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The Modulation of Brain Dopamine and $GABA_A$ Receptors by Estradiol: A Clue for CNS Changes Occurring at Menopause

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SUMMARY

1. Tardive dyskinesia is more important in postmenopausal women than men of comparable age and a peak of first episodes of schizophrenia is observed in postmenopausal women. The effect of ovariectomy (2 weeks or 3 months) in rats was investigated as a model of decreased gonadal function associated with menopause.

2. Frontal cortex D1 receptor density and affinity were similar in intact male compared to intact female rats and progressively decreased in density with time after ovariectomy, with no change of affinity. Striatal D1 and D2 receptors also decreased in density after ovariectomy for both receptor subtypes, with no change of affinity. Striatal D1 receptor density and affinity were similar in intact male and female rats, whereas the density of D2 receptors was higher in females. Treatment with estradiol for 2 weeks restored the D2 but not the D1 receptor changes.

3. In the substantia nigra pars reticulata, striatum, nucleus accumbens, and entopeduncular nucleus, a progressive increase in [^{3}H]flunitrazepam specific binding associated with $GABA_A$ receptors was observed as a function of time following ovariectomy; this was corrected with estradiol treatment. In contrast, the opposite was observed for [^{3}H] flunitrazepam binding in the globus pallidus,

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where ovariectomy decreased binding, which was corrected with estradiol replacement therapy.

4. Low prefrontal cortex dopamine activity with implications of D1 receptors in negative symptoms of schizophrenia is hypothesized. Furthermore, GABAergic overactivity in the internal globus pallidus–substantia nigra pars reticulata complex is hypothesized in tardive dyskinesia.

5. The present data suggest that gonadal hormone withdrawal by reducing brain dopamine receptors and producing an imbalance of $GABA_A$ receptors in the output pathways of the striatum may predispose to schizophrenia and dyskinesia.

INTRODUCTION

Gender differences in schizophrenia have been observed repeatedly in clinical and epidemiological studies (McCabe, 1975; Seeman, 1982; Loranger, 1984; Flor-Henry, 1985; Seeman, 1985; Angermeyer and Kühn, 1988; Angermeyer *et al.*, 1989; Häfner *et al.*, 1989; Bardenstein and McGlashan, 1990; Goldstein and Tsuang, 1990; Gureje, 1991; Häfner *et al.*, 1991; Iacono and Beiser, 1992; Nicole *et al.*, 1992; Deister and Marneros, 1993) and these differences remain when the present criteria for schizophrenia are applied (Angermeyer, 1982; Goldstein and Tsuang, 1990). Age at onset is 4 to 7 years earlier in men than women, with a second peak larger and later in women after 40–45 years (Hambrecht *et al.*, 1992). In the long-term follow-up of schizophrenia, women tend more than men to deteriorate more specifically in the perimenopausal period (Childers and Harding, 1990; Opjordsmoen, 1991). After menopause, women seem to require larger doses of neuroleptics and to be more at risk to develop tardive dyskinesia; generally women develop more and more severe forms of dyskinesias (Seeman, 1985; Yassa and Jeste, 1992).

Many levels of hypothesis have been formulated to account for the gender differences in schizophrenia (Lewine, 1985; Pogue-Giele and Zubin, 1988; Dworkin, 1990; Goldstein *et al.*, 1990; Ring *et al.*, 1991). More specifically, the focus here is on clinical changes occurring at menopause with respect to schizophrenia and tardive dyskinesia. The aim of the present study was thus to model gonadal hormone withdrawal occurring at menopause with ovariectomy in animals and investigate its effect on brain neurotransmitters. Dopamine (DA) and γ-amino-*n*-butyric acid (GABA) receptor systems were chosen because of their implications in schizophrenia and tardive dyskinesia. Estradiol replacement therapy was also included in order to investigate the reversibility of the effect of ovariectomy and the steroid implicated.

MATERIALS AND METHODS

Animals and Surgery. Adult female and male Sprague–Dawley rats were purchased from Charles River Canada Inc. (St-Constant, Québec, Canada). These rats weighing 200–250 g were housed two per cage and maintained at 22–23°C

for 3 months on a 14:10 light/dark cycle (lights on from 0500 to 1900). They received rat chow and water ad libitum. Rats were ovariectomized under anesthesia (1.5% halothane–air mixture). Female rats were divided into three groups; in one group, rats were ovariectomized at the beginning of the experiment (ovariectomized, 3 months); in another group, rats were ovariectomized 2 weeks before sacrifice (ovariectomized, 2 weeks); and the third group of rats remained intact and was at random stages of the estrous cycle (control). Half of the ovariectomized rats received estradiol replacement therapy (17β-estradiol, 10 μg b.i.d. SC, for 2 weeks) starting 2 weeks before sacrifice. Rats were killed by decapitation; their brains were rapidly removed, flash-frozen in isopentane over dry ice, individually wrapped in aluminum foil, and kept at −80°C until dissection and assay.

Binding Assays. Striata and frontal cortexes from two rats of each group were dissected, homogenized with a glass–Teflon homogenizer in 100 vol (w/v) of 15 m*M* Tris–HCl, pH 7.4, and centrifuged at 50,000*g* for 15 min at 4°C. Supernatants were discarded, and the pellets resuspended and centrifuged under the same conditions. Supernatants were discarded and the final pellets were resuspended in 100 vol of incubation buffer: 15 m*M* Tris–HCl, pH 7.4, 120 m*M* NaCl, 20 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 0.1 m*M* EDTA, and 0.01% ascorbic acid. To estimate D1 and D2 receptor densities (B_{max}) and affinities (K_d), [^{3}H]SCH23390 (eight concentrations, 0.05 to 0.75 n*M*; 79 Ci/mmol, Amersham) and [^{3}H]spiperone (eight concentrations, 0.025 to 0.05 n*M*; 100 Ci/mmol; Amersham) saturation binding isotherms were performed respectively on appropriate homogenates, as described previously (Di Paolo *et al.*, 1982; Lévesque *et al.*, 1989). In these assays, 200 μl of membranes (100–125 μg of protein) was incubated in a final volume of 2 ml for 60 min at room temperature. Incubation was stopped by rapid filtration (Cell Harvester M-48R, Brandel Co., Gaithersburg, MD) with three rapid 3-ml washes of cold buffer through Whatman GF/C fiber-glass filters. Filters were placed in scintillation counting vials with 10 ml scintillation cocktail (Formula-989, NEN-Dupont). Nonspecific binding was estimated using 1 μ*M* SKF38393 for the D1 or 1 μ*M* (+)-butaclamol for the D2 assay. Ketanserin (50 n*M*) was also added in the D2 assay to block 5-HT_2 binding sites. Radioligand binding was quantified in a LKB beta-counter with 60–65% efficiency. Protein determination was performed by the method of Lowry *et al.* (1951).

$GABA_A$ Benzodiazepine Binding Site Autoradiography. Brains from six animals per group of female rats were immersed in Tissue-Tek (o.c.t. compound, Miles Inc., Elkhart, IN) at −18°C, mounted on cryostat chucks, and cut into 10-μm-thick coronal slices. Four consecutive slices per brain region were thaw-mounted on chromalun/gelatin-coated microscope slides. Slides were vacuum-desiccated at 4°C for 12 hr and stored at −80°C. Autoradiography of the $GABA_A$ benzodiazepine binding site with [^{3}H]flunitrazepam (75 Ci/mmol; Amersham) was performed as described by Canonaco *et al.* (1989) with minor modifications. Slide-mounted brain sections were preincubated for 20 min in incubation buffer: 50 m*M* Tris-HCl, pH 7.4. The slides were then incubated with 10 n*M* [^{3}H]flunitrazepam during 45 min at room temperature. To determine nonspecific binding, 10 μM clonazepam was included in the incubation buffer.

After incubation, slides were placed in racks and washed twice for 2 min in buffer at 4°C. Slides were then dipped in distilled water at 4°C during 10 sec and allowed to air-dry during 12 hr. Sections were apposed to Amersham Hyperfilm-^{3}H with calibrated standards (microscale, Amersham) and exposed during 14 days; they were then revealed in Kodak D-19 developer and fixed in Kodak rapid fixer. Films were analyzed by computer-assisted videodensitometry (RAS 1000, Amersham), binding data being determined from the film optical density.

Statistics. Data were analyzed by analysis of variance (ANOVA) using Staview 4.0 for MacIntosh computer.

RESULTS

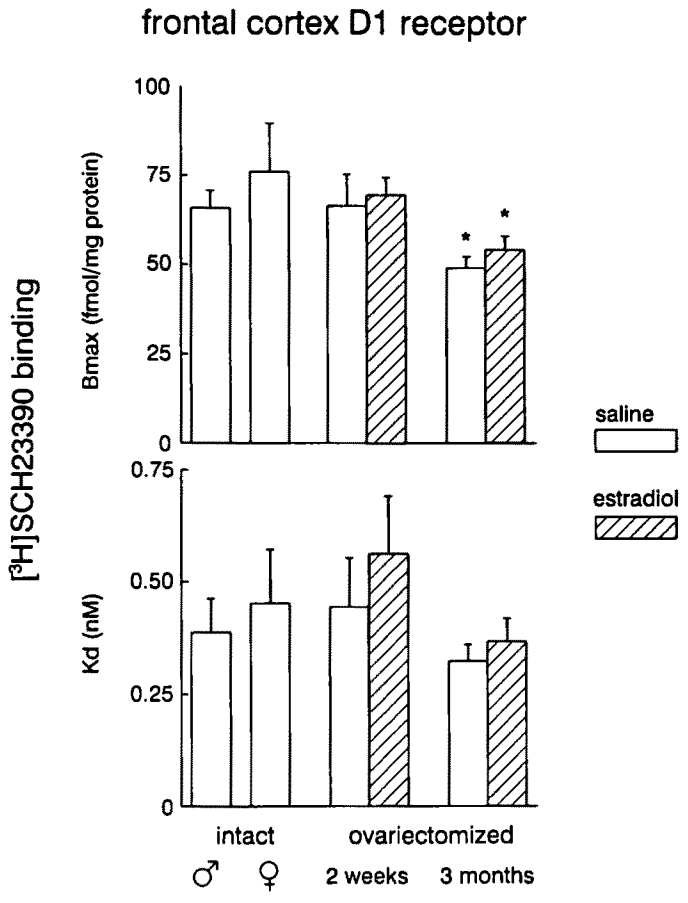
Saturation experiments using [^{3}H]SCH23390 and [^{3}H]spiperone binding to striatal and/or cortical homogenates gave linear Scatchard plots, indicating interaction with a single receptor population (not shown). Ovarian hormone withdrawal after ovariectomy led to a decrease, which was significant after 3 months, in frontal cortex and striatal D1 receptor densities compared to intact female rats (Figs. 1 and 2). Striatal D2 receptor density was lower after 2 weeks or 3 months of ovariectomy compared to intact rats (Fig. 3). Ovariectomy did not significantly change striatal or frontal cortex binding affinity of [^{3}H]SCH23390 for D1 receptors or [^{3}H]spiperone to D2 receptors (Figs. 1–3). No significant difference was observed between intact male and female rats for D1 receptors, whereas a higher density of striatal D2 receptors was observed in females. Frontal cortex or striatal D1 receptor changes following ovariectomy were not corrected by a 2-week estradiol treatment, whereas this steroid treatment corrected striatal D2 receptor changes.

In contrast to DA receptors, autoradiography of $GABA_A$ benzodiazepine binding sites showed, in general, that ovariectomy progressively increased [^{3}H]flunitrazepam binding in the substantia nigra pars reticulata (SNr), striatum, nucleus accumbens (NAc), and entopeduncular nucleus (EP); this was corrected by estradiol treatment (Fig. 4). However, the opposite was observed for the globus pallidus (GP), where [^{3}H]flunitrazepam binding decreased, and this was corrected with estradiol treatment.

No significant change was observed in the total striatum by [^{3}H]flunitrazepam binding; however, analysis of lateral, medial, dorsal, and ventral subregions showed significant effect of ovariectomy concentrated in the dorsolateral striatum. The balance between the two output pathways of the striatum as estimated with the ratio of [^{3}H]flunitrazepam binding in the GP over the EP–SNr complex (Fig. 5) showed a decrease after ovariectomy which was compensated by estradiol treatment.

DISCUSSION

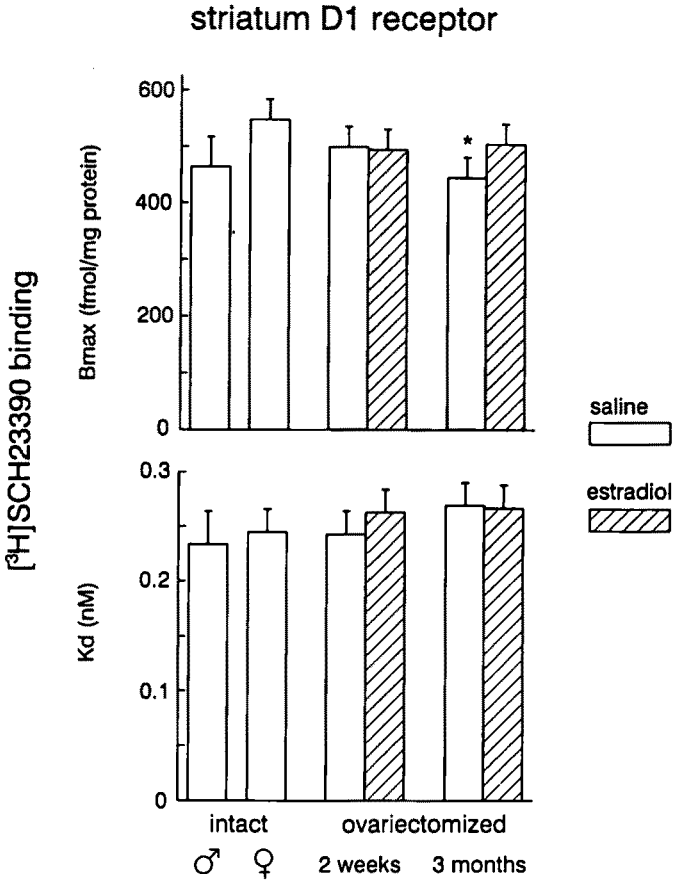
The present data have shown that brain D1 and D2 receptors progressively decreased after ovariectomy, whereas the opposite was generally observed for $GABA_A$ receptors. These changes were generally corrected with estradiol



* $p<0.05$ vs intact ♀

Fig. 1. [³H]SCH23390 binding to D1 receptors in the frontal cortex of intact male and intact female rats as well as ovariectomized rats for 2 weeks or 3 months with or without 17β-estradiol treatment for 2 weeks (10 μg b.i.d.). Results shown are the means ± SE of 14 values from 28 female rats and 7 values from 14 male rats per group.

treatment for $GABA_A$ and D2 receptors but not for D1 receptors. A gender difference was also observed, female rats having a higher striatal D2 receptor density than male rats. This is the first observation that ovariectomy decreases frontal cortex D1 receptors and that this is not corrected by estradiol treatment. This response of D1 receptors in the frontal cortex to gonadal hormone withdrawal and estrogen treatment is similar to the response of this receptor in the striatum. Furthermore, this is in agreement with our previous observation for striatal D1 receptors after 2 weeks or 1 month of ovariectomy and estradiol treatment in rats (Lévesque *et al.*, 1989; Lévesque and Di Paolo, 1990; Di Paolo, 1994). Similarly to D1 receptors, we also observed that striatal D2 receptor

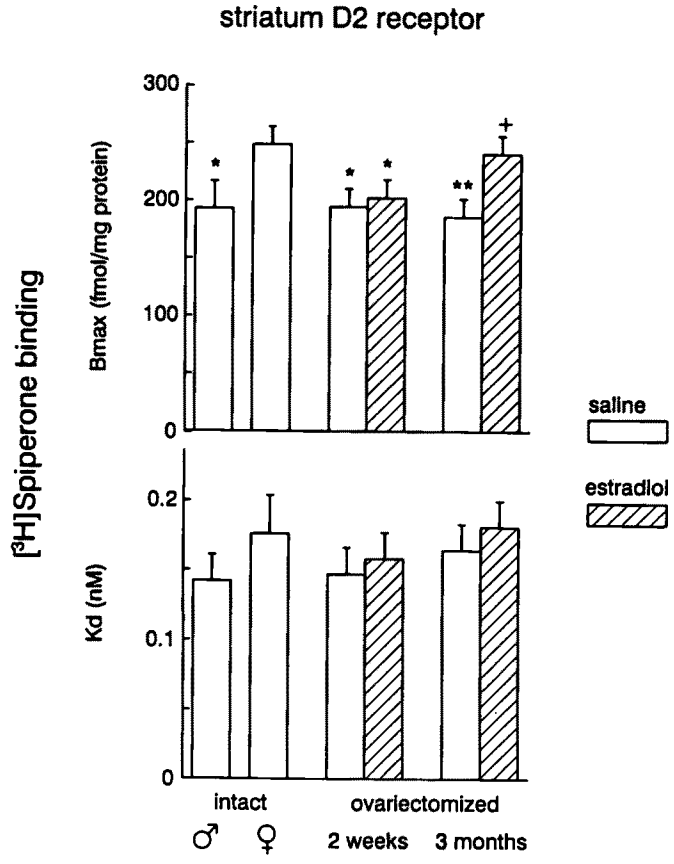


* $p<0.05$ vs intact ♀

Fig. 2. [^{3}H]SCH23390 binding to D1 receptors in the striatum of intact male and intact female rats as well as ovariectomized rats for 2 weeks or 3 months with or without 17β-estradiol treatment for 2 weeks (10 μg b.i.d.). Results shown are the means ± SE of 16 values from 28 female rats and 7 values from 14 male rats per group.

density decreased after ovariectomy, with no change of affinity; however, contrary to D1 receptors, the D2 receptors remain responsive to estradiol treatment. In rats ovariectomized for 3 months behavioral supersensitivity to dopamine agonists is observed (Gordon and Fields, 1989; Fields *et al.*, 1991) and these authors have associated this increased behavior with increased striatal D2 receptors. The present data report the opposite, that is, decreased striatal D1 and D2 receptors after ovariectomy. However, behavioral supersensitivity in animals and, more specifically, dyskinesia are now believed to be more complex than only striatal D2 receptor changes.

The earlier hypothesis to explain the development of tardive dyskinesia is



* p<0.05, ** p<0.01 vs intact ♀ , + p<0.05 vs respective OVX

Fig. 3. [^{3}H]Spiperone binding to D2 receptors in the striatum of intact male and intact female rats as well as ovariectomized rats for 2 weeks or 3 months with or without 17β-estradiol treatment for 2 weeks (10 μg b.i.d.). Results shown are the means ± SE of 16 values from 28 female rats and 7 values from 14 male rats per group.

based on the concept of supersensitivity at the D2 receptor level (review by Gerlach, 1988). This supersensitivity is thought to develop as a compensatory response to the chronic blockade of these receptors by neuroleptics that are D2 receptor blockers. The theory has been supported by various clinical observations, e.g., decreasing or discontinuing the neuroleptic drug aggravates dyskinesia (Crane and Naranjo, 1971), whereas readministration of neuroleptics ameliorates them (Gerlach, 1988). However, this theory proved to be too simplistic and is incongruous with a number of observations made in patients with tardive dyskinesia (Fibiger and Lloyd, 1984; Gerlach, 1988). For example, no increase in the number of D2 receptors studied in the postmortem brains of tardive dyskinesia patients compared to the brains of the nontardive dyskinesia patients

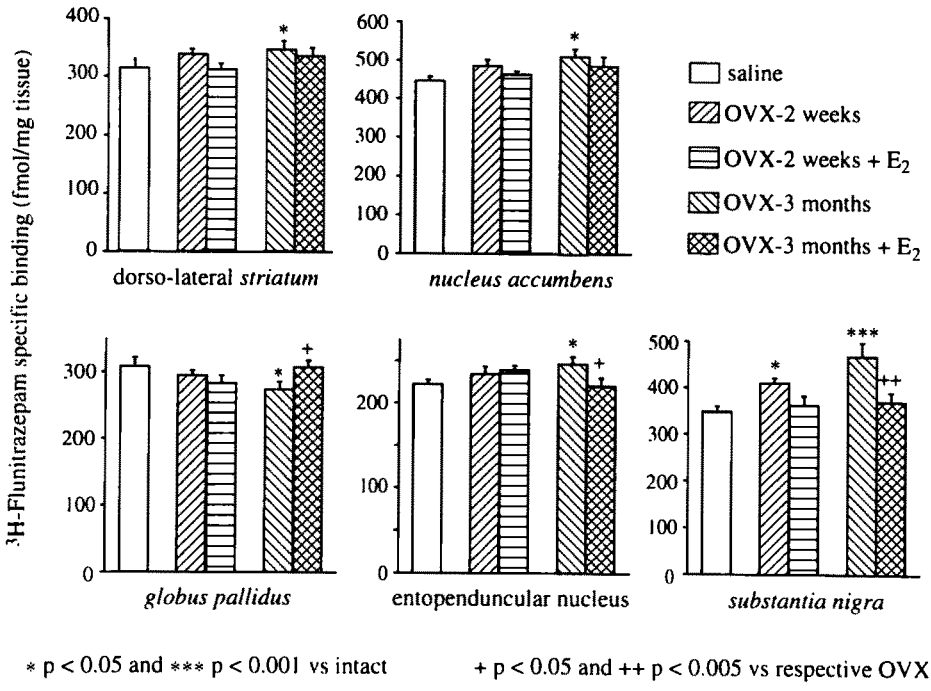


Fig. 4. [^{3}H]Flunitrazepam (10 n*M*) binding to the benzodiazepine site associated with $GABA_A$ receptors in the substantia nigra pars recitulata, dorsolateral striatum, nucleus accumbens, entopeduncular nucleus, and globus pallidus of intact female rats as well as ovariectomized rats for 2 weeks or 3 months with or without 17β-estradiol treatment for 2 weeks (10 μg b.i.d.). Results shown are the means ± SEM from six rats per group.

can be proven. By altering the D1/D2 receptor homeostasis using selective receptor antagonists, several groups have been able to induce dyskinetic syndromes in animals. For example, Rosengarten *et al.* (1983) and Diana and Collu (1990) have shown that stimulation of D1 receptors induces vacuous chewing in rats and dyskinesias in monkeys. Hence, although D2 receptor function is shut down by neuroleptics, D1 receptors will be stimulated by endogenous dopamine. Am imbalance in D1/D2 receptor function in the nigrostriatal system could then be responsible for the induction of tarvide dyskinesia. Indeed, when both SCH23390 (a D1 antagonist) and raclopride (a D2 antagonist) are coadministered for 21 days in rats, no apomorphine-induced stereotypy is observed, suggesting no behavioral supersensitivity (Marin *et al.*, 1993). In schizophrenics, PET scan studies show about 65–89% of D2 receptor and no D1 occupancy with classical neuroleptics, whereas the atypical neuroleptic clozapine binds to both D1 and D2 receptors with a high affinity (Farde *et al.*, 1989). Furthermore, this same group also observes that schizophrenic patients with extrapyramidal side effects have a significantly higher D2 receptor occupancy than those without (Nordstrom *et al.*, 1993). Hence, from the above human and animal studies, it seems that dyskinesia is more likely to occur when D1 and D2

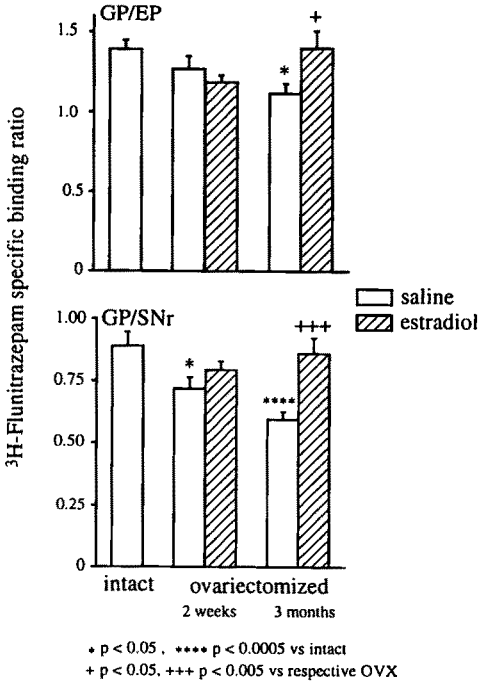


Fig. 5. Effect of ovariectomy on the balance between the two output pathways of the striatum as estimated with the ratio GP/EP and GP/SNr of [^{3}H]flunitrazepam binding. Ratios were computed from data in Fig. 4. Results are the means ± SE of six values from six rats in each group.

receptors are not equally blocked, further supporting the importance of a D1/D2 imbalance in tardive dyskinesia.

Experimental observations have given rise to the concept of two distinct pathways from the striatum to the main output station, the globus pallidus–SNr complex, both using GABA as a neurotransmitter (Penney and Young, 1986; Albin *et al.*, 1989). DA receptors are located principally on the GABAergic striatal medium spiny output neurons, which compose more than 95% of all striatal neurons (Gerfen, 1992). Interestingly, DA receptors appear to be, to a certain degree, segregated in that D1 receptors are localized mostly on striatal neurons projecting to the globus pallidus zona interna Gpi–SNr (the direct pathway), while D2 receptors are more abundant in the indirect pathway (Gerfen *et al.*, 1990; Harrison *et al.*, 1990). The Gpi in human and nonhuman primates is the equivalent of the EP nucleus in rodents. This DA receptor segregation in the basal ganglia has been recently challenged (Surmeier *et al.*, 1993) with evidence that D1 and D2 family receptors are not strictly segregated in the somatodendritic membrane but are indeed segregated in terminal regions. Based on the presently available evidence, one can hypothesize that both the direct and the indirect output systems of the striatum are normally operating in balance and that, after chronic neuroleptic treatment (most of them with predominantly D2 antagonistic activity), the equilibrium is lost. Hence, in tardive dyskinesia, it appears that the D1 response is increased and D2 activity is decreased.

Therefore, the striatal D1/D2 receptor imbalance caused by ovariectomy, which decreases slightly more D2 than D1 receptors, may favor the direct output

pathway from the striatum to the EP–SNr to the detriment of the indirect pathway. This then could influence GABAergic activity in the SNr. Indeed, we have shown that ovariectomy increases [^{3}H]flunitrazepam binding in the EP–SNr, whereas a decrease is observed in the GP. Hence, hormone withdrawal could affect SNr $GABA_A$ receptors directly or indirectly through changes in the striatum. A direct hormonal effect on the $GABA_A$ receptor complex is also possible since the progesterone metabolite 3α-hydroxy-5α-dihydroprogesterone can affect *in vitro* [^{3}H]flunitrazepam binding in the SNr (Canonaco *et al.*, 1989, 1993).

The increase after ovariectomy of SNr $GABA_A$ receptors has not been reported previously. However, it has been observed in the SNr of ovariectomized rats that estradiol treatment decreases [^{3}H]muscimol binding to $GABA_A$ receptors (O'Connor *et al.*, 1988). This is in accordance with our results supporting a tonic inhibitory role of gonadal steroids in the SNr on $GABA_A$ receptors. An increase in $GABA_A$ receptors after ovariectomy which is corrected by estradiol is observed in four of the five brain regions studied. The brain areas investigated were chosen because of their involvement in the control of movement. A similar pattern has been observed where ovariectomy increases and estradiol/progesterone treatment restores to intact rat [^{3}H] muscimol binding density values in other brain areas, namely, the preoptic brain area, the mediobasal hypothalamus, the corticomedial amygdala, and the cerebral cortex (Jüptner *et al.*, 1991). Hence, previous observations and the present data suggest that the effect of ovariectomy and estradiol treatment on $GABA_A$ receptors is not restricted to areas containing cytosolic estradiol receptors. The opposite response of the GP benzodiazepine site to gonadal hormone withdrawal and replacement therapy compared to the other brain areas tested is novel and yet to be explained.

In ovariectomized MPTP monkeys, we recently found (Calon *et al.*, 1994) that [^{3}H]flunitrazepam binding is increased in the GPi in those animals that developed dyskinesia following long-term pulsatile administration of L-DOPA or U-91356A (a D2 agonist). In ovariectomized MPTP monkeys and ovariectomized monkeys bearing a midbrain electrolytic lesion, estradiol can inhibit L-DOPA-induced dyskinesia (Gomez-Mancilla *et al.*, 1993) or prevent haloperidol-induced dyskinesias (Bédard and Boucher, 1986).

Evidence from human and animal studies suggests that all forms of choreic dyskinesia imply a (transient) lowered gabaergic output from the GPi to the thalamus (Albin *et al.*, 1989; Crossman, 1990; DeLong, 1990). The thalamus then innervates the motor cortical regions with increased (glutamatergic) tonus since it is less inhibited, thus inducing a state of hyperkinesia. We propose that in ovariectomized rats (as a model of menopause) the D1/D2 receptor imbalance in the striatum and/or the increase in $GABA_A$ receptors in the EP–SNr complex leads to a decreased GABAergic output to the thalamus, which, is then less inhibited and thus is overactive, sending excessive glutamatergic signals rendering susceptible to vacuous chewing movements (or dyskinesia in humans) (Fig. 6).

To support this hypothesis, rats with vacuous chewing movement induced with haloperidol as a model of dyskinesia have an increase in [^{3}H]flunitrazepam

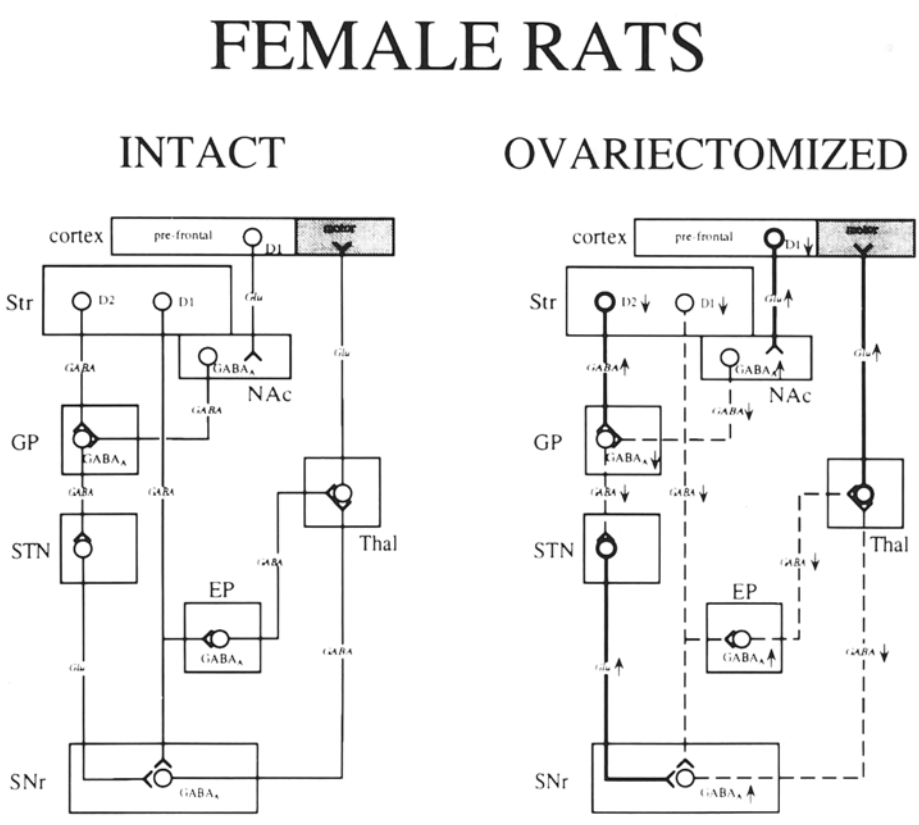


Fig. 6. Schematic representation of basal ganglia neurotransmitters in intact and ovariectomized rat, with an emphasis on the imbalance caused by gonadal hormone withdrawal. EP, entopeduncular nucleus; GABA: γ-amino-*n*-butyric acid; Glu, glutamate; GP, globus pallidus; NAc, nucleus accumbens; NMDA, *N*-methyl-D-aspartic acid; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; STR, striatum; Thal, thalamus; VTA, ventral tegmental area, (· · · · · ·) Decreased activity; (——) normal activity; (——) increased activity.

binding in the SNr (Shirakawa *et al.*, 1993) such as is observed here after long-term ovariectomy.

Low prefrontal cortex dopamine activity in schizophrenia is suggested to cause deficit symptoms (Davis *et al.*, 1991). We observe here a decrease in D1 receptors in the frontal cortex of ovariectomized rats. By analogy to menopause, this decrease in D1 receptors could bring a predisposing contribution in the second peak of incidence of schizophrenia in women at menopause (Hambrecht *et al.*, 1992). In addition, the lowered cortical dopaminergic activity caused by ovariectomy may also promote the glutamate output to the NAc. As glutamate is known to enhance release of dopamine in the NAc, dopaminergic hyperactivity in the NAc may also be observed after ovariectomy and contribute to schizophrenic symptoms.

In summary, ovariectomy, as a model of menopause, decreased brain D1 and D2 receptors as well as $GABA_A$ receptors in the GP, whereas increased $GABA_A$

receptors were observed in the striatum, NAc, EP, and SNr. This brings an imbalance of neurotransmitter systems upon gonadal hormone withdrawal, which may predispose susceptible individuals to schizophrenia and dyskinesia. Hence, ovariectomy would favor the direct output pathway from the striatum to the EP–SNr, in detriment to the indirect pathway through the GP and the subthalamic nucleus toward the SNr. A better understanding of steroid–dopamine and steroid–GABA interactions may help improve dopaminergic drug treatments by taking into account the person's gender and endocrine status.

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