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Estradiol and Dehydroepiandrosterone Potentiate Levodopa-Induced Locomotor Activity in 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine Monkeys

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Six monkeys were rendered hemiparkinsonian with a unilateral injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. These monkeys displayed ipsilateral circling under basal conditions, and after dopaminergic stimulation with levodopa they decreased their ipsilateral circling and started turning to the contralateral side of their lesion. The effect of 17β-estradiol and dehydroepiandrosterone (DHEA) was investigated in these animals. 17β-Estradiol (0.1 mg/kg) added to a threshold dose of levodopa significantly potentiated contralateral circling (mean/30 min) compared to saline or threshold levodopa treatment whereas the duration of circling remained unchanged. DHEA (1–15 mg/kg) alone induced contralateral circling, compared to saline treatment, for 90 min. In addition, DHEA (1–15 mg/kg) potentiated the contralateral circling (mean/30 min) induced by a threshold dose of levodopa and did not change the duration of levodopa circling. A maximal response was observed with 1 or 5 mg/kg of DHEA combined with levodopa depending on the monkey. No correlation was found between the dose for the maximal DHEA response and baseline circling or threshold dose of levodopa. These results suggest that 17β-estradiol or DHEA is able to potentiate locomotor activity of hemiparkinsonian monkeys. The DHEA doses investigated are similar to those presently used in humans. DHEA may be an alternative to 17β-estradiol to modulate dopaminergic activity.

Key Words: Estradiol; dehydroepiandrosterone; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine monkeys; Parkinson disease; levodopa; circling.

Introduction

Estrogens and androgens exert profound effects on brain differentiation, neural plasticity, and central neurotransmission during development *(1,2)*. In adult men and women, accumulating evidence supports a modulatory role of these steroids in the brain *(3)* and their prime importance in the normal maintenance of brain function during aging *(4)*.

Beneficial effects of sex steroids are reported in mental diseases such as schizophrenia, premenstrual syndrome, and postnatal depression, as well as in neurodegenerative diseases such as Alzheimer and Parkinson *(5)*. Parkinson disease is the second most common neurodegenerative disorder mainly characterized by the progressive and selective depletion of dopamine (DA) neurons in the substantia nigra *(6)*. A greater prevalence and incidence of Parkinson disease is described in men than in women *(7–15)*, although no such gender difference is also reported *(16)*. Gender differences on evolution of symptoms and responses to levodopa treatment are reported *(17)*. Symptoms of Parkinson disease and levodopa-induced dyskinesias are shown to be modulated by estrogens *(18–21)*. An antidopaminergic effect of estrogens on parkinsonian symptoms is reported *(20)*. However, a recent study suggests that estrogen replacement therapy might be beneficial to women with early Parkinson disease prior to initiation of levodopa *(22)*. Estrogen is shown to improve motor disability in parkinsonian postmenopausal women with motor fluctuations *(23)* whereas a slight antiparkinsonian effect *(24)* or no effect *(25)* is also reported. Hence, in humans, the issue of whether estrogens stimulate or, rather, inhibit the dopaminergic system is still open. Animal studies also show pro- and antidopaminergic activities of estrogens *(18)*. This could hypothetically be dissociated using other estrogenic compounds.

In the search for estrogenic compounds specific for the brain with prodopaminergic activity, we investigated precursors of estradiol. Hence, dehydroepiandrosterone (DHEA) was chosen because it is a precursor of both estradiol and testosterone. DHEA and the sulfate derivative of DHEA, DHEA-S, are also termed *neurosteroids* because they have been shown to be synthesized in the brain *(26)*. No significant

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differences in cerebrospinal fluid DHEA and DHEA-S are found between patients with Parkinson disease and age-matched control subjects *(27)*. Plasma DHEA and DHEA-S decrease extensively during normal aging in both men and women *(28,29)*. A 1-yr clinical study with DHEA in normal aged humans has shown no important stimulation of the gonads *(30)*. In light of the Women's Health Initiative trial on the risk of estradiol with progesterone replacement therapy *(31,32)*, DHEA is an interesting alternative.

The modulation of dopaminergic neurotransmission in the basal ganglia by estrogens is now well established *(18, 33,34)*. DA synthesis and release, DA receptors, DA uptake sites, as well as catechol-*o*-methyltransferase expression have been shown to be regulated by ovarian steroids *(18,35)*. Studies in animal models and cell cultures also support a neuroprotective role of estrogens on dopaminergic neurons *(5,36)*. Although less numerous than for estrogens, DHEA is reported to modulate dopaminergic activity. Hence, DHEA is reported to stimulate dopamine release *(37)* and was shown to protect against the dopamine depletion caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) *(38)*.

We hypothesize that DHEA such as 17β-estradiol can potentiate striatal dopaminergic activity. This was tested functionally in hemiparkinsonian MPTP monkeys with DHEA alone as well as with DHEA and 17β-estradiol combined with a threshold dose of levodopa (T.L-Dopa).

Results

The hemiparkinsonian MPTP monkeys under basal conditions or after saline treatment displayed ipsilateral circling behavior. After dopaminergic stimulation with DHEA alone, DHEA combined with T.L-Dopa, or 17β-estradiol combined with T.L-Dopa, they decreased their ipsilateral circling and started turning to the contralateral side relative to the lesion.

17β-Estradiol (0.1 mg/kg) was added to the T.L-Dopa, as shown in Fig. 1. Estradiol significantly potentiated contralateral circling (mean/30 min) compared to saline or T.L-Dopa treatment whereas the duration of circling remained unchanged (data not shown).

DHEA alone investigated at different doses was also able to induce contralateral circling, as shown in Fig. 2. DHEA (1–15 mg/kg) significantly and similarly increased contralateral circling (mean/30 min) compared to saline treatment. The DHEA effect lasted 90 min. The effect of DHEA at different doses with T.L-Dopa is shown in Fig. 3. The smallest dose of DHEA (1 mg/kg) added to T.L-Dopa significantly increased contralateral circling (mean/30 min) compared to saline or T.L-Dopa treatment alone. T.L-Dopa combined with higher doses of DHEA (5, 10, and 15 mg/kg) significantly increased contralateral circling (mean/30 min) compared to saline treatment. DHEA treatment did not change the duration of levodopa circling (data not shown). Higher doses of DHEA (25–100 mg/kg) alone or in combination with T.L-Dopa gave similar results (data not shown).

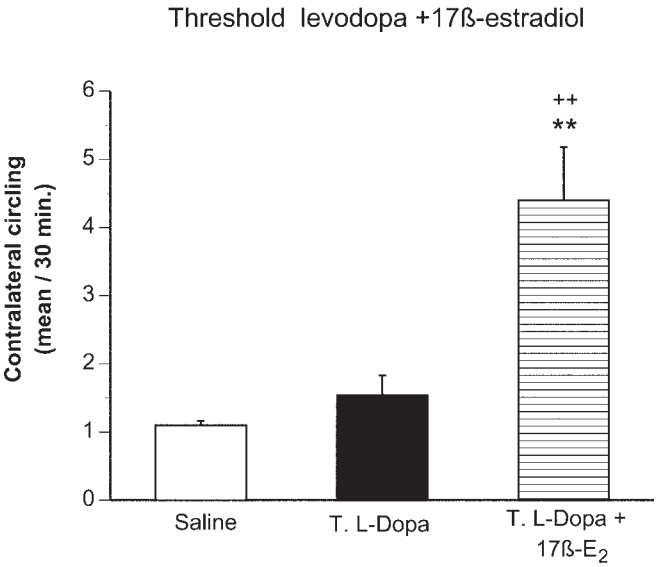

Fig. 1. Effect of 17β-estradiol (17β-E_2) (0.1 mg/kg) in combination with a threshold dose of levodopa (T.L-Dopa) compared to T.L-Dopa alone or saline treatment in hemiparkinsonian MPTP monkeys on contralateral circling behavior. Results shown are the mean circling ± SEM of six monkeys measured two to four times each. $^{**}p < 0.01$ vs saline-treated and $^{++}p < 0.01$ vs T.L-Dopa-treated MPTP monkeys.

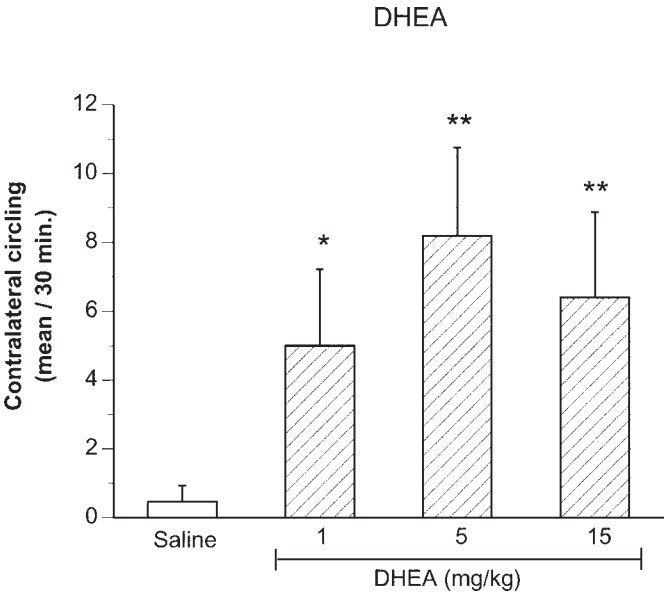

Fig. 2. Effect of DHEA (1, 5, and 15 mg/kg) compared to saline treatment in hemiparkinsonian MPTP monkeys on contralateral circling behavior. Results shown are the mean circling ± SEM of six monkeys measured two to four times each. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs saline-treated MPTP monkeys.

DHEA alone or combined with levodopa did not produce a significant dose response on circling behavior. A maximal response was obtained with different doses in the monkeys tested for DHEA alone or combined with levodopa. Figure 4 shows the mean of the optimal dose of DHEA for each monkey for ipsilateral, contralateral, and their dif-

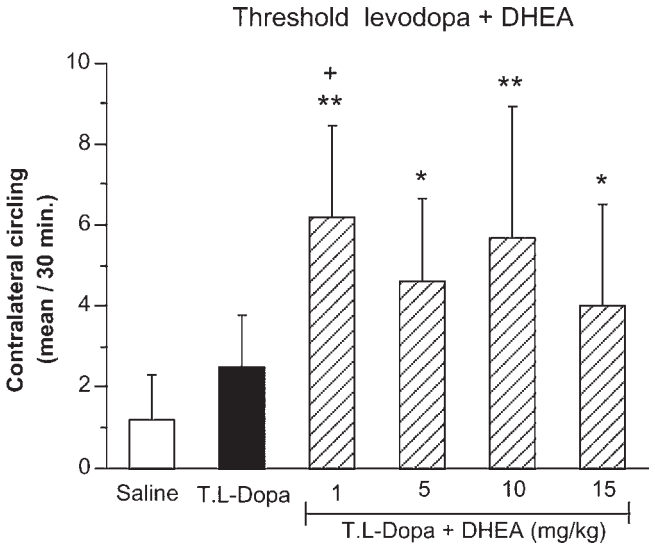

Fig. 3. Effect of DHEA (1, 5, 10, and 15 mg/kg) in combination with a threshold dose of levodopa (T.L-Dopa) compared to T.L-Dopa alone or saline treatment in hemiparkinsonian MPTP monkeys on contralateral circling behavior. Results shown are the mean circling ± SEM of six monkeys measured two to four times each. $*p < 0.05$ and $**p < 0.01$ vs saline-treated and $^{+}p < 0.05$ vs T.L-Dopa-treated MPTP monkeys.

ference (ipsilateral-contralateral). Levodopa increased contralateral and decreased ipsilateral circling, thus reducing overall ipsilateral-contralateral circling. The optimal dose of DHEA combined with DHEA significantly potentiated this effect. A maximal response was observed with 1 or 5 mg/kg of DHEA combined with levodopa depending on the monkey. No correlation was found between the dose for the maximal response and baseline circling or threshold dose of levodopa (data not shown).

Discussion

The present study showed that 17β-estradiol potentiates levodopa-induced locomotor activity in MPTP monkeys. Hence, in this animal model of Parkinson disease, estradiol has prodopaminergic activity. This is in agreement with a clinical study in postmenopausal women with mild to moderate Parkinson disease showing that after 10 d of treatment with transdermal 17β-estradiol, there was a significant reduction in the threshold dose of iv levodopa *(39)*. Furthermore, dyskinesia scores were unaltered during 17β-estradiol treatment compared to placebo *(39)*.

DHEA was next investigated in order to seek the estrogenic activity of other steroids in this model. DHEA also significantly potentiated levodopa-induced locomotor activity and stimulated circling alone. DHEA motor activity in MPTP monkeys could be associated with its binding to an allosteric site on $GABA_A$ receptors. Indeed, DHEA-S behaves as a negative modulator of the $GABA_A$ receptor complex

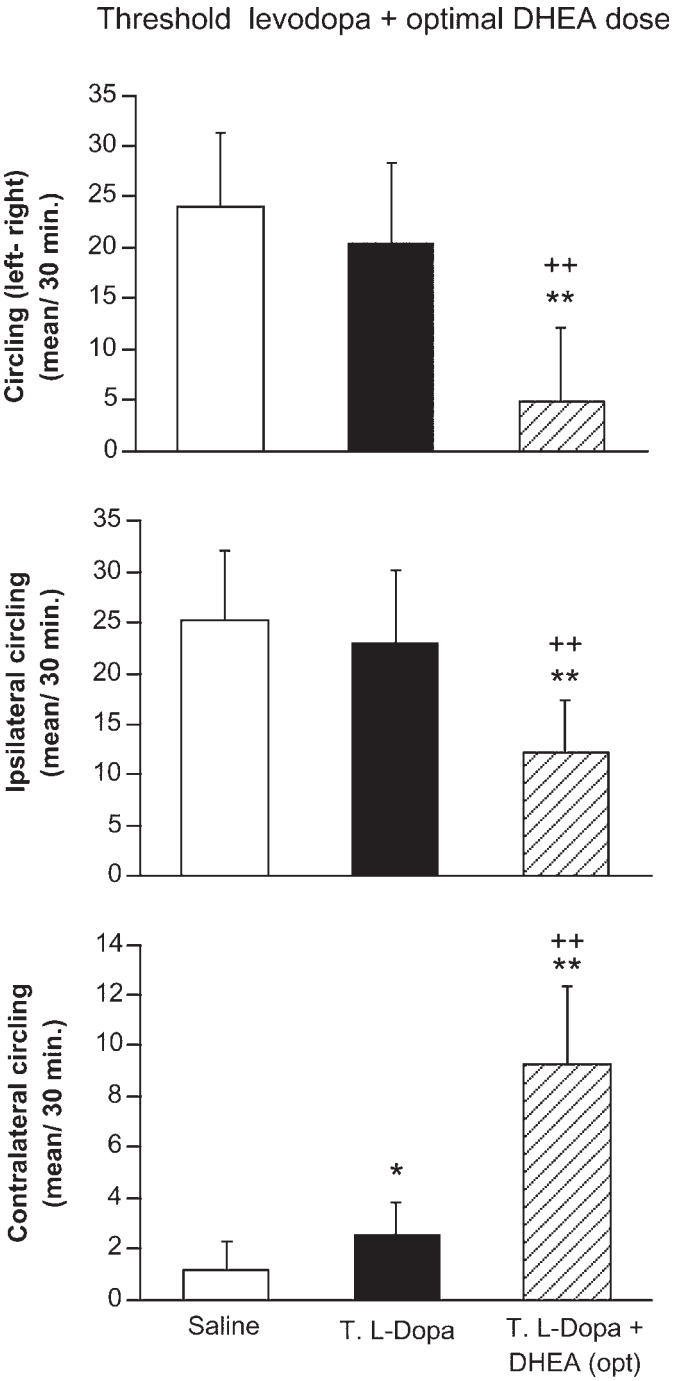

Fig. 4. Effect of DHEA (optimal dose: 1 or 5 mg/kg) in combination with a threshold dose of levodopa (T.L-Dopa) compared to T.L-Dopa alone or saline treatment in hemiparkinsonian MPTP monkeys on ipsilateral and contralateral circling as well as their difference (ipsilateral-contralateral) in behavior. Results shown are the mean circling ± SEM of six monkeys measured two to four times each. $*p < 0.05$ and $**p < 0.01$ vs saline-treated and $^{++}p < 0.01$ vs T.L-Dopa-treated MPTP monkeys.

(40). Inhibition by DHEA-S of $GABA_A$ receptor function could result in increased neuronal excitability *(41)*. $GABA_A$ receptors are present in the basal ganglia in the striatum as

well as in its direct and indirect output pathways; they have been shown to be functionally implicated in motor behavior *(42)*. However, the present results with DHEA alone or in combination with levodopa do not show a clear dose-response effect. Hence, the DHEA locomotor–activating activity cannot be simply linked to its binding to $GABA_A$ receptors. Nevertheless, this possibility cannot be ruled out and will require a detailed evaluation of these receptors throughout the basal ganglia to assess their contribution to behavior.

Because DHEA is a precursor of 17β-estradiol, and has shown in the present paradigm similar functional activity, it could be acting as a prodrug. Indeed, enzymes involved in the transformation of DHEA to 17β-estradiol are present in the brain *(26)*. Hence, DHEA could be used as a prodrug for estradiol and could bring more estrogenic activity for the brain compared to peripheral tissues. In this respect, a clinical study by Baulieu et al. *(30)* in healthy elderly (69–79 yr old) men and women showed that DHEA (50 mg/d) for 1 yr did not lead to a harmful accumulation of DHEA-S and active steroids. DHEA did not give important gonadal stimulation. Therefore, DHEA could act more as an estrogen-like compound in the brain compared to its activity in peripheral tissue. By analogy to selective estrogen receptor modulators such as raloxifene, DHEA could have more selective estrogenic activity for the brain through a different extent of metabolization into estradiol in various tissues. Indeed, in humans DHEA and DHEA-S are weakly transformed in the periphery following administration *(30)*. The doses of DHEA reported in the present paradigm as having locomotor-activating activity are in the range used in humans. In fact, the effective doses reported here for monkeys (1 and 5 mg/kg, thus 2.8–32.5 mg/[monkey·d] depending on the weight of the monkey) as well as the maximal dose tested (100 mg/kg, thus maximal of 280–650 mg/[monkey·d] depending on the weight of the monkey) are well in the range used safely in humans for 1 yr (50 mg/d *[30]*) or a month to a year (maximal of 1600 mg/d *[43,44]*). DHEA administered for 4 wk to 6 mo (30–90 mg/d) was associated with a significantly greater decrease in Hamilton depression scale rating than placebo *(45,46)*. In elderly men, Wolf et al. *(47)* reported that DHEA (50 mg/d for 2 wk) improved electrophysiologic indices of central nervous system stimulus processing, but these effects did not appear to be strong enough to improve memory or mood.

In conclusion, DHEA such as 17β-estradiol potentiates levodopa-induced locomotor activity in an MPTP monkey model of Parkinson disease. The effective doses of DHEA are in the range used safely in humans. The present paradigm used minimal threshold doses of levodopa. Hence, adding DHEA to levodopa therapy could enable reduction of the dose of levodopa and hence protect against the side effects associated with this drug, which are dose related. In future experiments, investigation of the effect of chronic DHEA is required to seek its beneficial effect in the long term.

Materials and Methods

Animals and Treatment

Six hemiparkinsonian ovariectomized female *Macaca fasicularis* monkeys weighing 2.8–6.5 kg were used. During induction of the parkinsonian syndrome, they received 3 mg of MPTP via an Alzet pump during a period of 1 to 2 wk depending on the monkey. The procedure consisted of inserting the pump to deliver MPTP in the left substantia nigra. MPTP lesioned the dopaminergic cells. The animals were used at least 5 mo after the induction of their hemiparkinsonian state, at which time they had stabilized.

These monkeys turn toward the ipsilateral side of the lesion (to the left) under basal conditions, and on dopaminergic stimulation this circling changes to the contralateral side (to the right). They all received the same treatments and were their own controls. Monkeys were first treated with levodopa for priming until a stable antiparkinsonian behavior was observed. During the investigation of DHEA administered alone, priming of the monkeys was continued with administration of levodopa/benserazide (50/12.5 mg) twice weekly. DHEA treatments were started on the third day after the last dose of levodopa. When treatments involved a combination of levodopa with steroids (DHEA or estradiol), the monkeys did not receive additional levodopa priming.

DHEA was administered at different doses (1, 5, and 15 mg/kg) by nasogastric gavage alone and in combination (DHEA: 1, 5, 10, and 15 mg/kg by nasogastric gavage) with sc T.L-Dopa. Threshold doses of levodopa (i.e., the minimal dose to obtain contralateral circling) were sought for each of the MPTP monkeys and varied from one monkey to another from 3 to 8 mg/kg (3, 4, 4.5, 5, 5, and 8 mg/kg). Higher doses of DHEA (25–100 mg/kg) alone or with T.L-Dopa were also tested in a similar paradigm. For comparison, combinations of sc T.L-Dopa (the same doses) with 0.1 mg/kg of 17β-estradiol were investigated.

Treatments were administered in the morning and each animal was recorded via a videocamera system. Duration of locomotor activation lasted for 90 min for all animals and treatments. Hence, behavior was measured for 90 min with left and right circling quantified for 30-min periods. Each treatment was repeated two to three times and averaged for each monkey. Saline and T.L-Dopa treatments were performed in the beginning and the end of each different hormonal treatment and were found not significantly different. Hence, these values were grouped.

Statistical Analyses

Experimental data were compared using an analysis of variance for repeated measures followed by post-hoc pairwise comparisons with the Fisher probability of least significant difference test. A value of $p < 0.05$ was required for the results to be considered statistically significant. Statistical comparisons of square root transformation of the data was used to stabilize variance when required *(48)*.

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