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Kinetic properties of the accumulation of ^{3}H-raclopride in the mouse brain in vivo

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Summary. The kinetic properties of the accumulation of ^{3}H-raclopride, a selective dopamine (DA) D-2 receptor antagonist, in mouse striatum in vivo was examined under various experimental conditions. The accumulation in striatum was saturable in contrast to that in cerebellum, which linearily increased with the dose. The specific binding of ^{3}H-raclopride in the striatum, defined as the difference in the accumulation in striatum and cerebellum 30 min after the injection was completely inhibited by the D-2 receptor antagonists spiperone and (+)-butaclamol [but not (−)-butaclamol] and the DA receptor agonist N-n-propylnorapomorphine. The mean B_{max} value of the specific binding was 40.7 ± 2.8 pmol/g tissue and the mean apparent K_D value, based on the dose injected, was 87.8 ± 11.5 nmol/kg i.v. (18 different experiments). Pretreatment of the mice with a single injection of reserpine 4 h or 3 days beforehand reduced the apparent K_D value which in part seemed to be due to the decreased concentration of synaptic DA. Similarly, γ-butyrolactone injected immediately before raclopride reduced the apparent K_D value, whereas amfonelic acid and (−)-amphetamine increased the observed K_D values. These findings indicate competition between endogenous DA and raclopride for the D-2 receptors. Both reserpine and γ-butyrolactone increased the apparent B_{max} value by about 50% which indicates a receptor pool of DA for which raclopride does not compete.

Key words: Dopamine D-2 receptors – In vivo binding – Mouse – Raclopride – Striatum

Introduction

We recently showed that the combination of the dopamine (DA) D-1 receptor agonist SKF 38393 and the D-2 receptor agonist quinpirole produced much greater locomotor stimulation in mice pretreated for 1 or 3 days with reserpine than in mice pretreated for 4 h (Ross et al. 1988). This supersensitivity was not accompanied by any change in the densities or affinities of the D-1 and D-2 receptors in the mouse striatum measured in in vitro binding experiments with the D-1 receptor antagonist ^{3}H-SCH 23390 and the D-2 receptor antagonist ^{3}H-spiperone. These negative findings do not exclude the possibility that changes in the receptors occur after reserpine but suggest that they are difficult to detect under in vitro conditions. In order to further examine the origin of the effect of the reserpine-induced supersensitivity response, we decided to study the effect of reserpine on the kinetic properties of the in vivo accumulation of ^{3}H-raclopride, a selective D-2 receptor antagonist (Köhler et al. 1985; Hall et al. 1988b). This compound has been used as a ligand in in vitro and in vivo studies of D-2 receptors (Köhler et al. 1985; Farde et al. 1985, 1986; Köhler and Radesäter 1986; Andersen 1988; Hall et al. 1988a, b).

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Materials and methods

Subjects. Male albino mice weighing 25–35 g were used. In the experiments performed in Australia the mouse strain was QS (Sydney University Animal Farm) and in those performed in Sweden (most of the experiments) the strain was NMRI (Alab Laboratorietjänst, Upplands-Väsby, Sweden). The animals were kept under constant temperature (22° C) and lighting (6 a.m. to 6 p.m.) and were allowed free access of food and water. The compounds were injected intravenously (i.v.), intraperitoneally (i.p.) or subcutaneously (s.c.) as stated for each experiment in a volume of 0.10 ml per 10 g body weight. The animals which were treated with reserpine were kept for 3 days at 25° C by means of thermostated infrared light. The mice were killed by cervical dislocation and the brain regions studied were immediately dissected on ice.

In vitro binding experiments. The metthod described by Köhler et al. (1985) was used. Pooled mouse striata were homogenized in 100 vol. (w/v) of ice-chilled 50 mmol/l Tris-HCl buffer, pH 7.4, with an Ultra-Turrax for 10 s. The homogenate was centrifuged at 39,000 × *g* for 10 min at +4° C. The pellet was rehomogenized in fresh buffer and centrifuged. The final resuspension was made in 100 vol. of of 50 mmol/l Tris buffer, pH 7.4, containing 0.1% ascorbic acid, 120 mmol/l NaCl, 5 mmol/l KCl, 2 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$ and 20 μmol/l pargyline. The incubation of 400 μl of the tissue suspension, 50 μl of the test compound and 50 μl of ^{3}H-raclopride producing the desired final concentration was performed at room temperature (21° C) for 1 h. The samples were thereafter rapidly diluted with 5 ml ice-chilled Tris-saline buffer and filtered through Whatman GF/B filters in a 24 channel cell harvester (Brandel, Gaithersburg, MD, USA). The filters were immediately washed twice with 5 ml saline buffer. The radioactivity trapped on the filters was counted in 5 ml Beckman Ready-Solve TM HP in a Beckman LS 8100 liquid scintillation spectrometer. (+)-Butaclamol (3 μmol/l) was used to define the specific binding of ^{3}H-raclopride to the DA receptors.

In vivo binding experiments. The method described by Köhler et al. (1985) was used. ^{3}H-Raclopride, about 4 μCi diluted with various amounts of unlabelled raclopride was injected in a tail vein (in some experiments s.c. in the neck). The mice were killed exactly 30 min after the injection (if otherwise not stated) and the cerebellum, striata and, in some experiments, olfactory tubercles were rapidly dissected and stored on ice. The tissues were weighed and dissolved in 0.5 ml Soluene-350 (Packard) at 60°C for 90 min. After addition of 5 ml scintillation liquid (Econfluor, NEN) the samples were stored for at least 10 h before counting because of interfering chemoluminescence. Tissues from non-treated animals were always taken as tissue blanks. Since more than 90% of the radioactivity in the brain was intact raclopride, the amount of raclopride in the tissue was calculated from the radioactivity counts from the various specific activities of ^{3}H-raclopride injected and expressed in pmol/g tissue. The specific binding of raclopride to the D-2 receptors was determined from the difference between the amounts in striatum or olfactory tubercle and cerebellum [for discussion see Köhler et al. (1985)]. The maximal number of specific binding sites (B_{max}) and the apparent dissociation constant (K_D) were determined by linear regression analysis of Hofstee plots of the specific binding either using the doses injected or the cerebellar concentrations of raclopride as the concentration parameter.

Thin layer chromatography (TLC). Ethanol extracts of striatum and cerebellum from mice treated with ^{3}H-raclopride 30 min before death were analysed with TLC, using silica gel plates (Polygram sil G/UV 254). The solvent was ethanol:acetic:acid:water (6:3:1).

Statistics. All comparisons between groups were made using Student's two-tailed *t*-test.

Compounds. Raclopride, (methoxy-^{3}H) (specific activities between 46.0 and 87.9 Ci/mmol) was obtained from NEN Research Products, DuPont, USA. Amfonelic acid, R-(−)-N-n-propylnorapomorphine and SCH 23390 [R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride], (+)- and (−)-butaclamol hydrochloride and spiperone were bought from Research Biochemicals Inc., Natick, MA, USA. (+)-Amphetamine sulfate and pargyline hydrochloride were purchased from Sigma, St. Louis, MO, USA. Unlabelled raclopride tartrate was synthesised at Astra Research Centre, Södertälje, Sweden. All other chemicals were of the highest purity available.

Results

In vitro binding

The kinetics of the high affinity binding of ^{3}H-raclopride to D-2 receptors, defined by 3 μmol/l (+)-butaclamol, to freshly prepared membranes from mouse striatum was determined. The B_{max} value obtained in two independent experiments was 34.9 (32.8, 36.9) pmol/g tissue, the apparent K_D value was 0.59 (0.55, 0.62) nmol/l and the Hill coefficient was 1.09 (1.05, 1.13). Treatment of the mice with a single injection of reserpine (5 mg/kg, s.c.) 3 days before death did not change the kinetics of the raclopride binding compared to the controls (Table 1). In this experiment the membranes were prepared from frozen tissue, stored at −70°C, which might explain the lower B_{max} and the higher apparent K_D compared to the values obtained with fresh preparations. DA inhibited the raclopride binding with inhibition curves best fitted by a two side model (LIGAND) (Munson and Rodbard 1980). The K_i^{high} value from two separate experiments was 48 (47, 48) nmol/l and the K_i^{low} was 910 (884, 935) nmol/l. The Hill coefficient was 0.70. The DA receptor agonist (−)-N-n-propylnorapomorphine was a potent inhibitor of the raclopride binding (K_i = 0.9 nmol/l, n_H = 0.78).

Table 1. Lack of effect of a single injection of reserpine on the in vitro binding of ^{3}H-raclopride to washed striatal membranes from the mouse. Reserpine, 5 mg/kg s.c. was injected in a single dose 3 days before the death of the mice. Striatum from groups of 5 mice were pooled and stored at −70°C until assayed. The kinetic constants of the ^{3}H-raclopride binding were determined from saturation curves based on 6 different concentrations (0.2–6.3 nmol/l) in duplicate. The non-binding at each concentration was determined in the presence of 3 μmol/l (+)-butaclamol. Each value is the mean ± SEM from 4 groups of pooled striata

Kinetic constants	Control	Reserpine
B_{max}, pmol/g tissue	24.7 ± 0.4	25.6 ± 1.6
K_D, nmol/l	1.2 ± 0.05	1.1 ± 0.03

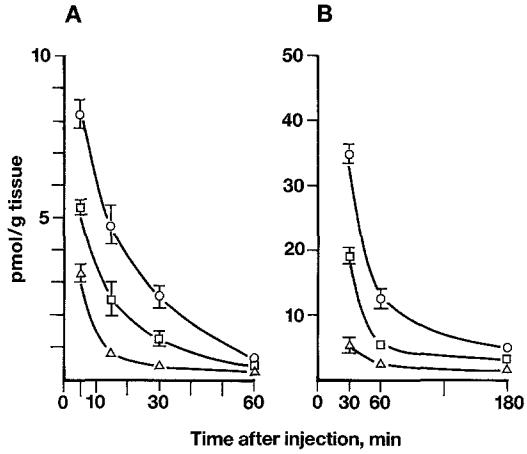

Fig. 1A, B. Time courses of the accumulation of ^{3}H-raclopride in striatum (○), olfactory tubercle (□) and cerebellum (△) after intravenous injection of ^{3}H-raclopride at two different doses. **A** 3.7 nmol/kg. **B** 45 nmol/kg. Each value is the mean ± SEM (*verticals bars*) from 4 mice

In vivo binding

Specific binding to D-2 receptors. The accumulation of raclopride in striatum and olfactory tubercle was considerably higher than that in cerebellum (Fig. 1), in accordance with previous findings in mice (Andersen 1988) and rats (Köhler et al. 1985). Examination of tissue extracts in ethanol with TLC showed that more than 90% of the radioactivity in striatum and cerebellum was unchanged raclopride (data not shown). The difference between the concentration of raclopride in striatum (or olfactory tubercle) and cerebellum was therefore taken as a measure of the specific binding

Table 2. Inhibition by DA antagonists and the DA agonist (−)-N-n-propylnorapomorphine (NPA) of the accumulation of ^{3}H-raclopride in the mouse brain in vivo. Spiperone, 5 mg/kg i.p. was injected 30 min and (+)- and (−)-butaclamol hydrochloride, 5 mg/kg i.p. 15 min before ^{3}H-raclopride, 25 nmol/kg i.v. (Exp. 1) or 40 nmol/kg i.v. (Exp. 2), 30 min before the death of the mice. Reserpine, 5 mg/kg s.c. was injected 24 h before the experiment. In Exp. 3 NPA, 1 mg/kg s.c. was given 15 min before ^{3}H-raclopride, 12 nmol/kg s.c. 45 min before the death of the animals. Each value is the mean ± SEM from n number of animals

Exp. Treatment	n	^{3}H-Raclopride accumulation, pmol/g tissue		
		Cerebellum	Striatum	Olfactory tubercle
1. Saline	4	2.3 ± 0.1	13.0 ± 1.9	6.9 ± 1.0
Spiperone	3	2.2 ± 0.06	2.5 ± 0.1**	2.8 ± 0.2**
2. Saline	4	6.3 ± 0.3	27.5 ± 2.1	21.7 ± 5.2
(+)-Butaclamol	5	5.9 ± 0.5	7.0 ± 0.4**	7.7 ± 0.5*
(−)Butaclamol	4	6.0 ± 0.5	23.9 ± 0.4	13.0 ± 1.1
Res + saline	4	24.4 ± 3.3	71.6 ± 5.2	40.1 ± 3.3
Res + (+)-butaclamol	5	20.8 ± 1.8	22.2 ± 2.5*	24.8 ± 2.4**
Res + (−)-butaclamol	4	23.8 ± 3.3	66.0 ± 3.4	45.4 ± 5.0
3. Saline	5	1.4 ± 0.1	5.3 ± 0.6	
NPA	4	1.4 ± 0.2	1.9 ± 0.2**	

* $P < 0.05$, ** $P < 0.01$ vs. saline-treatment (Student's t-test)

of raclopride in these regions (Köhler et al. 1985). This binding was completely inhibited by pretreatment of the animals with the selective D-2 antagonist spiperone, the stereoselective DA receptor antagonist (+)-butaclamol, but not by (−)-butaclamol (Table 2). The DA receptor agonist (−)-N-n-propylapomorphine was also an effective inhibitor of the raclopride binding in vivo. The time courses of the raclopride binding at two different doses showed that the cerebellar concentrations of raclopride were quite low 30 min after the i.v. injection compared with those in striatum and olfactory tubercle (Fig. 1). This time point was therefore used in the kinetic experiments.

Saturation kinetics. The specifc binding of ^{3}H-raclopride in striatum (Fig. 2) and olfactory tubercle (data not shown) in vivo followed saturation kinetics. The mean B_{max} value from 18 experiments calculated from Hofstee plots either based on the doses injected or on the cerebellar concentrations of raclopride was 40.7 ± 2.8 and 38.2 ± 2.0 pmol/g tissue, respectively. The mean apparent K_D value expressed in nmol/kg i.v. raclopride injected was 87.8 ± 11.5. When expressed in the cerebellar concentration of raclopride it was 8.5 ± 0.6 pmol/g tissue. The mean values of the linear regression coefficient of the Hofstee plots on which these values are based were 0.927 ± 0.008 and 0.924 ± 0.013, respectively. There was no difference between the results obtained with the two different mouse strains used (data not shown).

Effect of reserpine. Pretreatment of mice with reserpine, 5 mg/kg s.c. 4 h or 3 days before raclopride injection increased the radioactivity both in striatum and cerebellum (Table 3). The radioactivity in the two regions was intact raclopride (> 90%) when analyzed with TLC. This effect of reserpine was probably due to inhibition of the metabolism of raclopride, since the plasma concentration was also increased. Reserpine, 5 mg/kg s.c. injected 4 h before raclopride tended to increase the B_{max} and reduce the apparent K_D value, particularly when these values were calculated on the basis of the raclopride doses injected (Table 4). The less marked changes observed when the constants were calculated from the cerebellar concentrations of raclopride, which were increased 3 times indicate that inhibition of the

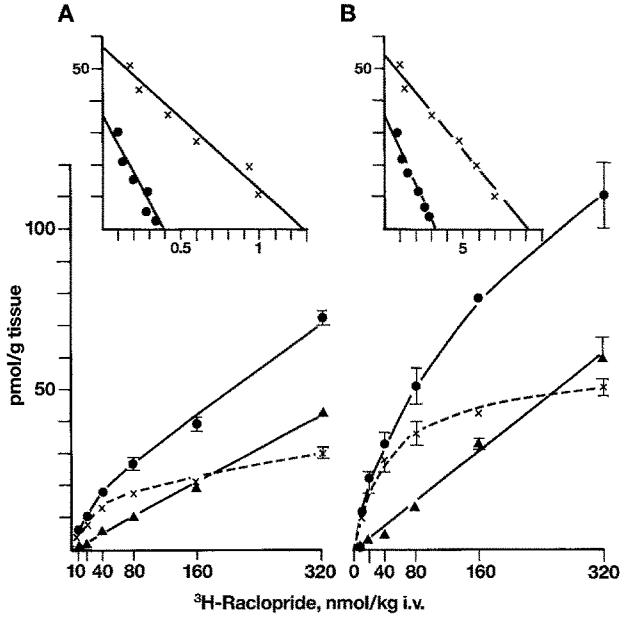

Fig. 2. Saturation curves of the accumulation of ^{3}H-raclopride in mouse striatum in vivo in naive (**A**) and reserpine pretreated (5 mg/kg s.c., once 3 days beforehand) (**B**) mice. Each point is the mean ± SEM from groups of 4 mice injected with various doses of ^{3}H-raclopride (11−321 nmol/kg i.v.) 30 min before the death. Striatum (●), cerebellum (▲) and the specific binding in striatum (striatum − cerebellum) (×). Significant ($P < 0.05$) differences between the specific binding in the treated and the control animals were obtained at all doses (Student's t-test). *Insets:* Hofstee plots. *Left panel:* specific binding (pmol/g tissue) vs this binding divided with the doses injected (nmol/kg). *Right panel:* specific binding (pmol/g tissue) vs. this binding divided with the cerebellar concentration of raclopride (pmol/g tissue). Control (●), reserpine (×)

raclopride metabolism caused a part of the decrease in the apparent K_D value. When reserpine was injected in a single dose 3 days before raclopride, a significant increase in B_{max} and decrease in K_D occurred, both when calculated from the doses and from the cerebellar concentration of raclopride (Fig. 2, Table 4). The nonspecific binding (cerebellar concen-

Table 3. The amount of radioactivity in blood plasma, cerebellum and striatum after injection of ^{3}H-raclopride in normal and reserpine pretreated mice. Reserpine, 5 mg/kg s.c. was given 4 h before the intravenous injection of ^{3}H-raclopride and the mice were killed 30 min later. The blood was collected in heparinized tubes. The radioactivity is expressed in pmol/g tissue. Each value is the mean ± SEM from 4 mice

Treatment	Dose ^{3}H-raclopride nmol/kg i.v.	Plasma	Cerebellum	Striatum
		pmol/g tissue		
Control	8.5	3.2 ± 0.2	0.8 ± 0.06	5.6 ± 0.9
Reserpine	8.5	8.4 ± 0.7*	3.4 ± 0.3*	16.3 ± 0.4*
Control	42	15.6 ± 0.6	4.3 ± 0.4	22.2 ± 1.3
Reserpine	42	45.1 ± 2.0*	21.5 ± 1.1*	61.5 ± 2.4*

* $P < 0.001$ vs. control (Student's t-test)

Table 4. Effects of compounds which reduce the synaptic DA concentration on the kinetics of the ^{3}H-raclopride binding in mouse striatum in vivo. Reserpine, 5 mg/kg s.c. was injected 4 h or 3 days, γ-butyrolactone (GBL) 400 mg/kg i.p. 15 min and α-methyl-p-tyrosine (α-MT), 400 mg/kg i.p. 4 and 1 h before the intravenous injection of 4–6 different doses of ^{3}H-raclopride (11–321 nmol/kg) to groups of 4 mice. They were killed 30 min thereafter and the concentration (pmol/g tissue) of raclopride in cerebellum and striatum were determined. The kinetic constants were calculated by linear regression analysis from Hofstee plots, either based on the doses injected (nmol/kg i.v.) or on the concentration of raclopride in cerebellum (pmol/g tissue). The ratios of the cerebellar concentrations in the drug treated and the control animals are also given. Each value is the mean ± SEM of the number of independent experiments noted (n). Controls were always analyzed simultaneously with the drug treated animals

Treatment	n	Kinetic constants determined from				Ratio of cerebellar concentration Drug/control
		the dose		cerebellar concentration		
		B_{max} pmol/g	K_D nmol/kg	B_{max} pmol/g	K_D pmol/g	
Control	4	38.4 ± 2.2	90.7 ± 7.3	37.5 ± 1.1	10.0 ± 0.8	
Reserpine, 3 days	4	57.5 ± 2.2**	35.2 ± 3.9**	55.1 ± 3.4**	5.3 ± 0.8**	2.0 ± 0.3
Control	3	49.5 ± 8.4	81.9 ± 22.6	43.8 ± 4.4	7.7 ± 1.7	
Reserpine, 4 h	3	70.6 ± 4.9	32.3 ± 5.9	56.8 ± 4.3	6.1 ± 2.4	3.1 ± 0.1
Control	4	33.6 ± 4.4	66.5 ± 13.4	34.4 ± 5.0	7.4 ± 1.7	
GBL	4	50.5 ± 4.6**	22.1 ± 1.4**	50.6 ± 4.7**	3.0 ± 0.5*	1.0 ± 0.1
Control	4	31.7 ± 3.0	66.8 ± 9.2	31.0 ± 2.5	7.4 ± 0.9	
α-MT	4	37.3 ± 2.4	39.6 ± 5.1*	36.9 ± 2.8	5.5 ± 0.3	1.7 ± 0.3

* $P < 0.05$, ** $P < 0.01$ vs control (Student's t-test)

tration) was also increased when reserpine was injected 3 days in beforehand but not to the same extent as 4 h after reserpine (Table 4).

Effect of γ-butyrolactone. γ-Butyrolactone, which stops the nerve activity in dopaminergic neurons (Walters and Roth 1972) was injected at a dose of 400 mg/kg i.p. 15 min before various doses of ^{3}H-raclopride. It significantly increased the apparent B_{max} value and decreased the apparent K_D value compared to the corresponding values in the control group (Fig. 3, Table 4). The cerebellar concentration of raclopride was unchanged.

Effect of α-methyl-p-tyrosine. α-Methyl-p-tyrosine, 400 mg/kg i.p. one and 4 h before raclopride tended to increase B_{max} and decrease K_D compared to controls but the changes did not reach statistical significance (Table 4).

Effects of amfonelic acid and (+)-amphetamine. Amfonelic acid and (+)-amphetamine increase the synaptic concentration of DA by inhibition of the DA uptake and release of DA, respectively (Ross 1979). Amfonelic acid, 4 mg/kg i.p. immediately before the injection of raclopride decreased the accumulation of raclopride in striatum in a competitive manner, i.e it increased the apparent K_D value without changing B_{max} (Fig. 4). (+)-Amphetamine sulfate, 5 mg/kg i.p. did not change significantly the kinetics of the raclopride binding in the mouse striatum in vivo (data not shown). However, when the dose was increased to 10 mg/kg i.p., injected immediately before various doses of raclopride the binding of raclopride, expressed in the cerebellum concentrations of raclopride was significantly decreased at the four lowest doses injected (11.5 to 81.4 nmol/kg i.v.) (Fig. 5).

Discussion

This study confirms previous findings that raclopride binds specifically to DA D-2 receptors in striatum and olfactory tubercle in vivo (Köhler et al. 1985; Andersen 1988). As discussed by Köhler et al. (1985) the specific binding in the DA rich regions can be expressed as the difference between the raclopride concentration in these regions and that in cerebellum, which contains very few D-2 receptors. This

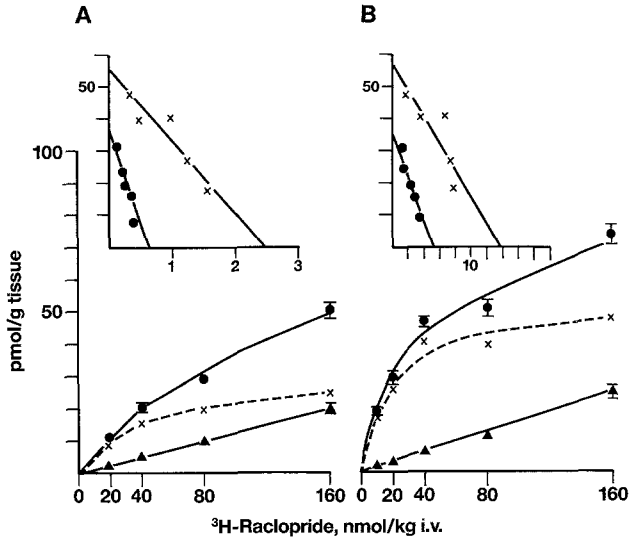

Fig. 3. Saturation curves of the accumulation of ^{3}H-raclopride in vivo in mice pretreated with water (**A**) and γ-butyrolactone (400 mg/kg i. p., 15 min) (**B**). Each point is the mean ± SEM (*vertical bars*) from groups of 4 mice injected with various doses of ^{3}H-raclopride (11–161 nmol/kg i.v.) 30 min before the death. Striatum (●), cerebellum (▲) and the specific binding in striatum (striatum minus cerebellum) (×). Significant ($P < 0.01$) differences between the striatal concentrations in the γ-butyrolactone-treated and the control animals were obtained at the doses 21, 41, 81 and 161 nmol/kg. *Insets:* Hofstee plots. *Left panel:* specific binding (pmol/g tissue) vs. this binding divided with the doses injected (nmol/kg). *Right panel:* specific binding (pmol/g tissue) divided with the cerebellar concentrations of raclopride (pmol/g tissue). Controls (●), γ-butyrolactone (×)

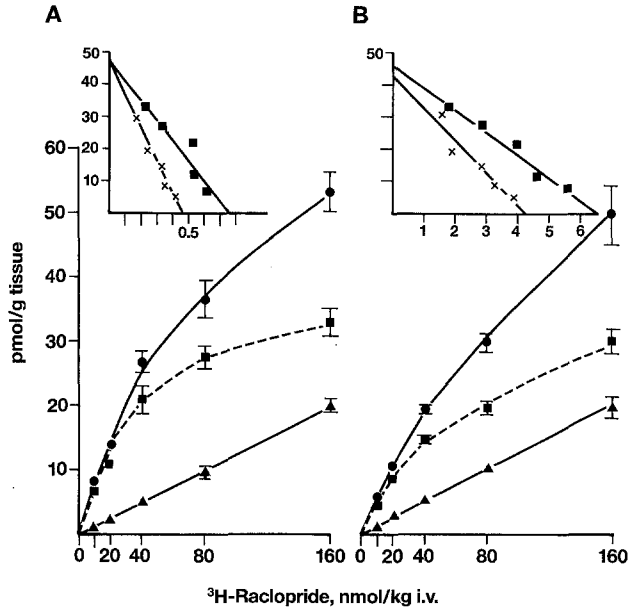

Fig. 4. Saturation curves of the accumulation of ^{3}H-raclopride in mouse striatum in vivo in pretreated with saline (**A**) and amfonelic acid (4 mg/kg i.p.) (**B**) immediately before the injection of various doses of ^{3}H-raclopride (11–161 nmol/kg i.v.) 30 min before the death. *Each point* is the mean ± SEM (*vertical bars*) from groups of 4 mice. Striatum (●), cerebellum (△) and the specific binding in striatum (striatum minus cerebellum) (■). Significantl ($P < 0.05$) difference between the stritial concentrations of raclopride in the amfonelic acid and saline treated animals were obtained at the doses 11–81 nmol/kg (Student's *t*-test). *Insets:* Hofstee plots. *Left panel:* specific binding (pmol/g tissue) vs. this binding divided with the doses injected (nmol/kg). *Right panel:* specific binding (pmol/g tissue) vs. this binding divided with the cerebellar concentration of raclopride (pmol/g tissue). Controls (■), amfonelic acid (×)

was justified by the finding that more than 90% of the radioactivity in these regions was unchanged raclopride and that the pretreatment of the animals with DA receptor antagonists reduced the accumulation of raclopride in these regions to that in cerebellum. Furthermore, the cerebellar concentration of raclopride can be used as a measure of the brain concentration in the calculations of the kinetic constants of the specific binding to D-2 receptors in these regions. The linearity of the raclopride concentration in cerebellum to the dose injected indicates that it is directly related to the free concentration in the brain. By using this technique, errors induced by individual or drug dependent variations in the metabolism and distribution of raclopride as well as failure of injection are reduced.

The aim of this study was to examine whether a single dose of reserpine causes any changes in the D-2 receptors which could explain the observation that reserpine injected 1–3 days before the combined injection of mice with SKF 38393 (or CY 208-243) and quinpirole resulted in an enhanced response (Ross et al. 1988). This enhancement did not appear to be due to increased number of D-1 or D-2 receptors, since no changes in the B_{max} or K_D values of the in vitro binding of ^{3}H-SCH 23390 or ^{3}H-spiperone to membrane preparations of striatum from reserpine pretreated mice could be detected (Ross et al. 1988). The failure of reserpine to change the in vitro binding properties of ^{3}H-raclopride in the present study confirms the previous observation. The possibility that the reserpine treatment caused changes in the D-2 receptors in vivo which were not detectable with in vitro techniques prompted us to examine

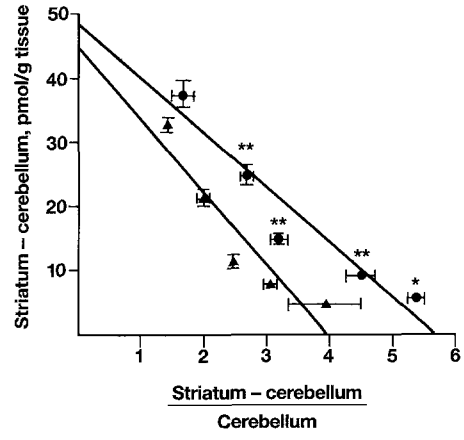

Fig. 5. Hofstee plot of the effect of (+)-amphetamine on the binding of ^{3}H-raclopride to mouse striatum in vivo. Groups of 4 mice were injected with saline (●) or (+)-amphetamine sulfate (▲), 10 mg/kg i.p. immediately before various doses (11.5–161.4 nmol/kg i.v.) of raclopride and killed 30 min thereafter. *Ordinate:* specific bound raclopride in striatum (striatum – cerebellum), means ± SEM (*verticals bars*). *Abscissa:* specific bound raclopride in striatum divided with the cerebellar concentration of raclopride, means ± SEM (*horizontal bars*). The asterisks denote statistical difference (* $P < 0.05$, ** $P < 0.01$, *t*-test) of these ratios between controls and amphetamine treated animals. The B_{max} and K_D values obtained in these plots were for the controls 48.1 pmol/g tissue and 8.5 pmol/g tissue, respectively, and for the amphetamine-treated mice 44.4 pmol/g tissue and 11.1 pmol/g tissue

the kinetic properties of the D-2 receptors in vivo. The use of ^{3}H-raclopride as a suitable ligand for studies of brain D-2 receptors in vitro and in vivo has been demonstrated in rats (Köhler et al.1985; Hall et al. 1988 b), mice (Andersen 1988), monkeys (Köhler and Radesäter 1986) and humans (Hall et al. 1988 a). ^{11}C-Raclopride is also a very suitable ligand in positron emission tomography (PET) of D-2 receptors in the living human brain (Farde et al. 1985, 1986; Hall et al. 1988 b).

The effect of reserpine on the accumulation of ^{3}H-raclopride in the mouse brain was rather complex. One obvious effect of the reserpine treatment was that more raclopride passed into the brain compared with the control animals. This was particularly pronounced 4 h after the reserpine injection but also apparent after 3 days. When the apparent K_D value was calculated from the dose injected a marked decrease in this value was therefore obtained. When the K_D value was estimated from the cerebellar concentration of raclopride the decrease was no longer significant in the 4 h pretreatment group but was still significantly reduced in the 3 day group. This indicates that the decrease in the apparent K_D value was not merely due to increased brain concentration of raclopride but another factor might be involved. Since reserpine depletes the brain of all the monoaminergic transmitters, this factor may be the decreased concentration of synaptic DA. If DA and raclopride compete for the same D-2 binding sites, the depletion of DA by reserpine should indeed decrease the apparent K_D value. Although the brain level of DA is decreased also 4 h after reserpine it might be more difficult to differentiate between the two factors at this stage than at day 3. The observation that acute treatment with γ-butyrolactone also decreased K_D without affecting the cerebellar concentration of raclopride supports this hypothesis. By inhibition of the firing in the dopaminergic neurons (Walters and Roth 1972) γ-butyrolactone decreases the synaptic concentration of DA. The failure of the synthesis inhibitor α-methyl-p-tyrosine to significantly decrease the K_D value could indicate that the synaptic DA concentration was less reduced than after reserpine and γ-butyrolactone.

Elevation of the synaptic DA concentration should, according to this hypothesis, increase the observed K_D values. This was in fact observed after the DA uptake inhibitor amfonelic acid, and after a high dose of the DA releasing agent (+)-amphetamine. Since DA has to compete with raclopride both for the high and low affinity states it is possible that the effect of elevated synaptic DA concentration on the raclopride binding is less pronounced than that observed at decreased DA concentration. Thus, the inhibitor constant of DA for the low affinity state (910 nmol/l) was almost 20 times larger than that for the high affinity state (48 nmol/l). Under in vitro conditions about half of the total number of D-2 receptors are in the high affinity state (Seeman 1980), but it is unknown if such also applies in vivo. In any case, the competition between the receptor agonist DA and the receptor antagonist raclopride for the D-2 binding sites in vivo is probably quite complex. This complexity may explain why both the apparent K_D value was reduced and the B_{max} value was increased when the synaptic DA concentration was reduced by reserpine or γ-butyrolactone. The increased B_{max} value observed may indicate that some D-2 receptor sites are not available for binding raclopride when occupied by DA under in vivo conditions (cf. Seeman et al. 1989).

The results obtained in the present study suggested that it might be possible to estimate changes in, and thereby also the normal level of DA, in dopaminergic synapses from changes in the apparent K_D values of te in vivo binding of D-2 receptor agonists. In a subsequent study the kinetic properties of the in vivo binding of the DA receptor agonist N-n-propylnoramorphine are reported showing that this in fact might be possible (Ross and Jackson 1989).

In conclusion, the accumulation of ^{3}H-raclopride in the mouse striatum in vivo was saturable due to specific binding to D-2 receptors. By reducing the synaptic dA concentration by reserpine and γ-butyrolactone the apparent K_D value was decreased, probably due to competition between DA and raclopride for these binding sites. The increase in the apparent B_{max} after DA depletion indicates that raclopride does not compete with DA for all D-2 receptors in the mouse striatum. Whether these changes explain the observation of the enhanced locomotor response by DA agonists after a single dose of reserpine is, however, uncertain.

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