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Differential Responsiveness of LH and Prolactin to *p*-Tyramine in Male and Female Rats (42714)

DAMASIA BECÚ-VILLALOBOS AND CARLOS LIBERTUN

Laboratorio de Neuroendocrinología, Instituto de Biología y Medicina Experimental, Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina

Abstract. The effect of *p*-tyramine, a natural amine which is found in the rat brain in trace amounts, was evaluated for its capacity to influence LH and prolactin secretion in male and female rats under different hormonal conditions. *p*-Tyramine (40 mg/kg ip) was ineffective in modifying LH levels in either female or male rats which had been gonadectomized for 2 days, but if the animals were injected with 12.5 μg of estradiol benzoate (EB) on the day of castration, *p*-tyramine was able to release LH in female but not in male rats. To evaluate whether early androgenization of brain structures which control LH secretion was involved in the sexual difference observed, *p*-tyramine was tested in female androgenized rats (200 μg of testosterone propionate on the day of birth), and in male rats castrated at birth. The trace amine was ineffective in altering LH levels in both experimental models, even if rats were pretreated with EB as control females. On the other hand, *p*-tyramine inhibited prolactin secretion in male rats pretreated with EB, and not in similarly treated female rats. The present results suggest that *p*-tyramine may be involved not only in prolactin regulation as it has been previously shown, but also in LH control, and that the hormonal response to this amine is sexually differentiated in the rat.

p-Tyramine belongs, together with phenylethylamine, octopamine, tryptamine and other amines, to a group of endogenous amines called "trace amines" (1) due to their low concentrations in the brain. These amines are related to catecholamines and 5-hydroxytryptamine both structurally and metabolically, and their pharmacological and physiological properties make them candidates for transmitter or neuromodulator roles in the mammalian brain (2, 3). *p*-Tyramine releases catecholamines from nerve terminals (4, 5), is an intermediate in the biosynthetic pathway of the catecholamines (2), and it may have properties and functions of its own (6–8).

In previous works we demonstrated that this trace amine was able to modify prolactin levels in male rats (9, 10); in the present experiments *p*-tyramine was assessed for its ability to modify LH secretion. The catecholamines, especially norepinephrine, are known to participate in LH regulation (11–13). Therefore, because of the relationship of *p*-tyramine and norepinephrine it was of interest to evaluate the effects of *p*-tyramine on LH release.

Materials and Methods. Male and female Sprague–Dawley rats (200–300 g body wt) of the Instituto de Biología y Medicina Experimental were used. They were housed in an air-conditioned room with controlled lighting (lights on from 0700 to 1900 hr) and given free access to laboratory chow and tap water. Experiments were always performed between 1000 and 1200 hr to prevent variation due to the circadian pattern of prolactin secretion (14).

Male and female rats were gonadectomized under ether anesthesia 2 days before the experiment, and some were injected with 12.5 μg of estradiol benzoate (EB) immediately after gonadectomy. A group of female rats was neonatally androgenized on the day of birth with a sc injection of 200 μg of testosterone propionate dissolved in oil. This treatment results in sterile adult rats (17). A group of male rats was orchidectomized under cold anesthesia on the day of birth; this prevented endogenous testosterone from masculinizing brain structures.

On the day of the experiment *p*-tyramine HCl (Sigma, St. Louis, MO), dissolved in NaCl 0.9%, was injected ip at a dose of 40 or 8 mg/kg in preliminary experiments (calculated as free base). Control animals were injected with saline solution. Ten minutes thereafter animals were decapitated and

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blood collected for RIA determinations of serum LH and prolactin. Prolactin was measured by a RIA kit provided by the NIADDK; results are expressed in terms of RP3 rat prolactin standard. LH was determined with the RIA developed by Niswender *et al.* (16), and results are expressed in terms of LH RP2 rat LH.

The data were analyzed by one-way analysis of variance (to study variation within each group). When F was significant, Duncan's test was used to compare the means. Results were considered significant when $P < 0.05$.

Results. *p*-Tyramine was ineffective in modifying LH levels in either female or male rats which had been gonadectomized for 2 days (Fig. 1, left); similar results were obtained if animals had been castrated for longer periods (data not shown). As it has been described (17, 19) after 2 days of gonadectomy LH levels rise abruptly in male rats, while in females the increase is more gradual and does not attain maximal castration levels at this time. If animals were injected with 12.5 μg of EB on the day of castration, *p*-tyramine was able to release LH in female but not in male rats (Fig. 1, right). The release in female rats was found to be dose dependent, the effect being greater with the dose of 40 mg/kg than with that of 8 mg/kg (control: 0.332 ± 0.05, $n = 7$; 8 mg/kg: 0.695 ± 0.071, $n = 7$, $P < 0.05$; 40 mg/kg: 0.904 ± 0.075 ng/ml, $n = 6$, $P < 0.05$). It was also time dependent as it could be evidenced at 10 minutes postinjection and had already disappeared at 30 min (0 min: 0.239 ± 0.108, $n = 5$; 10 min: 0.613 ± 0.080, $n = 7$, $P < 0.05$; 30 min: 0.302 ± 0.050 ng/ml, $n = 6$). The relatively high doses needed as well as the short duration of the effect are probably related to the rapid inactivation of *p*-tyramine injected systemically (20).

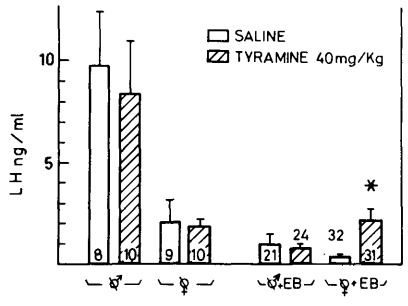

FIG. 1. Effect of *p*-tyramine ip on LH release in gonadectomized male (♂) and female (♀) rats (left side). Castration was performed 2 days before experiment; and in male and female rats treated with estradiol benzoate (EB) 12.5 μg sc, at the moment of castration (right side). In this and the following figures, the height of the bar represents the mean, the line on top represents 1 SE, and the number within bars represents the number of rats.* $P < 0.05$.

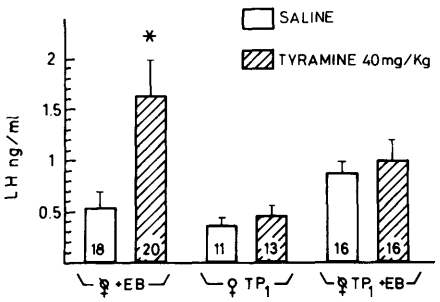

FIG. 2. Effect of tyramine on LH release in female rats castrated and treated with EB; in perinatally adrogenized female rats (♀ TP_1); and in female androgenized rats castrated in adulthood and treated with EB (♀ TP_1 + EB).

To evaluate whether early androgenization of brain structures which control LH secretion was involved in the sexual difference observed, the effect of *p*-tyramine was tested in female androgenized rats. *p*-Tyramine released LH in female ovariectomized rats which had been injected with EB (Fig. 2), but androgenized females of the same litter failed to respond to *p*-tyramine with an LH increase, whether intact or ovariectomized as adults and injected with 12.5 μg of EB. Male rats which had been castrated at birth and injected with EB as adults did not show an increase after *p*-tyramine injection either (7.08 + 1.25 vs 8.79 ± 1.28 ng/ml). EB was not as effective in lowering LH titers in neonatally orchidectomized rats as in adult orchidectomized rats (7.08 ± 1.25 vs 1.52 ± 0.46 ng/ml, Fig. 1).

As *p*-tyramine has been shown to modify prolactin levels in male rats (9, 10) prolactin was also measured in all animals. It was found that though *p*-tyramine could lower prolactin levels in male rats (orchidectomized +EB), it had no hypoprolactinemic effect in females given EB, and a suggestion

of a weak prolactin releasing effect was observed (Fig. 3). In a second set of experiments this effect was found to be significant, and it could be abolished if females were androgenized at birth (Fig. 4). On the other hand, in male rats orchidectomized at birth and given EB as adults *p*-tyramine did not modify prolactin levels.

Discussion. Though *p*-tyramine has been classically considered as an indirectly acting sympathiomimetic amine (4, 5), some data indicate that it may possess direct receptor stimulating properties (6, 7), and it can be considered as a neuromodulator in the mammalian brain (3). In previous studies (9, 10), we demonstrated that *p*-tyramine was able to modify prolactin secretion in the male rat. In the present experiments its relation to LH secretion was investigated, and its effects on prolactin in female and male rats, which had been treated with estradiol, were evaluated.

The present results show that *p*-tyramine can alter LH and prolactin secretion differentially in female and male rats. The fact that *p*-tyramine released LH in female rats that had been pretreated with estradiol and not in ovariectomized rats could be related to its indirect action of increasing noradrenaline availability within the brain. In this regard it is interesting to note that in contrast to the inhibitory effect of noradrenaline on LH release seen in ovariectomized rats (21–23), priming with estrogen and progesterone completely shifts the hypothalamic microenvironment wherein an excitatory α-adrenergic activity predominates (21, 23–26). Thus, the steroid environment of the animal may modulate the manner in which LHRH neurons respond to noradrenaline (27, 28). As noradrenaline stimulation of LH has been related to the phasic release of the gonadotropin (12), it is not surprising to find that *p*-tyramine was ineffective in male rats, whether intact or treated with estradiol, as well as in androgen-sterilized female rats which lack the ability to produce LH peaks in response to estradiol (29). Furthermore, in male rats no change in LH secretion was found after infusion of low doses of noradrenaline into the third ventricle, (30). Nevertheless it must be stated that the effect of *p*-tyramine on LH secretion may be related to the function of other neurotransmitter systems or to intrinsic properties of the amine.

The failure of estrogen to suppress effectively LH titers in male rats that had been castrated at birth has been previously documented (31). The presence of testicles (until 25 days of age) appears to prevent high secretory levels of LH (32). On the other hand, sex differences exist in the feedback effects of estrogen on LH secretion, which induce a rapid elevation of LH levels after orchidectomy (8 to 18 hr) and a slower rise (3 to 5 days) after ovariectomy (17–19), as we confirmed in the present experiments.

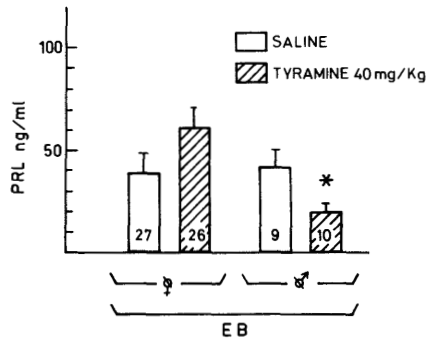

FIG. 3. Effect of tyramine on prolactin (PRL) release in male and female rats castrated for 2 days and treated with EB.

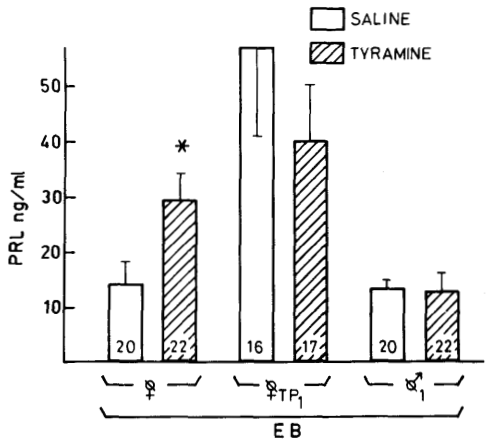

FIG. 4. Effect of tyramine on prolactin release in female ovariectomized rats, female androgenized rats ovariectomized in adulthood, and in male rats castrated at birth (δ_1). All groups were treated with EB.

With regard to *p*-tyramine modulation of prolactin secretion in rats pretreated with estrogen unexpected results were obtained. In the male rat pretreated with estrogen, *p*-tyramine was able to lower prolactin values, as it has been described in the male rat under different hyperprolactinemic states (e.g., after stress, haloperidol, serotonin, TRH, median eminence lesion, *in vivo* as well as *in vitro*) (9, 10). However, in the female rat treated with estradiol this effect could not be evidenced, and even a weak releasing effect was apparent. This effect was absent in androgen-sterilized female rats. Noradrenaline has been shown to increase prolactin in ovariectomized rats primed with estradiol and progesterone, and to decrease or not to change prolactin levels in male rats (25, 33–34). It is interesting to note that similar results are herein obtained using *p*-tyramine.

On the other hand in male rats castrated at birth, in which presumably testosterone has not differentiated the hypothalamus to a masculine pattern, *p*-tyramine was ineffective in releasing LH. High testosterone levels which in the male rat occur at 18 days of gestation (35) may have already masculinized brain structures, or perhaps small amounts of early estrogen may be necessary for the full development of this female type response to *p*-tyramine in adulthood.

The present results suggest that *p*-tyramine may be involved not only in prolactin but also in LH regulation, and that the hormonal response to this amine in the rat is sexually differentiated. Nevertheless, since high doses of *p*-tyramine were used, the physiological importance of the present observations needs to be studied.

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