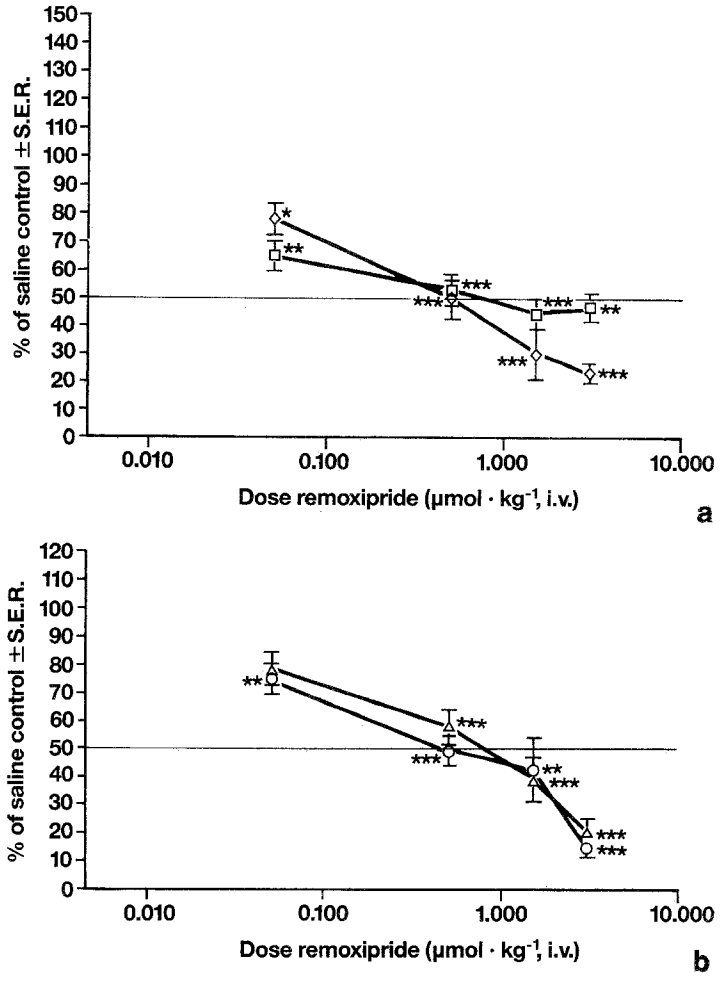

Fig. 6. Dose-response curves showing the blockade by remoxipride of specific in vivo binding of [^{3}H]-raclopride in the anterior (□) and intermediate (◇) lobes of the pituitary gland (**a**) and in the caudate nucleus (△) and olfactory tubercle (○) (**b**). The values are expressed as mean percent of saline treated control rats. Bars indicate S.E.R. * $p<0.05$; ** $p<0.01$; *** $p<0.001$, Student's t-test

A closer analysis of the ability of different enantiomers of D-2 antagonists to prevent the specific in vivo [^{3}H]-raclopride binding showed clear stereospecific effects by S- and R-sulpiride, remoxipride and FLA 731(+) and raclopride and FLB 472 with the active enantiomer being 10 to 125 times more potent than the inactive [e.g. R-sulpiride, FLB 731(+) and FLB 472] ones, depending upon the compound and the particular pituitary lobe under study (see Table 2). Among the active D-2 antagonists, large differences in potencies were noted between spiperone, domperidone, raclopride and S-sulpiride (Table 2 and Figs. 3–5). Of these compounds, S-sulpiride and spiperone were among the most potent drugs tested in preventing binding of [^{3}H]-raclopride in different parts of the pituitary gland (Table 2 and Figs. 2 and 4). They differed markedly,

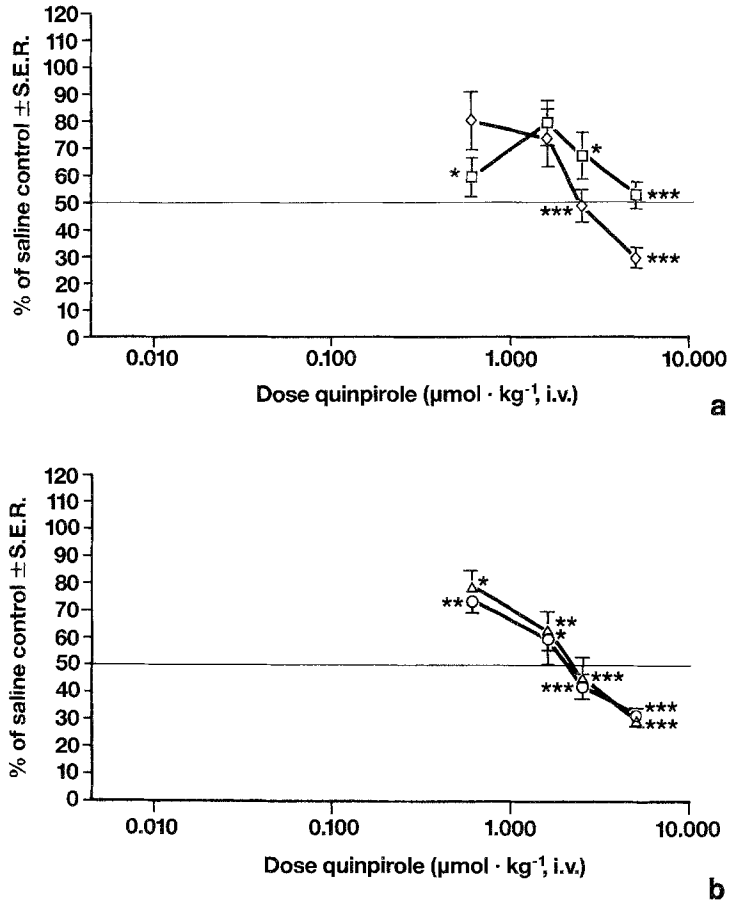

Fig. 7. Dose-response curves showing the blockade by the D-2 agonist quinpirole of specific in vivo binding of [^{3}H]-raclopride in the anterior (□) and intermediate (◇) lobes of the pituitary gland (**a**) and in the caudate nucleus (△) and olfactory tubercle (○) (**b**). The values are expressed as the mean percent of saline treated control rats. Bars indicate S.E.R. * $p<0.05$; ** $p<0.01$; *** $p<0.001$, Student's t-test

however, in their relative potencies in the caudate nucleus and olfactory tubercle. Thus, the following rank order was noted with regard to the potencies of different compounds in preventing binding in the caudate nucleus; spiperone > raclopride > remoxipride > domperidon > sulpiride. Relatively large differences existed for several of the drugs tested, with regard to their potencies in the pituitary gland and the brain. Thus, the ratios between the ED_{50} values for blockade of [^{3}H]-raclopride binding in the caudate nucleus or olfactory tubercle and within individual lobes of the pituitary gland, were very high for drugs such as S-sulpiride and domperidone, indicating that these drugs are far more potent in blocking in vivo [^{3}H]-raclopride binding in the pituitary gland than in the brain, while the ratios for remoxipride, raclopride and spiperone ranged between 1–3 (Table 3), indicating near equipotency in the brain and in

the pituitary gland. However, the two D-2 agonists NPA and quinpirole were slightly more potent in preventing in vivo [^{3}H]-raclopride binding in the brain than in the pituitary gland (Table 3).

Discussion

The present study has shown that [^{3}H]-raclopride, a novel substituted benzamide compound with high selectivity for dopamine D-2 receptors in the brain (Köhler et al., 1985; Ögren et al., 1986) can be used to label DA receptors in the rat pituitary gland in vivo. The present pharmacological analysis, together with previous (Hall and Wedel, 1986; Köhler et al., 1985; Ögren et al., 1986) characterization of [^{3}H]-raclopride binding in vitro as well as in vivo (Köhler and Karlsson-Boethius, 1988) suggest that DA D-2 receptors are labelled by raclopride also under in vivo conditions. The in vivo binding of [^{3}H]-raclopride to the anterior and intermediate lobes of the pituitary gland is rapid, saturable, reversible and of high specificity as shown in competition experiments with (+)butaclamol. The binding capacity (B_{max}) was found to be similar in the intermediate lobe and the caudate nucleus in the same animals, but the affinity of binding was slightly lower in brain than in the pituitary gland. Dopamine D-2 antagonists (e.g., S-sulpiride, remoxipride, raclopride, spiperone, domperidone) and agonists (NPA and quinpirole) potently prevent the in vivo [^{3}H]-raclopride binding in the pituitary gland as well as in the brain. This is in contrast to drugs shown to act selectively at the DA D-1 receptor in vitro (e.g., SCH 23390 and SKF 38393-A) (Andersen et al., 1986; Billard et al., 1984; O'Boyle and Waddington, 1984) as well as drugs acting at serotoninergic or noradrenergic receptors, none of which blocked the in vivo binding of [^{3}H]-raclopride in the pituitary gland (present study; see also Köhler et al., 1985).

In the pituitary gland, the highest amount of specifically bound [^{3}H]-raclopride was detected in the intermediate lobe, while the anterior and posterior lobes harboured far fewer binding sites. This is in line with the findings of previous in vitro and in vivo studies of the localization of pituitary D-2 receptors using [^{3}H]-spiperone as the ligand (DeSouza and Kuhar, 1984; Köhler and Fahlberg, 1985; Lightman et al., 1982; Pazos et al., 1985). In the present study the posterior lobe was found to contain a slightly larger amount of bound [^{3}H]-raclopride than the anterior lobe. This finding is somewhat puzzling since earlier studies have shown larger number of [^{3}H]-spiperone labelled D-2 receptors in the anterior than in the posterior lobe (Pazos et al., 1985). Indeed, our own in vivo (present study) and in vitro (Köhler, unpubl. obs.) autoradiographic studies indicated that the posterior lobe is virtually devoid of specific [^{3}H]-raclopride binding sites. One likely explanation for the relatively high values in the posterior lobe noted here may be that, during dissection of the posterior part, small tissue-fragments of the intermediate lobe remained attached to the posterior lobe, thereby contributing to a relatively large number of binding sites in this part of the gland. In light of this possibility, we have excluded the posterior lobe from the present pharmacological analysis.

All D-2 antagonists tested caused a dose-dependent blockade of the in vivo [^{3}H]-raclopride binding in the anterior and intermediate lobes of the pituitary gland. Large differences existed, however, with regard to their relative potencies as well as their maximal effects on the binding in the individual lobes. For example, S-sulpiride and domperidone, two drugs known to have profound polactin releasing properties (Besser et al., 1980; Melzer et al., 1979), differed in their relative potencies to block [^{3}H]-raclopride binding in the anterior lobe. Remoxipride, which causes a relatively modest increase in prolactin levels in rats (Ögren, pers. comm.) was found to be equipotent with regard to the blockade of D-2 receptors in the anterior lobe and in the caudate nucleus and olfactory tubercle, in spite of the fact that the actual levels of remoxipride is most probably higher in the anterior lobe of the pituitary gland than in the brain. S-sulpiride, which like remoxipride shows a high separation between blockade of apomorphine induced hyperactivity and stereotypies (Ögren et al., 1984) and which penetrates poorly into the brain, was found to block far more [^{3}H]-raclopride labelled D-2 receptor sites than remoxipride at the lowest behaviorally relevant doses tested. These findings, thus, show clear differences between S-sulpiride and remoxipride in the pattern of D-2 receptor blockade in brain and pituitary gland. If this difference derives from differences in receptor affinities or pharmacokinetic differences between the two drugs remains unknown. It could, however, support the idea (see Sokoloff et al., 1984) that different D-2 receptor subclasses exist in the pituitary gland and the caudate nucleus.

The most potent drug tested with regard to blockade pituitary D-2 receptors labelled by [^{3}H]-raclopride was spiperone. Previous studies have shown that raclopride is relatively poor in preventing the in vivo binding of [^{3}H]-spiperone in the anterior or intermediate lobes of the pituitary gland (Köhler and Fahlberg, 1985). This could indicate that [^{3}H]-spiperone and [^{3}H]-raclopride bind to partly overlapping sites in the pituitary gland while some binding-sites are different. Similar suggestions have been made previously for the in vivo binding of the two ligands in the caudate nucleus (Köhler and Karlsson-Boethius, 1988) and is supported by findings of [^{3}H]-spiperone and [^{3}H]-raclopride binding to homogenates of rat striatum (Hall and Wedel, 1986) as well as by in vitro autoradiographic studies (Köhler, in prep.) using these ligands in the pituitary gland.

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Authors' address: Dr. Ch. Köhler, Department of Neuropharmacology, Astra Alab AB., S-15185 Södertälje, Sweden.

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