Pharmacology Biochemistry & Behavior, Vol. 33, pp. 123–125. Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

A Permanent Dopamine Receptor Up-Regulation in the Ovariectomized Rat

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Received 7 April 1988

GORDON, J. H. AND J. Z. FIELDS. *A permanent dopamine receptor up-regulation in the ovariectomized rat.* PHARMACOL BIOCHEM BEHAV **33**(1) 123–125, 1989.—Although a permanent supersensitivity to dopamine agonists can be induced by lesions of the nigrostriatal dopamine tract, the ovariectomized rat is the first animal model of a permanent hypersensitivity to dopamine agonists in which the neurons survive. This permanent behavioral hypersensitivity to direct acting dopamine agonists is accompanied by an increase in D2 dopamine receptor density in the striatum.

D2 dopamine receptors Dopaminergic hypersensitivity Stereotypy Apomorphine Spiroperidol Ovariectomy Tardive dyskinesia

POSTMENOPAUSAL women have one of the highest incidences of tardive dyskinesia (TD) relative to the general population of psychiatric patients receiving neuroleptic drugs (28). This suggests that loss of ovarian function may predispose individuals to this iatrogenic syndrome. Since an excess of neurotransmission in dopaminergic pathways has been implicated in TD (1, 2, 4, 7, 8, 11, 17–20, 21, 26, 27, 29), we have studied the possible modulating influence of estrogen on nigrostriatal neuropharmacology and neurochemistry in animal (rat) models of TD (8, 13, 27).

In previous reports we have demonstrated that estrogen administration would attenuate neuroleptic-induced dopamine receptor hypersensitivity in rats (8,13). During the course of those studies we observed serendipitously that the neuroleptic-induced dopamine receptor hypersensitivity that developed in ovariectomized (OVX) rats appeared to be a permanent condition, as the neuroleptic-treated OVX animals never returned to pre-OVX levels of behavioral sensitivity. Indeed OVX itself was noted to result in the development of an apparently permanent behavioral hypersensitivity to dopamine receptor agonists (15). The present study was undertaken 1) to document the existence of a permanent behavioral hypersensitivity to apomorphine in the OVX animal, 2) to evaluate its developmental time course and 3) to determine whether changes in D2 dopamine receptor binding correlate with the permanent increase in striatal dopamine receptor sensitivity indicated by the changes in behavioral responses.

METHOD

Female Sprague-Dawley rats (King Labs, Madison, WI) were housed in group cages (6/cage) with free access to food and water in a 12:12 light/dark cycle (lights on at 6 a.m.). Animals at 90 days of age were ovariectomized (OVX) under ether anesthesia.

From 0.25 to 12 months following OVX or sham operation, the incidence of apomorphine-induced (0.25 mg/kg, IP) stereotyped sniffing was determined on individual rats (10,14). Stereotyped sniffing was defined as intense 8–9 Hz sniffing (9) directed towards either the sides or floor of the observation cage. All animals were observed for 10 separate 10-second intervals during the 10–20 min postinjection period, and the presence of stereotyped sniffing recorded. The incidence of stereotyped sniffing was compared statistically across groups using the Chi Square statistic. Animals were sacrificed by decapitation and the brains were rapidly removed and placed on solid CO_2. Frozen brains were then wrapped in aluminum foil and stored frozen until dissection (16) and assay.

The binding of [^{3}H]spiroperidol ([^{3}H]spiro) to striatal membranes was performed as described previously (14). Twelve concentrations of [^{3}H]spiro (range 5–500 pM) were used to label both the high and low affinity binding sites. Tissue samples were homogenized in 100 volumes of phosphate buffer (100 mM, pH 7.4), using a Brinkmann polytron. The homogenate was centrifuged at 40,000 × g for 15 minutes and the supernatant discarded. The pellet was washed twice by resuspending the tissue pellet in 100 volumes of buffer and centrifuging. The final pellet was resuspended in 100 volumes of phosphate buffer and 200 μl (i.e., 2.0 mg original tissue weight) was added to each assay tube. Assay tubes were incubated for 45 min at 37°C in a final volume of 2.0 ml. Nonspecific binding was defined with 1×10^{-6} M (+)-butaclamol. Agonist competition assays were performed as described previously (5). Assay parameters were: 2 mg tissue (original wt.) incubated in 2.3 ml phosphate buffer containing 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA and 90–100 pM [^{3}H]spiro. Unlabeled displacer (dopamine) concentrations ranged from 1×10^{-10} to 1×10^{-4} M.

Binding parameters (B_{max} and K_d) were estimated for [^{3}H]spiro binding to D2 dopamine receptors in striatal membranes using a nonlinear least squares regression analysis program (23) based on the independent site models and assumptions of Feldman (6). The parameter estimates for the agonist affinity states ($D2^{hi}$, $D2^{low}$) of the D2 dopamine receptor (R^{hi}, R^{low}, Ki^{hi}, Ki^{low}) were obtained

TABLE 1

INCIDENCE OF APOMORPHINE-INDUCED STEREOTYPED SNIFFING IN OVARIECTOMIZED RATS

	Months Postovariectomy					
	0.25	1.0	3.0	6.0	9.0	12.0
Sham	18	35	28	29	23	22
OVX	26	37	58*	61*	57*	64*

Effects of apomorphine (0.25 mg/kg) IP on the incidence of stereotyped sniffing in OVX (ovariectomized) and sham-operated rats. The values represent the total number of observations in which stereotyped sniffing was observed. Each experimental group consisted of 10 animals, and 10 observations were scored on each animal during the 10–20 min postinjection period. *Indicates significant difference from sham animals (Chi Square statistic, $p<0.05$).

with a simplex program (3) written for the IBM-PC that utilized the assumptions and equations published by McGonigle *et al.* (24). An F-test (25) was used to reject or accept various binding models for both the saturation and the agonist displacement isotherms.

RESULTS

The incidence of apomorphine-induced stereotypic sniffing for the 12-month period following OVX is shown in Table 1. Clearly, by 3 months post-OVX the animals are hypersensitive to the behavioral effects of apomorphine. Moreover this OVX-induced increase in sensitivity to apomorphine appears permanent as no decrease in sensitivity was noted during the 12 months post-OVX period.

The D2 dopamine receptor density was increased in striatal homogenates from similarly treated, permanently hypersensitive OVX rats (Table 2). The number (i.e., density) of D2 dopamine receptors was increased 35% in the permanently hypersensitive animals. Agonist (dopamine) displacement of [^{3}H]spiro from striatal membranes was biphasic, as expected, suggesting the presence of two agonist affinity states for the D2 dopamine receptor in the striatum (Table 2). The percentage of D2 dopamine receptors in these two distinct agonist affinity states was similar in both the OVX and the sham-operated controls. In addition OVX animals showed no significant changes in the affinity of either state for the agonist (dopamine).

TABLE 2

[^{3}H]SPIROPERIDOL BINDING TO STRIATAL MEMBRANES

	Antagonist Binding Parameters		
	B_{max} fmol/mg tissue	% Increase	K_d (pM)
Sham	13.8 ± 0.6	—	24 ± 4
OVX	18.7 ± 1.2*	35	20 ± 6
	Agonist Binding Parameters		
	Ki^{hi} (nM)	Ki^{low} (μM)	Fraction as R^{hi} (%)
Sham	5.9 ± 4.9	2.4 ± 1.2	30 ± 3
OVX	4.8 ± 1.5	3.9 ± 3.9	32 ± 3

Each vlaue represents the Mean and S.E. of 6 individual animals. OVX (Ovariectomized) and Sham-operated rats were sacrificed and brains frozen between 3–4 months postsurgery. *Significant increase over sham control; *t*-test ($p<0.05$).

DISCUSSION

TD in humans can become irreversible, especially in the elderly and as such presents a difficult problem. Primates will develop "persistent TD like" symptoms with chronic neuroleptic administration (4, 17–20). One of the perplexing problems encountered when studying rodent models of TD has been the inability to produce a "permanent" dopamine receptor hypersensitivity. Without a rodent model, the compensatory neurochemical changes associated with a permanent dopamine receptor hypersensitivity cannot be characterized and/or studied in an economical fashion.

The behavioral results (Table 1) confirm our previous observation that the OVX-induced increase in sensitivity to apomorphine requires 3 months to develop (15). The behavioral data also indicate that, once developed, the increase in apomorphine sensitivity is maintained for a prolonged period of time, perhaps permanently.

The positive correlation between D2 dopamine receptor density and apomorphine-induced stereotypy behavior (8, 12–15, 27), suggests that increases in receptor density and apomorphine-induced stereotypy are associated phenomenon. The [^{3}H]spiro binding to striatal membranes from the OVX and sham-operated rats are consistent with this, as the increased behavioral sensitivity to apomorphine was associated with an increased density of D2 dopamine receptors in the striatum.

Interpretation of the agonist binding to D2 dopamine receptors is more complex since agonist displacement of [^{3}H]spiro was clearly biphasic, which suggests a complex or multisite binding model. The statistical comparison, F-test (25), of binding models supports this assumption as the displacement curve was best predicted by a two site model. A two site agonist displacement curve is thought to be indicative of two agonist affinity states for the D2 dopamine receptors ($D2^{hi}$ and $D2^{low}$). In the current study the proportion or percentage of D2 dopamine receptors in the high affinity agonist state was not changed in the OVX animals; similarly the affinity of the agonist (dopamine) for either of these two states of the D2 receptor was not altered in the OVX animals. Thus, the behavioral hypersensitivity seen in the OVX animals appears to be a result solely of an increase in the total number of D2 dopamine receptors. This is not an unexpected observation as changes in the proportion of $D2^{hi}$ receptors appear to be a short-term or immediate compensatory response to modulate receptor sensitivity (5). Similarly, adjustments or changes in agonist affinity have usually been observed as short-term compensatory mechanisms (22) not as long-term adjustments.

Until our observation that the OVX rat developed a permanent dopamine receptor hypersensitivity (15), the only animal models reported to have a permanent increase in dopamine receptor density involved either neurotoxic or electrolytic lesions of the nigrostriatal dopamine tract. Both 6-hydroxydopamine and electrolytic lesions of the substantia nigra will destroy nigrostriatal dopamine neurons. The resulting loss of the endogenous dopamine and its down-regulating influences on dopamine receptors is then translated, by compensatory mechanisms, into an increase in apomorphine sensitivity and an increase in dopamine receptor density. Although these lesions produce a permanent increase in receptor density and behavioral responses, such models of receptor up-regulation are inappropriate for the study of how an intact nigrostriatal dopamine system would attempt to adjust to, or compensate for, the permanent changes in receptor sensitivity and/or density. The OVX animals appear, for the first time, to have circumvented this problem as no neuronal death is involved

in this permanent up-regulation of the striatal D2 dopamine receptors. This model may prove useful, not only in understanding TD, but also in schizophrenia research since schizophrenia also appears to involve a permanent dopamine receptor up-regulation.

ACKNOWLEDGEMENTS

Supported in part by the Tourette's Syndrome Association, NS26449 V.A. Medical Research, V.A. Hospital, Hines, IL and by Scottish Rite Schizophrenia Research Programs, N.M.J., USA.

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