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L-DOPA Reverses Castration-Induced Disruption of Dishabituation Responses to Female Chemical Cues in Male Rats

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GUAN, X.-B. AND D. E. DLUZEN. *L-DOPA Reverses castration-induced disruption of dishabituation responses to female chemical cues in male rats.* PHARMACOL BIOCHEM BEHAV 48(2) 515–519, 1994.—In the present experiment, habituation/dishabituation behavioral tests were conducted to measure discriminatory olfactory recognition responses to chemical cues among control, castrated, and castrated + L-3,4-dihydroxyphenylalanine (L-DOPA)-treated male rats. Castration produced a disruption of dishabituation responses to female urine, and this effect was reversed by treatment with L-DOPA. In the posterior olfactory bulb, 3,4-dihydroxyphenlacetic acid (DOPAC) levels were significantly increased in L-DOPA-treated animals compared with the vehicle-treated control and castrated groups. No significant differences in olfactory bulb norepinephrine or dopamine concentrations among the three treatment groups were obtained. The restoration of behavioral dishabituation responses following L-DOPA treatment suggests that the catecholaminergic system of the olfactory bulb may play a critical role in the recognition and possibly attractions for or preferences to female chemical cues.

Olfactory bulb Odorant recognition Norepinephrine L-DOPA Dopamine DOPAC Castration Rats

IT was demonstrated over 40 years ago that the male rat shows significant preferences for estrous versus diestrous female rats (20). That these preferences are primarily mediated by the olfactory system are indicated by experiments in which male rats show similar preferences to estrous female urine (21,27) and the abolition of preferences following removal of the olfactory bulb (OB) (11). Preferences for estrous females can similarly be abolished by castration of the male rat (3), and it has been reported that bulbectomy reduces plasma testosterone levels (26), suggesting an important relationship between gonadal steroids and OB function as related to these preferences associated with reproduction.

The OB receives a rich supply of noradrenergic input from the locus coeruleus (25,29), and it appears that this OB norepinephrine (NE) may also be involved with recognition of and preference/attraction responses related to reproduction. This follows from data demonstrating that depletion of OB-NE reduces preferences of male rats for chemical cues of estrous females (4) and that changes in OB-NE content and release are observed in response to social and chemical cues related to reproduction (6–9). We have recently reported that treatment of male rats with the noradrenergic neurotoxin DSP-4 results in a selective reduction in behavioral dishabituation responses for female chemical cues, an effect which is associated with 50% reductions in OB-NE (16), and it has been shown that dishabituation responses of male rats to intact and castrated male urine are lower than those to estrous and diestrous urine (2), suggesting an important association between recognition and attraction/preference responses. A final piece of evidence linking OB-NE, preferences, and gonadal steroids are the data which show that castration results in reductions of OB-NE concentrations (4) and release (17).

If the abolition of olfactory recognition and preferences or attractions to female chemical cues following castration is related to the reduction in OB-NE, then a restoration of OB catecholamines should bypass these effects of castration. In the present experiment we addressed the issue of restoration of function in this system. Specifically, we attempted to restore OB catecholamines in castrated males by administering the catecholamine precursor L-3,4-dihydroxyphenylalanine (L-

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DOPA). L-DOPA is converted to dopamine (DA) by L-aromatic amino acid decarboxylase and can be further converted to NE by dopamine-β-hydroxylase depending upon the processing enzyme in different tissues. Therefore, in this experiment a group of castrated male rats with L-DOPA treatment was compared with vehicle-treated castrated and intact control males to examine the effects of L-DOPA treatment on behavioral habituation/dishabituation responses to chemical cues (urine). To evaluate the neurochemical effects of these treatments upon the OB catecholaminergic system, concentrations of NE, DA, and DOPAC were measured in the anterior and posterior OB immediately following behavioral tests.

METHODS

Animals and L-DOPA Treatment

Twenty-one adult male Sprague–Dawley rats (250–300 g) were used in the experiment and divided into three groups of seven animals in each group (control, castration, and castration + L-DOPA). Animals were caged individually and maintained under a 12-h light–dark cycle (lights on 0600) in a temperature-controlled room with food and water ad lib. The castration was performed 20 days prior to the experiment using a midline scrotal approach while the male rats were under ketamine anesthesia (ketamine–acepromazine solution, 10 : 1, at the dose of 100 mg/kg). Animals were treated with L-DOPA (castration + L-DOPA group) or vehicle (saline, control, and castration group). L-DOPA methyl ester (Sigma Chemical Co., St. Louis) was dissolved in saline at a concentration of 500 mg/ml and was administered IP at 50 mg/kg body weight. Identical amounts of saline according to body weight were given to the intact control and vehicle-treated castrated animals. The behavioral test described below was conducted 45 min after the administration of L-DOPA or vehicle. The behavioral test took 55 min, and animals were sacrificed immediately after completion of behavioral testing. Since it took 8 min to sacrifice each animal and remove the OB areas and up to three L-DOPA-treated animals were included on each day of behavioral testing, the time period between the administration of L-DOPA and decapitation was 100–116 min.

Odor Discrimination Tests

All animals were subjected to an odor habituation–dishabituation test which was described previously (16). Briefly, a cotton-tip applicator with 40 μl of urine was placed approximately 1–1.5 cm lateral to the nostril of the rat. The amount of time the rat spent investigating each odor presentation in a trial was recorded using a stopwatch. The amount of investigation directed to the urine-treated cotton-tip applicator was recorded until the animal withdrew for a 2-s interval, at which time the trial was terminated. Each animal was tested in its home cage with urine from three different sources. Three trials with urine from Sprague–Dawley males was followed by three trials using urine from Zucker males, and then three trials using urine from Zucker females. Therefore, there were a total of nine odor presentations for each subject, with an intertrial interval of 6 min.

Urine Collection

Urine was collected from a separate group of animals (Sprague–Dawley males, Zucker males and females) maintained under laboratory conditions identical to those described for the experimental animals. Individual rats were placed in a clean plastic cage and urine was collected into a plastic tube using a pipet. Urine obtained within each of the three stimulus conditions was pooled and frozen (−20°C) in aliquots of 50 μl. Urine collected from female rats was pooled across the estrous cycle to diminish the possible effect of any change in urine composition across the estrous cycle. It should be noted that urine collected on estrous may not be superior to urine from other phases of the cycle in its abilities to elicit habituation–dishabituation (2) or preference responses (12). On the day of an experiment the urine was thawed to room temperature and a fresh 40-μl aliquot of urine was used for each trial.

Brain Tissue Collection

After the behavioral tests were completed, animals were decapitated. Brains were quickly removed and anterior and posterior OBs were dissected from each animal. These two areas were separated to determine whether a localized change in catecholamine activity would be obtained, as we have observed for OB-NE release (17). The tissue samples were placed in 500 μl of cold (4°C) 0.1-N perchloric acid, sonicated, and centrifuged, and an aliquot was removed for catecholamine determination.

Catecholamine Assay

Catecholamine determinations were accomplished by using high-performance liquid chromatography with electrochemical detection (HPLC-EC) as described previously (14). Briefly, a model 420 HPLC pump, analytical cell 5011, EC detector 5100A (ESA, INC., Bedford, MA), and integrator SP 4270 (Spectra-Physics, San Jose, CA) with A 100 × 4.6-mm, 5-μ C-18 reverse phase column (Biophase ODS, BAS Inc., West Lafayette, IN) was used for this assay. The mobile phase consisted of 5.75 g citric acid, 4.1 g sodium acetate, 20 mg sodium octyl sulfate, and 35 mg ethylenediaminetetraacetic acid (EDTA) in 1.0 l of deionized water with a pH adjusted to 4.5 by sodium hydroxide. The buffer was filtered through a millipore membrane (0.45 μm), degassed, and mixed with methanol (7% methanol). NE, DOPAC, and DA (Sigma) standards were stored in 0.1-N $HClO_4$ (2 μg/ml). Five doses (31.25, 62.5, 125, 250, and 500 pg/20 μl) were used to build up a standard curve.

Statistical Analysis

For the behavioral data, a 3 × 9 two-way ANOVA with one repeated measurement (Treatment × Trials) was used to test the significance of investigation times in the habituation/dishabituation tests. In addition, paired t tests were used to evaluate differences within a treatment condition between some selected trials. Separate one-way ANOVAs were used to test differences for each of the neurochemicals among the three groups. A Fisher's least significant difference test was used for post hoc analysis to delineate pairwise differences. A $p < .05$ was required for the results to be considered statistically significant.

RESULTS

Behavioral Test

The behavioral data for the chemical cue tests are presented in Fig. 1. Statistical analysis of these data revealed that the trial variable, $F(8, 144) = 91.58$, $p < 0.01$, and the interaction variable, $F(16, 136) = 2.00$, $p < 0.05$, were significantly

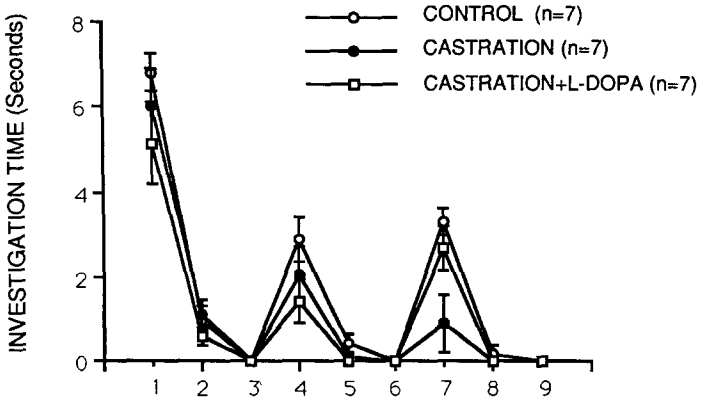

FIG. 1. Investigation time in seconds (mean ± SEM) to urine odors. Intact, castrated, and castrated + L-DOPA-treated male Sprague-Dawley (SD) rats were given three trials (1-3) with male SD urine, followed by three trials (4-6) with male Zucker (Z) urine, followed by three trials (7-9) with female Z urine. Castration altered the dishabituation responses to female Z urine, and this effect was reversed by the treatment of L-DOPA.

different. In general, all groups showed clear habituation responses with repeated exposures to the same stimulus. A salient difference in the performance of the castration group was obtained on trial 7. In the castration group, no significant dishabituation response was observed to Zucker female urine (trial 7). The statistical basis for this statement is that no significant differences in investigation scores were obtained between trials 6 and 7 in the castration group, $t(6) = 1.31$, $p > .05$. By contrast, both the controls, $t(6) = 11.2$, $p < .001$, and castrate + L-DOPA animals, $t(6) = 4.94$, $p < .01$, showed a statistically significant increase on trial 7 versus trial 6. In addition, the investigation times on trial 7 were significantly lower ($p < .01$) in the castration group versus the control and L-DOPA-treated castrated animals, with these latter two groups failing to differ from one another.

Neurochemicals

In both the anterior OB (Fig. 2A) and the posterior OB (Fig. 2B) the DOPAC levels were substantially increased in the L-DOPA-treated group compared with the other two groups. Among them, there was a 292.6% increase of DOPAC concentration compared with castrated animals and a 313.5% increase compared with control group in the anterior OB. These differences approached but failed to achieve statistical significance, $F(2, 18) = 3.11$, $p > .05$. In the posterior OB the increases of DOPAC levels in the L-DOPA-treated group were 317.0% ($p < 0.05$) versus the castrated and 374.6% ($p < 0.05$) versus the control group, $F(2, 18) = 4.11$, $p < .05$. No significant differences were observed in NE and DA concentrations in these two areas.

DISCUSSION

The present results demonstrate a selective habituation/dishabituation deficit in response to urinary chemical cues following castration. This was indicated by the poor dishabituation responses to female urine observed in the castrated animals. It appears that these animals show attenuated attraction/preferences and/or recognition of female urinary cues as the result of castration, results which are comparable to those of other paradigms in which castration abolished preferences for female rats (3). Interestingly, we have reported that intact male rats whose OB-NE concentrations were reduced by 40-60% as achieved by treatment with the noradrenergic neurotoxin DSP-4 also showed a poor dishabituation response to female urine (16). It seems possible that the OB-NE system may play an important role in the demonstration of these olfactory preferences/attractions and/or recognitions (1) of chemical cues. The OB-NE system may be particularly sensitive to female chemical cues, as relatively slight perturbations of this system selectively reduce dishabituation responses only to female chemical cues (16), while more severe depletions of OB-NE resulting from 6-OHDA lesions interrupt dishabituation responses to both female and male urinary chemical cues (15).

The fact that the behavioral deficit observed in castrated animals was restored by L-DOPA treatment further implicates a role for catecholaminergic activity involvement in this effect. In an attempt to gain some insights into this possibility, we assayed selective areas of the OB for concentrations of NE, DA, and DOPAC immediately following behavioral testing. Within the OB, significant increases in DOPAC concentration were obtained for the posterior OB; however, no changes in NE or DA were obtained. While we were somewhat surprised to find no changes in these parent catecholamine compounds, it should be noted that under certain circumstances, specifically when there is more NE than DA, DOPAC can be and has been used to reflect NE neuron activity. For example, it has been demonstrated that electrical stimulation of the ascending noradrenergic pathway of the locus coeruleus increases DOPAC levels in the hippocampus, an effect which was prevented by lesion of noradrenergic pathways (28). In addition, since DA is the precursor of NE and can be converted to DOPAC in NE neurons (5,28), changes in DOPAC concentrations may then also be reflective of noradrenergic activity (31). These changes in DOPAC concentrations may result following saturation of dopamine-β-hydroxylase, as can be achieved when there is too great a noradrenergic impulse or too much DA precursor such as from administration of L-DOPA (28). That such an effect can in fact alter NE metabolism is indicated by the report that L-DOPA increases the turnover (19) and release (13) of NE in the CNS. Since the

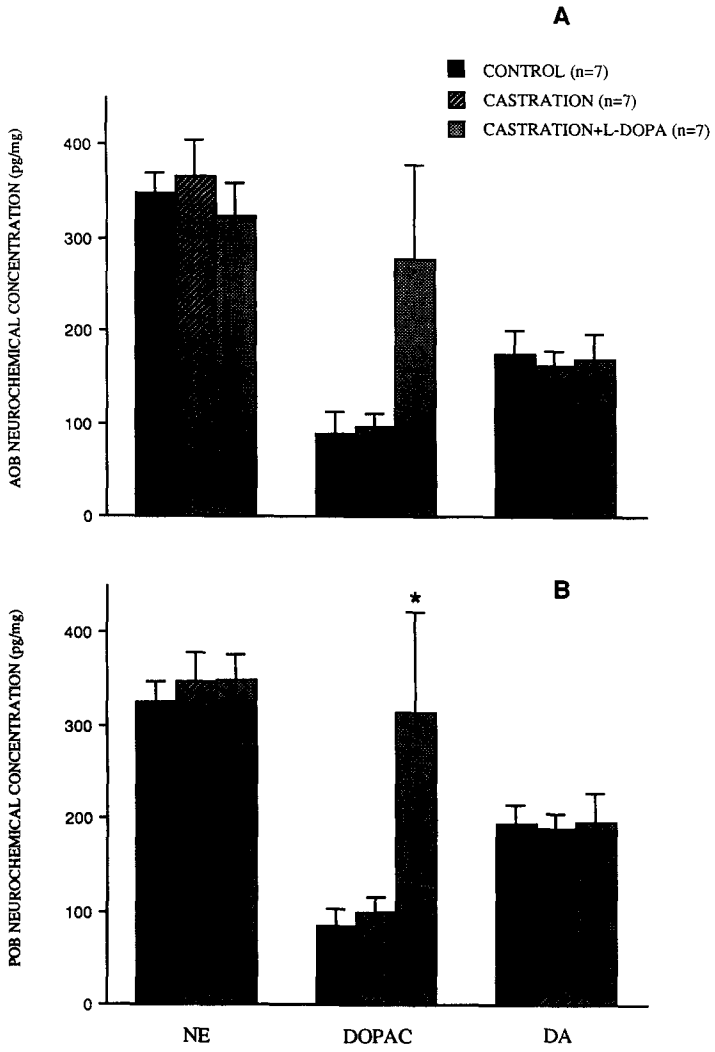

FIG. 2. (A) Anterior olfactory bulb (AOB) and (B) posterior olfactory bulb (POB) neurochemical contents (norepinephrine [NE], DOPAC, and dopamine [DA], mean ± SEM, pg/mg) of control, castrated, and castrated + L-DOPA-treated rats. No effect of castration on AOB and POB neurochemicals was observed, whereas L-DOPA significantly increased DOPAC levels in the posterior OB. $*p < 0.05$ compared with control group. $**p < 0.01$ compared with control group. $*p < 0.05$ compared with castrated group.

concentration of OB-NE is about twice that of OB-DA, it is possible that the DOPAC concentration may in fact be reflective of NE activity. In this way, although no changes in OB-NE were observed, an increase of OB-NE activity may be present, as indicated by such high levels of DOPAC concentrations observed in the posterior OB in L-DOPA-treated animals. Such an increase in OB catecholaminergic activity may in part be related to the restoration of behavioral responses.

In general, the content of a neurochemical may not reliably reflect its activity. This can be due to the potential for a number of factors, including synthesis, release, and metabolism to change simultaneously as a function of some treatment (e.g., castration) and obscure the meaning of a change in the determination of a static, single point concentration value. Some of the apparent inconsistencies with regard to the effects of castration upon OB-NE concentrations in male rats may be related to this issue (4,10). This indicates the need to supplement this information with alternative methods to evaluate OB catecholaminergic function between intact and castrated male rats. We have reported that a dynamic estimate of OB noradrenergic activity as determined with in vitro superfusion of OB tissue fragments reveals that reductions in NE release rates are obtained in castrated versus intact male rats (17). This castration-dependent decrease in dynamic NE activity and its potential restoration with L-DOPA may be related to the alterations in dishabituation responses that accompany these manipulations.

Clearly, the effects of the L-DOPA treatment in the present experiment are not limited to the OB, but are producing relatively diffuse effects within the CNS that may also account for the changes in behavioral responses. For example, there are reports showing that L-DOPA can alter sexual behavior,

with both increases (23,24,30) and decreases (14,18) in copulatory performance being reported. This effect is evidently dose-dependent because low doses facilitate whereas high doses inhibit the copulatory behavior in castrated male rats (22). These additional CNS sites which are activated with L-DOPA to alter sexual behavior may likely be involved with the changes in behavioral responses of the present experiment. Therefore, the present results do not permit us to demonstrate unequivocally a specificity of L-DOPA's effects within the OB. Nonetheless, this experiment establishes that L-DOPA can be used to restore dishabituation responses to female urine in castrated male rats.

In conclusion, this experiment demonstrates that castration abolishes the dishabituation response of the male rat to female urine and this effect is reversible by giving L-DOPA. We have speculated that the OB-NE system may play an important role in this process; however, additional work is required to determine the exact neurochemical mechanisms and sites involved in this effect.

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