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Effects of Gonadal Steroids on Tuberoinfundibular and Tuberohypophysial Dopaminergic Neuronal Activity in Male and Female Rats (42384)

JOSEPH W. GUNNET, KEITH J. LOOKINGLAND, AND KENNETH E. MOORE

Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan 48824

Abstract. The activities of tuberoinfundibular and tuberohypophysial dopamine (DA) neurons were estimated by measuring the turnover of DA in terminals of these neurons in the median eminence and in the neural and intermediate lobes of the pituitary, respectively. The rate of DA turnover (α-methyltyrosine-induced decline of DA) in the median eminence was two to three times faster in females than in males, but no sexual differences in DA turnover rates were noted in the neural and intermediate lobes. Two weeks following gonadectomy the rate of DA turnover in the median eminence was increased in the male but decreased in the female. These effects were reversed by testosterone and estrogen replacement in gonadectomized males and females, respectively. Neither gonadectomy nor steroid replacement altered DA turnover in the neural or intermediate lobes of either males or females. These results indicate that estrogen stimulates and testosterone inhibits tuberoinfundibular DA neuronal activity while neither steroid affects tuberohypophysial DA neuronal activity.

Perikarya of both tuberoinfundibular (TI) and tuberohypophysial (TH) dopamine (DA) neurons are located within the periventricular-arcuate region of the rat hypothalamus. Axons of TIDA neurons project to the external layer of the median eminence (ME) and terminate near the primary capillary loops of the hypophysial portal system (1, 2). DA released from these neurons is carried by the portal blood to the anterior pituitary where it inhibits the release of prolactin (3). Axons of the THDA neurons project through the ME and terminate in the intermediate lobe (IL) and the neural lobe (NL) of the posterior pituitary (1). In the IL these neurons have been implicated in the release of β-endorphin and α-melanotropin (4, 5) while in the NL they have been postulated to modulate the release of vasopressin, oxytocin, and prolactin (6, 7).

TIDA and THDA neurons respond differently to various pharmacological and environmental manipulations. The activity of TIDA neurons is not altered by the acute administration of DA agonists or antagonists but is increased by prolactin and decreased by restraint stress and suckling (8–10). THDA neurons, on the other hand, are unresponsive to the actions of prolactin and immobilization stress, but are selectively activated by dehydration and salt loading (9, 10). Recently, differences in the responses of THDA neurons that terminate in the IL and NL have also been noted (11, 12); THDA neurons that terminate in the NL are similar to TIDA neurons in that they are unresponsive to the action of DA agonists and antagonists and are inhibited by morphine. In contrast, those THDA neurons that project to the IL respond to DA agonists and antagonists but not to morphine.

In the present study, the effects of gonadal steroids on the activities of TIDA neurons in the ME and THDA neurons in the IL and NL were compared in male and female rats. Neuronal activities were estimated biochemically by measuring the rate of decline of DA concentrations in the ME, IL, and NL following the administration of α-methyltyrosine (αMT), an inhibitor of tyrosine hydroxylase.

Materials and Methods. Adult male and female Long-Evans rats (200–225 g) were obtained from Charles River Laboratories (Wilmington, Mass.) and were maintained under conditions of controlled temperature and lighting (illumination 0600–1800 hr) with food and water supplied *ad libitum.* Vaginal lavage was performed daily to monitor estrous cyclicity. Intact females were sacrificed on the morning of the first day of diestrus. Gonads were removed under diethylether anesthesia, 2 weeks prior to sacrifice. For testosterone re-

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placement, orchidectomized male rats were implanted subcutaneously with Silastic capsules (1.57 mm i.d., 3.18 mm o.d., 30 mm length) that were either empty or filled with crystalline testosterone (Sigma Chemical, St. Louis, Mo.) immediately following castration. Silastic capsules of this size produce serum testosterone concentrations identical to those of intact male rats (13). For estrogen replacement, ovariectomized females were implanted with Silastic capsules (1.57 mm i.d., 3.18 mm o.d., 20 mm exposed length) containing either corn oil or 17β-estradiol benzoate in corn oil (150 μg/ml) 2 days before sacrifice. Capsules such as these restore the serum estrogen levels of ovariectomized females to diestrous levels within 2 days (14).

After appropriate treatments, animals were decapitated and the brains and whole pituitary glands were removed and frozen. The brains were sliced into 600 μm frontal sections on a cryostat and the ME removed using a modification (15) of the punch technique described by Palkovits (16). The IL and NL were dissected from the frozen pituitary glands using a technique previously described (11). Tissue samples were placed in either 15 μl (NL, IL) or 35 μl (ME) of 0.2 *N* perchloric acid containing 10 mg% EGTA and stored at −20°C until assayed. On the day of assay the samples were thawed, sonicated for 3 sec, and centrifuged for 1 min in a Beckman Microfuge. DA was determined in the supernatants by radioenzymatic assay (17); tissue pellets were dissolved in 1.0 *N* NaOH and assayed for protein (18).

The turnover rates of DA were estimated according to the method of Brodie *et al.* (19). Groups of rats were decapitated 60 min after an ip injection of the water vehicle (1 ml/kg; zero time controls) and 30 and 60 min after the ip administration of α-methyl-DL-*p*-tyrosine methylester HCl (αMT, Sigma Chemical Co.; 250 mg free base/kg). Following inhibition of tyrosine hydroxylase with αMT, DA concentrations decline exponentially with time as a function of the rate of amine release. The slope of the line resulting from plotting the log DA concentration versus time after αMT administration was calculated from one "best fit" by the method of least squares, and from this a single rate constant of amine decline (k) was derived. The rate constant expressed in reciprocal hours (1/hr) was used as an index of DA turnover since this measure directly reflects the activity of DA neurons without regard to the density of their regional innervation.

Statistical analyses were conducted using either two-tailed Student's t test or Bonferroni's multiple t test (20). Differences were considered significant if the probability of error was less than 5%.

Results. DA concentrations and rate constants for the decline of DA in ME, IL, and NL of intact male and diestrous female rats are summarized in Table I. There were no sex differences in the DA concentrations of the ME, IL, or NL. In both males and females, the DA concentration in IL is two to three times greater than that in the NL, as previously reported for the male (11). The rate constant for DA decline in the ME of the female was

TABLE I. CONCENTRATIONS AND RATE CONSTANTS OF DOPAMINE IN THE MEDIAN EMINENCE AND THE NEURAL AND INTERMEDIATE LOBES OF THE PITUITARY OF INTACT MALE AND DIESTROUS FEMALE RATS

	DA concentration[a] (ng/mg protein)		DA rate constant[b] (1/hr)	
Tissue	Male	Female	Male	Female
Median eminence (ME)	83.8 ± 3.2	91.8 ± 5.4	−0.38 ± 0.04	−0.94 ± 0.07[c]
Intermediate lobe (IL)	13.9 ± 0.7	14.4 ± 0.7	−0.55 ± 0.03	−0.47 ± 0.05
Neural lobe (NL)	5.4 ± 0.4	5.3 ± 0.4	−0.45 ± 0.04	−0.43 ± 0.07

[a] Concentration values represent means ± 1 SE of 23–25 animals per group.

[b] Rate constants (k ± 1 SE) represent means from three experiments; each rate constant being determined from 22 to 26 rats.

[c] Significantly different from intact male rats ($P < 0.05$).

TABLE II. EFFECTS OF CASTRATION AND STEROID REPLACEMENT ON THE CONCENTRATIONS OF DOPAMINE IN THE MEDIAN EMINENCE AND THE NEURAL AND INTERMEDIATE LOBES OF THE PITUITARY OF MALE AND FEMALE RATS

Tissue	Dopamine (ng/mg protein)[a]		
	Median eminence	Intermediate lobe	Neural lobe
Male[b]			
Intact	89.4 ± 6.2	12.7 ± 0.6	4.4 ± 0.6
Orchidectomized	86.4 ± 6.0	11.4 ± 0.5	5.8 ± 0.8
Orchidectomized + testosterone	73.3 ± 4.4	10.6 ± 1.2	5.4 ± 0.7
Female[c]			
Intact	93.7 ± 7.6	14.8 ± 1.3	4.3 ± 0.6
Ovariectomized	116.4 ± 8.3	15.5 ± 1.1	4.0 ± 0.2
Ovariectomized + estrogen	98.8 ± 5.8	13.0 ± 2.3	4.5 ± 0.5

[a] Values represent means ± 1 SE of 5–8 determinations. There are no statistically significant differences in DA concentrations in any region among the three groups of male and female rats.

[b] Males were orchidectomized and implanted subcutaneously with either empty or testosterone-filled capsules 2 weeks prior to sacrifice.

[c] Intact females were killed on the first day of diestrus. Ovariectomized females (2 weeks) were implanted with capsules containing corn oil or estradiol benzoate in corn oil 2 days prior to sacrifice.

two- to threefold greater than that of the male, in agreement with previous reports (21, 22). There were no differences among the DA rate constants in the NL and IL of male and female rats.

The steady-state DA concentrations in the ME, IL, and NL were not altered by orchidectomy or testosterone replacement (Table II). Two weeks following orchidectomy, the DA rate constant in the ME was significantly increased over that seen in intact males (Fig. 1). Testosterone replacement, begun at the time of castration, reversed the effects of castration and maintained the rate of DA turnover in the ME at a value similar to that of intact males. Neither castration nor testosterone replacement had any effect on the DA rate constants in the IL or NL.

In female rats, neither ovariectomy nor estradiol treatment altered DA concentrations in ME, IL, or NL (Table II). On the other hand, the DA rate constant in the ME was significantly lower in ovariectomized than in diestrous females (Fig. 2). Two days of estrogen replacement increased the DA rate constant in the ME of the ovariectomized females to a value similar to that of diestrous females. The DA rate constants in the IL and NL were unaffected by ovariectomy or estrogen replacement.

Discussion. TIDA and THDA neurons modulate the release of hormones from the anterior and posterior pituitary glands. Sexual differences in the release of pituitary hormones may, therefore, be related to male–female differences in the activity of TIDA and THDA neurons. This relationship has been demonstrated for the release of prolactin from the

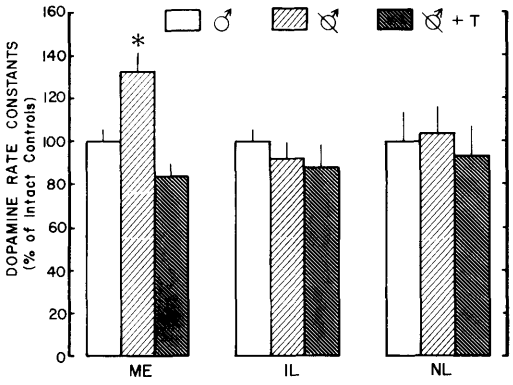

FIG. 1. Effects of orchidectomy and testosterone replacement on the DA rate constants in the ME, IL, and NL of male rats. αMT was given as described in Table I. Each column and vertical line represent the rate constants (k) ± 1 SE expressed as a percentage of the intact male rate constant. See legend to Table II for additional details. *Significantly different from intact males ($P < 0.05$).

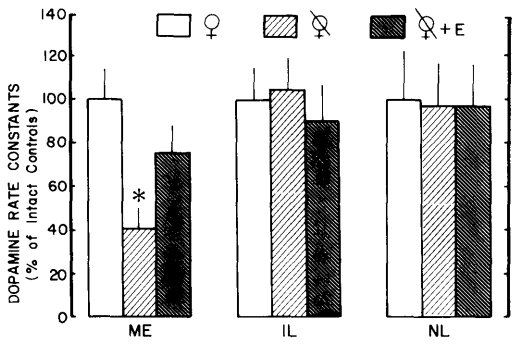

FIG. 2. Effects of ovariectomy and estrogen replacement on the DA rate constants in the ME, IL, and NL of female rats. Each column and vertical line represent the rate constants (k) ± 1 SE expressed as a percentage of the diestrous female rate constant. See legend to Table II for additional details. *Significantly different from diestrous females ($P < 0.05$).

anterior pituitary. Prolactin secretion exhibits a marked sexual difference (23) and is directly inhibited by DA tonically released from TIDA neurons (3). The activity of TIDA neurons is greater in females than in males as shown in the present study and in other reports using various techniques to estimate the activity of these neurons (21, 22, 24, 25). There appear to be at least two mechanisms responsible for this sexual difference; a reduced responsiveness of TIDA neurons in the male to the stimulatory actions of prolactin (24), an effect of neonatal androgenization (21), and the actions of estrogen and testosterone in the adult animal.

The gonadal steroids of both sexes affect TIDA neuronal activity. In female rats, the turnover rate of DA in the ME was reduced 2 weeks after ovariectomy and returned to diestrous levels by estrogen replacement. These results agree with those of other studies which have demonstrated the stimulatory effect of estrogen on TIDA neuronal activity (9, 22, 26–29). This effect is not due to a direct action of estrogen on TIDA neurons but instead is mediated by prolactin whose secretion is increased by estrogen (26, 27). The results of studies concerning the effects of castration and steroid replacement on TIDA neurons in the male rat are not as consistent as those described above for the female rat. Using the αMT technique, Kizer *et al.* (30) found that orchidectomy increased the rate of DA turnover in the ME suggesting that testosterone inhibits TIDA neuronal activity. In contrast, others have reported no effect after castration but an increase in the rate of DA turnover in the ME following testosterone administration to castrated male rats (29, 31). In the present study, 2 weeks after orchidectomy the rate of turnover of DA in the ME was increased, and this effect was reversed by testosterone. The same pattern of effects was observed when the activity of TIDA neurons was estimated by measuring the rate of DA synthesis in the terminals of these neurons (22). The increased DA turnover in the ME of orchidectomized rats seen in the present study and the reversal of this effect by testosterone replacement do not appear to be due to prolactin since as administered in the present study, testosterone does not alter serum levels of this hormone (32).

The DA concentrations and the rates of DA turnover in the IL and NL are similar in male and female rats. In the present study neither gonadectomy nor steroid replacement had any effect on the THDA neurons in the IL and NL. These data contradict a recent report in which estrogen increased both the steady-state concentration of DA in the IL and the rate of DA turnover in the IL and NL (33). The gonadal steroids can alter the release of vasopressin and β-endorphin from the posterior pituitary (34, 35); results from the present study suggest that these steroid effects on posterior pituitary hormone secretion do not involve changes in THDA neuronal activity. Given the similarities and differences between TIDA and THDA neurons, the lack of response to gonadectomy and steroid replacement in THDA neurons terminating in both the IL and NL further demonstrates the individual characteristics of DA neurons innervating the ME, IL, and NL.

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