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HYPERPROLACTINAEMIA DOES NOT ALTER SPECIFIC STRIATAL ^{3}H-SPIPERONE BINDING IN THE RAT

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Abstract—We have investigated the effect of single injections (1 mg/animal i.v.) of prolactin or vehicle, and repeated depot (0.125–1.0 mg/animal/day) or bolus (1.0 mg/animal/day) administration of prolactin or vehicle for 6 days to adult male rats. The density (B_{max}) and affinity (K_d) of specific striatal [^{3}H]spiperone binding was not changed by any of the prolactin treatment schedules used. Allogeneic transplants of anterior pituitary glands resulted in an increased concentration of circulating prolactin but did not alter the density or affinity of specific striatal [^{3}H]spiperone binding in male rats, measured 2 weeks following the operation. Prolactin did not displace specific striatal [^{3}H]spiperone binding when incorporated *in vitro*. Any effect of prolactin on striatal dopamine receptor function appears to be exerted only by high, non-physiological concentrations, and such effects are difficult to reproduce.

It has been claimed that hyperprolactinaemia produces changes in striatal dopamine receptor numbers as measured by specific [^{3}H]spiperone binding. In particular, Hruska *et al.* [1] showed that administration of ovine prolactin at doses of 1 mg/animal/day s.c. for 6 days to either normal or hypophysectomised male rats caused an increase in the density of striatal dopamine receptors (B_{max}), as measured by [^{3}H]spiperone binding on the day following the final administration of prolactin. Further, Eva *et al.* [2] showed that in male rabbits a single intravenous dose of ovine prolactin (1 mg/kg) induced an increase in the density of specific striatal [^{3}H]spiperone binding sites measured at 15 min and 2 hr after prolactin administration. Hyperprolactinaemia induced by pituitary homografts also increased the density of specific striatal [^{3}H]spiperone binding sites in intact male or ovariectomised female rats, measured 2 weeks following surgery [3]. However, prolactin does not appear to have a direct action on striatal dopamine D-2 receptors, since prolactin did not displace specific striatal [^{3}H]spiperone binding *in vitro* [1, 2].

Administration of neuroleptic drugs [4] or oestradiol [5] also produces hyperprolactinaemia. The increase in striatal dopamine receptor density due to haloperidol or oestradiol administration has been attributed, at least in part, to the hyperprolactinaemia which is a consequence of these treatments. Thus, in some experiments, hypophysectomy has been shown to prevent the increase in specific striatal [^{3}H]spiperone binding produced by chronic oestradiol [6] or chronic haloperidol [7] administration. However, we and others have been unable to show that the increase in striatal dopamine receptor density produced by repeated neuroleptic treatment is mediated via the pituitary. Thus, the increase in specific striatal [^{3}H]spiperone binding produced by repeated haloperidol treatment was not abolished by prior hypophysectomy in our experiments [8], or in those of Gordon and Diamond [9]. Similarly, Di Paolo *et al.* [3] showed that the increase in striatal dopamine receptor density measured by specific [^{3}H]spiperone binding produced by chronic oestradiol treatment of ovariectomised female rats was also independent of pituitary function.

There appear to be discrepancies between the effects on striatal dopamine receptor function of hyperprolactinaemia induced by administration of prolactin and implantation of pituitary tissue, as compared to the effects observed with neuroleptic drugs. Consequently we have re-examined the effect of hyperprolactinaemia induced by pituitary homografts or by administration of ovine prolactin on the specific binding of [^{3}H]spiperone to rat striatal membranes.

MATERIALS AND METHODS

Animals and drug treatment. Male Wistar rats (195–210 g at the start of treatment, Bantin & Kingman Ltd) were housed in groups of six under standard conditions of temperature (23 ± 2°) and lighting (12 hr light/dark cycle, 06.00 hr–18.00 hr). Free access was allowed to food and water.

Specific [^{3}H]spiperone binding to striatal preparations. For the determination of specific [^{3}H]spiperone binding to striatal tissue preparations, washed membrane preparations were prepared from tissue samples according to the technique of Leysen *et al.* [12] using a final tissue dilution of 600 volumes in 50 mM Tris HCl (pH 7.6) containing 120 mM sodium chloride. The specific binding of [^{3}H]spiperone (17 Ci/mmole; Amersham International, Amersham, U.K.) was determined in triplicate using six concentrations between 0.03 and 1.0 nM. Specific binding was defined by the incorporation of (±)-

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sulpiride (10^{-5} M, Delagrange). In control experiments in these laboratories it has been shown that this concentration of (±)-sulpiride is that which is required to fully displace specific [^{3}H]spiperone binding to rat striatal membranes, over the range of concentrations of [^{3}H]spiperone used in the assay. Samples were incubated for 15 min at 37° and the reaction was terminated by filtration under vacuum (15–20 mm Hg) over Whatman GF/C glass fibre filters, which were then washed twice under vacuum with 2 × 5 ml of ice cold incubation buffer. Filters were then placed in plastic scintillation vials and were solubilised in 5 ml of scintillant (ES299, Packard Ltd, Caversham, U.K.). Samples were counted for tritium for 4 min at an efficiency of 48–49% (Minaxi Tricarb, Packard Ltd). Specific [^{3}H]spiperone binding was determined in membrane preparations obtained from individual animals, using 6–7 animals per treatment group.

The specific binding data from individual animals was analysed using both Scatchard and Eadie–Hofstee transformations, in order to obtain estimates of the number of specific binding sites (B_{max}; pmole/g wet weight of tissue) and the dissociation constant (K_d; nM). These estimates, together with the original data, were then analysed by a computer program which derived values of the parameters of binding by an iterative method, applying these values to the original saturation isotherm, and calculating the fit of the data by calculation of the error mean square value. The final estimates of B_{max} and K_d obtained by this analysis were used to calculate group means and standard errors.

The effect of in vitro *incorporation of ovine prolactin on specific [^{3}H]spiperone binding to striatal preparations.* [^{3}H]Spiperone (0.1 nM) was incubated with striatal membrane preparations, using a final tissue dilution of 600 volumes in 50 mM Tris–HCl (pH 7.6) containing 120 mM sodium chloride. Displacement of [^{3}H]spiperone binding to striatal membranes was determined by the incorporation of ovine prolactin (10^{-10}–10^{-5} M, dissolved in deionised water).

Prolactin was either added at the same time as [^{3}H]spiperone or was preincubated with the striatal membrane preparation for 30 min at 37°, prior to the addition of [^{3}H]spiperone. Incubation of samples with [^{3}H]spiperone, and further processing were as described above.

In order to determine whether prolactin could interact with specific striatal [^{3}H]spiperone binding indirectly, the displacement of binding by dopamine itself and by dopamine antagonists was determined, alone and after preincubation of membrane preparations with ovine prolactin. Thus, [^{3}H]spiperone (0.1 nM) was incubated with striatal membrane preparations, as described above. Displacement of binding was determined by the incorporation of (±)-sulpiride (10^{-10}–10^{-4} M, Delagrange), haloperidol (10^{-11}–10^{-5} M, Janssen Pharmaceutica) and dopamine (10^{-10}–10^{-3} M, Sigma Ltd, London, U.K.) in membrane preparations preincubated with ovine prolactin (10^{-8} M, dissolved in deionised water), or with vehicle, for 30 min at 37° prior to addition of [^{3}H]spiperone.

Inhibition curves for sulpiride, haloperidol and dopamine were determined by a graphical method. Percentage specific binding was plotted versus log concentration of drug. Displacement curves were fitted by eye to the experimental data, and IC_{50} values for each compound were obtained from the displacement curve. IC_{50} values were determined in three separate experiments, and mean and standard error values were calculated.

Drug treatment. Ovine prolactin (Sigma Ltd) was dissolved in 0.9% sodium chloride solution (normal saline) or was dissolved in saline and then suspended in sesame oil (saline : oil 1/10, v/v). Prolactin was administered to rats following three different treatment schedules. In experiment 1, animals received a single intravenous injection of ovine prolactin (1 mg/kg). This treatment was shown by Eva *et al.* [2] to increase the density of specific striatal [^{3}H]spiperone binding in rabbits 2 hr following injection. In our study, 2 hr following administration of prolactin animals were killed by cervical dislocation and decapitation, and the paired corpora striata were dissected out onto ice and the tissue utilised for [^{3}H]spiperone binding analysis as described below.

In experiment 2, animals received ovine prolactin (0.125–1.0 mg/animal/day) as a depot injection in saline/oil suspension, subcutaneously in the neck region, or an equivalent volume of saline/oil suspension (0.1 ml/100 g), daily for 6 days, between 09.00 and 10.00 hr. On the day following the final prolactin treatment, animals were killed by cervical dislocation and decapitation, and the paired corpora striata were dissected onto ice and utilised for [^{3}H]spiperone binding analysis. This experiment used a similar treatment regime to that of Hruska *et al.* [1], who found that a depot dose of ovine prolactin of 1 mg/animal/day for 6–7 days induced an increase in specific striatal [^{3}H]spiperone binding in the rat on the day following the final prolactin treatment.

In experiment 3, the effect of repeated bolus injections of prolactin was studied, in order to compare the effect of this treatment with that of the depot prolactin administration. Animals received ovine prolactin (1.0 mg/animal/day) dissolved in saline, subcutaneously in the neck region, or an equivalent volume of saline (0.1 ml/100 g), daily for 6 days between 09.00 and 10.00 hr. On the day following the final prolactin treatment animals were killed by cervical dislocation and decapitation and the paired corpora striata were dissected out onto ice and utilised for [^{3}H]spiperone binding analysis.

Implantation of pituitary tissue. Male Wistar rats (190–210 g; Bantin & Kingman Ltd, Hull, U.K.) were killed by cervical dislocation and decapitation. The brain was removed from the skull, and the membranes surrounding the pituitary gland were rapidly dissected away. The intact pituitary was removed from the base of the skull and the anterior and posterior lobes were separated. Anterior pituitaries were retained on ice for not more than 5 min.

Recipient male Wistar rats (190–210 g; Bantin & Kingman Ltd) were anaesthetised using etorphine hydrochloride 10 μg/kg i.v. and methotrimeprazine 2 mg/kg i.v. (Immobilon; C-Vet Ltd). Two anterior pituitaries were implanted under the left kidney capsule of each recipient rat [10]. In sham-operated animals a similar amount of abdominal smooth mus-

cle was implanted under the left kidney capsule. Animals were revived using diprenorphine hydrochloride 0.5 mg/kg i.v. (Revivon, C-Vet Ltd). All animals received 50 mg/kg i.p. ampicillin trihydrate (Penbritin, Beecham Ltd). Two weeks following surgery animals were killed by cervical dislocation and decapitation. Trunk blood samples were collected into heparinised tubes and the plasma was separated by centrifugation at 2500 rpm (Sorvall RC3B) for 30 min at 4°. Plasma samples were stored at −20° for subsequent determination of prolactin concentrations. The paired corpora striata were dissected out onto ice and utilised for [^{3}H]spiperone binding analysis.

Prolactin assay. Plasma prolactin concentrations in sham-operated and pituitary-implanted animals were estimated using 10 μl aliquots of plasma. Samples were assayed in triplicate by a homologous double antibody radioimmunoassay [11] using reagents provided by NIADDK (Baltimore, MD). Prolactin concentrations were expressed as ng/ml of rat prolactin standard (NIADDK-rPrl-RP-3). The sensitivity limit of the assay was 5 ng/ml of NIADDK-rPrl-RP-3 and the mean inter-assay coefficient of variation was 14% in plasma pools with low concentrations of prolactin.

RESULTS

The effect of in vitro *incorporation of prolactin on specific [^{3}H]spiperone binding to striatal membranes*

When added together with [^{3}H]spiperone (0.1 nM) or when pre-incubated with striatal membranes for 30 min at 37°, prior to the addition of [^{3}H]spiperone, ovine prolactin did not alter [^{3}H]spiperone binding at concentrations up to 10^{-7} M (Fig. 1a).

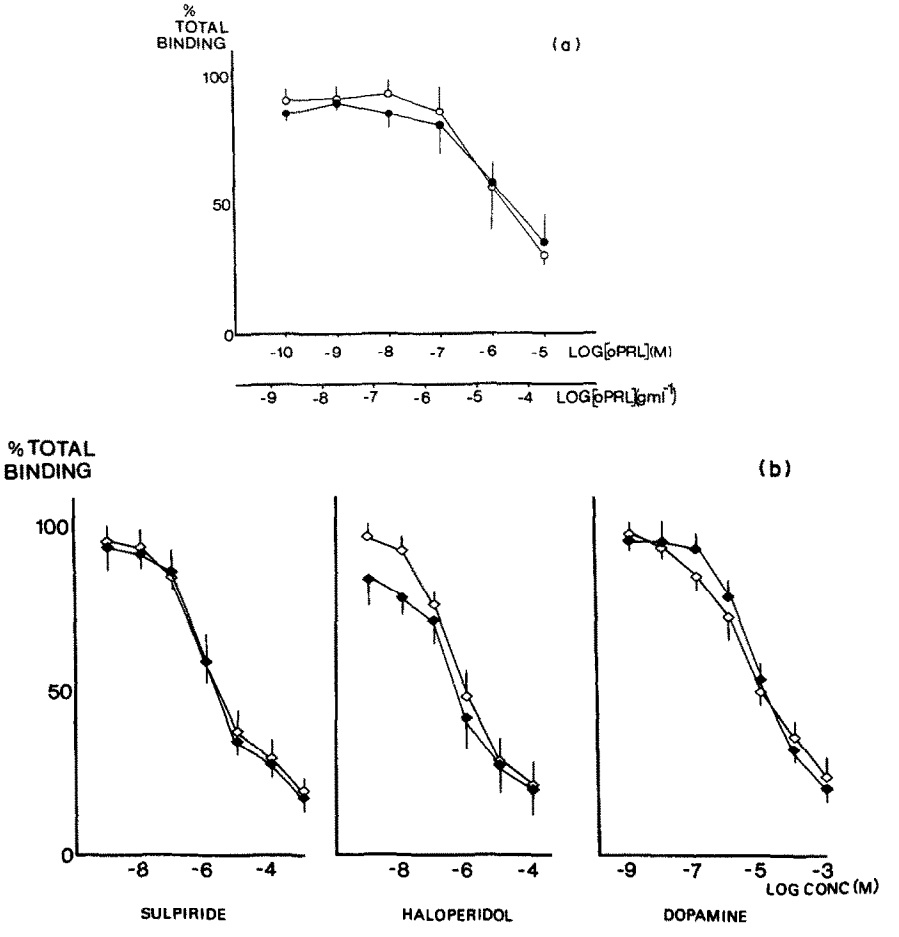

Fig. 1. (a) The effect of *in vitro* incorporation of ovine prolactin upon specific [^{3}H]spiperone binding to male rat striatal membranes. [^{3}H]spiperone (0.1 nM) was incubated with rat striatal membranes for 15 min at 37°. Specific binding was defined by the incorporation of 10^{-5} M (±)-sulpiride. Prolactin was added at the same time as [^{3}H]spiperone (●) or was preincubated with membranes for 30 min at 37° (○). Each point is the mean ± S.E.M. of at least three determinations conducted in triplicate. (b) The effect of ovine prolactin on the displacement of [^{3}H]spiperone binding by *in vitro* incorporation of haloperidol, sulpiride and dopamine. [^{3}H]spiperone (0.1 nM) was incubated with rat striatal membranes for 15 min at 37°. Ovine prolactin (10^{-8} M) (◆) or vehicle (◇) was preincubated with membranes for 30 min at 37°. Each point is the mean ± S.E.M. of at least three determinations conducted in triplicate.

Table 1. The effect of ovine prolactin treatment on specific striatal [^{3}H]spiperone (0.03–1 nM, defined using 10^{-5} M (±)-sulpiride) binding in male rats

Treatment	B_{max} (pmole/g)	K_d (nM)
Acute (single injection)		
Saline	23.7 ± 0.8	0.058 ± 0.009
oPrl 1 mg/kg i.v.	22.9 ± 0.6	0.065 ± 0.009
Repeated (6 days)		
Depot injections (s.c.):		
Saline/oil	23.4 ± 1.6	0.086 ± 0.004
oPrl 0.125 mg/day	20.2 ± 1.4	0.084 ± 0.009
0.25 mg/day	20.7 ± 0.6	0.082 ± 0.018
0.5 mg/day	19.3 ± 3.9	0.074 ± 0.001
1.0 mg/day	21.2 ± 1.2	0.096 ± 0.032
Bolus injections (s.c.):		
Saline	14.2 ± 1.8	0.074 ± 0.010
oPrl 1.0 mg/day	14.9 ± 0.6	0.086 ± 0.010

Values are the mean ± S.E.M. of 6–8 individual determinations. Binding was assessed 2 hr after the single injection of prolactin or 24 hr after the final injection of the repeated treatments. The density of binding (B_{max}) determined in control and prolactin-treated animals in the bolus injection study was lower than that determined in the other experiments. This may be because in this study a different batch of [^{3}H]spiperone was used.

The effect of in vitro *incorporation of prolactin on the displacement of [^{3}H]spiperone binding to striatal membranes by dopamine, sulpiride and haloperidol.*

Ovine prolactin (10^{-8} M) was preincubated with striatal membranes for 30 min at 37°, prior to the addition of [^{3}H]spiperone. This concentration of prolactin was used because it did not itself alter [^{3}H]spiperone binding. The displacement of [^{3}H]spiperone binding produced by the incorporation of sulpiride (10^{-10}–10^{-4} M), haloperidol (10^{-11}–10^{-5} M) and dopamine (10^{-10}–10^{-3} M) was not altered by ovine prolactin (10^{-8} M) (Fig. 1b). The IC_{50} values of each compound in displacing striatal [^{3}H]spiperone binding *in vitro* in the absence and presence of prolactin respectively were: dopamine 1.12×10^{-5} M, 1.58×10^{-5} M; sulpiride 3.16×10^{-7} M, 2.51×10^{-7} M; haloperidol 8.91×10^{-9} M, 5.01×10^{-9} M.

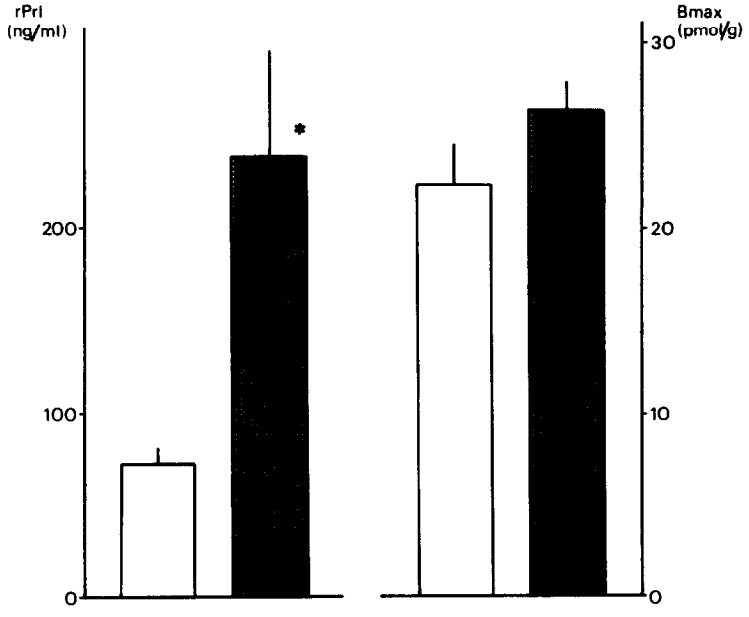

Fig. 2. The effect of implantation of anterior pituitary tissue upon plasma prolactin levels (left columns) and specific [^{3}H]spiperone (0.03–1 nM, defined using 10^{-5} M (±)-sulpiride) binding in male rat striatum (right columns). Values are the mean ± S.E.M. of 5–6 individual determinations. Binding and circulating plasma prolactin levels were assayed two weeks after the implantation of pituitary tissue. Open columns: sham-operated; hatched columns: pituitary-implanted. *$P < 0.05$, Student's *t*-test.

The effect of a single intravenous injection of prolactin on specific [^{3}H]spiperone binding to striatal membranes

Ovine prolactin administered as a single intravenous bolus dose (1.0 mg/kg) did not alter the number (B_{max}) or affinity (K_d) of specific binding sites for [^{3}H]spiperone in male rat striatum (Table 1).

The effect of repeated depot injections of prolactin on specific [^{3}H]spiperone binding to striatal membranes

Repeated administration of ovine prolactin as subcutaneous depot injections (0.125–1.0 mg/animal/day) for 6 days did not alter the number (B_{max}) and affinity (K_d) of specific binding sites for [^{3}H]spiperone in male rat striatum (Table 1).

The effect of repeated bolus doses of prolactin on specific [^{3}H]spiperone binding to striatal membranes

Repeated administration of ovine prolactin as subcutaneous bolus injections (1.0 mg/animal/day) for 6 days did not alter the number (B_{max}) and affinity (K_d) of specific binding sites for [^{3}H]spiperone in male rat striatum (Table 1).

The effect of pituitary implants on specific [^{3}H]spiperone binding to striatal membranes

Implantation of anterior pituitary tissue produced hyperprolactinaemia in recipient rats, as judged by the elevation of circulating prolactin concentrations measured two weeks following implantation (Fig. 2). However, there was no difference in either the value of B_{max} or K_d for specific [^{3}H]spiperone binding to striatal membranes in recipient rats at two weeks following implantation as compared to the values determined in sham-operated animals (Fig. 2).

DISCUSSION

The results of the present study do not support the contention that hyperprolactinaemia in the rat increases the density of striatal dopamine D-2 receptors, as measured by [^{3}H]spiperone binding.

Prolactin is not potent in displacing specific striatal [^{3}H]spiperone binding *in vitro* and has no effect on the ability of dopamine or neuroleptics to displace specific striatal [^{3}H]spiperone binding *in vitro*. These results agree with the findings of Hruska *et al.* [1] and Eva *et al.* [2], who also found that prolactin was unable to displace [^{3}H]spiperone binding *in vitro*. Therefore, prolactin does not appear to interact directly with the striatal dopamine D-2 receptor, nor does it alter the interaction of agonist and antagonist compounds with the receptor.

Our investigation of the *in vivo* effects of prolactin firstly considered the effect of a single intravenous injection of ovine prolactin. This was to reproduce the study of Eva *et al.* [2], where an intravenous dose of 1 mg/kg ovine prolactin increased the density of striatal dopamine receptors in intact female rabbits, as soon as 15 min or 2 hr after a single injection. However, in rats we found 1 mg/kg i.v. prolactin to have no effect on the density or affinity of striatal dopamine receptors in male rats 2 hr after injection.

We then investigated the effect of repeated administration of prolactin in a subcutaneous saline/oil suspension, upon specific striatal [^{3}H]spiperone binding in male rats. We found that this treatment did not alter the density or affinity of [^{3}H]spiperone binding. This result contrasts with that of Hruska *et al.* [1], who showed depot injections of ovine prolactin (1 mg/animal/day for 4–6 days) to cause a 20% increase in the density of striatal dopamine receptors as judged by [^{3}H]spiperone binding.

In the experiment described above, prolactin was administered in a saline/oil suspension. We have also administered prolactin at the highest dose used in the previous experiment, as a repeated daily dose in solution in saline. The previous study was intended to produce a sustained increase in prolactin levels, whilst this treatment was used in order to produce short pulses of very high prolactin concentrations. It may be that the threshold level for the effect of prolactin was not achieved when prolactin was administered in suspension. However, we again found that this treatment had no effect on the density or affinity of striatal [^{3}H]spiperone binding in male rats.

Thus, in our hands, administration of ovine prolactin as an acute or chronic treatment did not alter the density or affinity of striatal [^{3}H]spiperone binding. However, Hruska *et al.* [1] reported that administration of rat prolactin significantly increased the density of striatal dopamine D-2 receptors in male rats, at a dose lower than that of ovine prolactin required to produce the same effect. Thus ovine prolactin may have little intrinsic activity in the rat.

Consequently we have examined the effect of hyperprolactinaemia induced by allogeneic transplants of anterior pituitary. Previously, Di Paolo *et al.* [3] showed that two weeks after the implantation of two adenohypophyses under the kidney capsule of intact male or ovariectomised female rats the density of striatal [^{3}H]spiperone binding was increased by about 30%, whilst circulating prolactin levels in ovariectomised females were increased from 20 to 270 ng/ml. However, in our study, in spite of a 200% increase in plasma prolactin levels produced by the pituitary implants, we found no change in the density or affinity of striatal [^{3}H]spiperone binding two weeks after the operation.

Other evidence also supports the inability of hyperprolactinaemia *per se* to alter striatal dopamine receptor numbers as judged by ligand binding assays. Prolonged hyperprolactinaemia induced by pituitary implants did not alter striatal dopamine receptor density in male rats over a 2-year period [13, 14]. Similarly, hyperprolactinaemia induced by domperidone, a dopamine antagonist drug which poorly penetrates into brain, also failed to alter striatal [^{3}H]spiperone binding in male rats [15] and mice [16].

There are also other reasons for not expecting prolactin to influence striatal dopamine receptor function. Thus, prolactin can increase striatal dopamine turnover and release [17–19]. Such events would not be expected to cause upregulation of dopamine receptor numbers. Also, prolactin does not appear necessary for maintenance of the normal striatal dopamine receptor density in male rats. Thus,

the B_{max} for specific striatal [^{3}H]spiperone binding is not altered by hypophysectomy [6–8].

If prolactin does have an influence upon striatal dopamine function it appears that fluctuations in prolactin concentrations within the normal range do not produce significant changes in dopamine receptor density. Any effect of prolactin must be exerted only by very high, perhaps non-physiological concentrations, and it appears that such effects are difficult to reproduce.

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