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Glutamic acid decarboxylase in rat olfactory bulb: effect of ovarian steroids or male pheromones

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Abstract

The effect of ovarian steroids and pheromones on the activity of glutamic acid decarboxylase, the enzyme that synthesizes γ-aminobutyric acid (GABA), was studied in the rat olfactory bulbs. The enzyme activity was measured in the main and accessory olfactory bulbs at 11:00 h and 17:00 h in ovariectomized rats, and in rats treated with ovarian steroids or exposed to male pheromones. The enzyme activity in both bulbs showed a diurnal fluctuation that was not affected in the accessory bulbs by the exposure to pheromones while the rhythm disappeared in the main bulbs. Estrogen and estrogen-progesterone treatments decreased the enzyme activity in both bulbs either in the morning or in the afternoon. The exposure of ovariectomized estrogen-primed rats to male pheromones reversed the effect of estrogen on the enzyme activity in the morning but not in the afternoon. Ovarian hormones plus pheromones prevented the steroid effect only in the morning. These results support the view that in olfactory bulbs, the GABAergic system can be modulated by endocrine and pheromonal factors.

Keywords: Male pheromone; Glutamic acid decarboxylase; Olfactory bulb; Ovariectomy; Estrogen; Progesterone; (Rat)

1. Introduction

Olfactory stimuli are relevant in reproductive processes such as estrous behavior (Donovan and Kopriva, 1965) or gonadotropin secretion (Beltramino and Taleisnik, 1983). We recently demonstrated that the activity of glutamic acid decarboxylase, the γ-aminobutyric acid (GABA) synthesizing enzyme in the olfactory bulbs of rats, changes in female rats in response to pheromonal stimuli from the pups (Munaro, 1990) and in male rats exposed to female pheromones (Navarro Becerra and Munaro, 1996). Estrogens can interact with glutamic acid decarboxylase; the enzyme activity in the hypothalamus increased after chronic but not after acute treatment with estrogen (Duvilansky et al., 1983; Munaro et al., 1986; O'Connor et al., 1988). The activity of glutamic acid decarboxylase is also modulated by estrogen and progesterone treatments (Wallis and Luttge, 1980). Combined treatments with estrogen and progesterone reduced glutamic acid decarboxylase concentration in the preoptic area (Leigh et al., 1990). In rats, ovariectomy increases GABA content in the brain, while steroid hormones can regulate GABAergic transmission (Saad, 1970). Furthermore, estrogen and progesterone can regulate GABA receptors in areas such as the olfactory bulbs (Maggi and Perez, 1984).

In the present study the effect of ovarian hormones and exposure to male pheromones on the activity of glutamic acid decarboxylase in olfactory bulbs was studied in the morning and in the afternoon in ovariectomized rats in order to investigate whether these factors modulate the GABAergic system.

2. Materials and methods

2.1. Animals

Experiments were performed on ovariectomized rats weighing between 200–270 g, maintained in a temperature-controlled room (20 ± 2°C) under a schedule of 14 h light and 10 h darkness (light period from 06:00 to 20:00 h). All animals had free access to food and water. Rats were used 30 days after ovariectomy. Ovariectomized rats were housed in individual cages and placed in an isolated room containing no other animals and maintained under

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these conditions for 3 days. They were killed either at 11:00 h or 17:00 h. Glutamic acid decarboxylase activity was assayed in the main and accessory olfactory bulbs as described below.

2.1.1. Effect of hormone treatment on glutamic acid decarboxylase activity

To study the effect of the hormone treatment on enzyme activity, ovariectomized rats were injected s.c. with estradiol benzoate, 5 μg/100 g body weight (Caligaris et al., 1971) and were killed 3 days later either in the morning or in the afternoon. Another group was injected s.c. with progesterone 2 mg/rat body weight (Caligaris et al., 1971) on the third day of the estrogen treatment and was killed 3 h later. Controls were ovariectomized rats that were injected with the same volume of vehicle as the experimental animals.

2.1.2. Effect of pheromone exposure on glutamic acid decarboxylase activity

The males providing the olfactory signals were housed in individual plastic cages and were kept in a second isolated room for at least 3 days. In the morning or afternoon, the inducible female rat was transferred into a clean transparent plastic cage. At this time, the cage was reversed so that the wire mesh screen formed the floor. Immediately thereafter, the reversed cage was placed for the whole period of observation (2 h) on top of the male's cage providing the olfactory signals arising from wood shaving soiled by male rats, so that both cages were separated by a double mesh screen. In the case of estrogen-treated rats, the exposure to the olfactory stimulus was on the third day after the hormone injection. In estrogen-progesterone treatments, the last hormone was injected 1 h before the rats were exposed to the male pheromones.

2.2. Tissue preparation

Olfactory bulbs were dissected from the brains of rats killed by decapitation. Brains were placed on ice and the main olfactory bulb was separated from the accessory olfactory bulb by a cut located obliquely 45° to the longitudinal axis of the bulb. The main bulb was separated from the anterior olfactory nucleus by a cut perpendicular to the oblique one. Weights were approximately 15 mg for the main bulb and 12 mg for the accessory bulb.

2.3. Glutamic acid decarboxylase estimation

Enzyme activity was determined according to the technique of Albers and Brady (1959) using a CO_2 trapping procedure. Tissue homogenates were prepared in 25 mM phosphate buffer pH 6.5. Ten microliters of homogenate were used to estimate glutamic acid decarboxylase activity. The reaction took place in test tubes and was initiated by the addition of a mixture containing [^{14}C]glutamic acid, pyridoxal phosphate, dithiothreitol and potassium glutamate. The ^{14}CO2 evolved was absorbed in 50 ml of hyamine hydroxide contained in a microtube connected to the test tube. After 30 min. incubation at 37°C, the reaction was stopped by injecting 50 μl of 5 M H_2SO_4. The tubes were returned to 37°C for 45 min to ensure maximum absorption of $^{14}CO_2$. The radioactivity retained was then estimated by liquid scintillation spectrometry. Blanks were prepared from a boiled homogenate of the tissue. Glutamic acid decarboxylase activity was expressed as nmol $^{14}CO_2$ formed/mg protein/h. Proteins were determined in the tissue homogenate by the method of Lowry et al. (1951).

2.4. Drugs

Estradiol benzoate (Progynon Schering) and progesterone (Proluton Schering) were used. The drugs were diluted in neutral peanut oil.

2.5. Statistical analysis

Data are expressed as group means ± S.E.M. Two-way and three-way analyses of variance were performed and the Student-Newman-Keuls procedure was used for individual group comparisons. Values of $P < 0.05$ or less were considered significant.

Table 1
Glutamic acid decarboxylase activity (nmol CO_2/mg protein/h) in the olfactory bulb of ovariectomized rats

Experimental condition	Accessory olfactory bulb		Main olfactory bulb	
	Morning	Afternoon	Morning	Afternoon
Control	223.86 ± 11.3 [a] (5)	194.56 ± 8.7 [b] (6)	235.22 ± 9.5 [a] (6)	178.18 ± 6.7 [b] (7)
Male odor	214.85 ± 9.0 [c] (6)	183.03 ± 8.8 [d] (5)	198.72 ± 5.0 [c] (6)	169.89 ± 6.4 (6)

Values are the means ± S.E.M. from 5–7 rats. Accessory olfactory bulbs: [a] vs. [b] $P < 0.05$; [c] vs. [d] $P < 0.05$. Main olfactory bulbs: [a] vs. [b] $P < 0.01$; [a] vs. [c] $P < 0.01$. All other comparisons were not significant. Three-way ANOVA and Newman-Keuls test were used for statistical analysis of the data.

3. Results

3.1. Glutamic acid decarboxylase activity in the main and accessory olfactory bulbs of ovariectomized rats

Glutamic acid decarboxylase activity in the accessory bulbs of ovariectomized rats was higher in the morning than in the afternoon ($P < 0.05$). A similar pattern of diurnal changes was seen in the main olfactory bulbs (Table 1).

3.2. Effect of ovarian steroids on glutamic acid decarboxylase activity in the olfactory bulbs

Estrogen treatment (5 μg/100 s.c.) of ovariectomized rats induced 3 days later a marked decrease in the enzyme activity in the accessory bulbs from rats killed in the morning ($P < 0.01$) but not in those killed in the afternoon. A similar response was seen in the main olfactory bulbs (Table 1 and Fig. 1 and Fig. 2). Progesterone injection (2 mg/rat, s.c.) into ovariectomized estrogen-primed rats decreased the enzyme activity in the accessory olfactory bulbs only in the afternoon ($P < 0.01$) whereas in the main olfactory bulbs a decreased activity ($P < 0.01$) was seen both in the morning and afternoon (Fig. 1 and Fig. 2).

3.3. Effect of the exposure to male pheromones on glutamic acid decarboxylase activity

The exposure of ovariectomized rats to male pheromones produced no changes in glutamic acid decarboxylase activity in the accessory bulbs and a decrease in the main bulbs of animals killed in the morning ($P < 0.01$) (Table 1). In ovariectomized estrogen-primed rats, pheromones increased the enzyme activity ($P < 0.01$) and decreased that in the afternoon ($P < 0.01$) in both the accessory and main olfactory bulbs (Fig. 1 and Fig. 2). Progesterone injection into ovariectomized estrogen-primed rats prevented the rise of the enzyme activity induced by male pheromones in the accessory and main olfactory bulbs ($P < 0.01$) observed in the morning and had no effect on the enzyme activity in the main bulbs in the afternoon, compared to the values obtained in ovariectomized estrogen-primed animals.

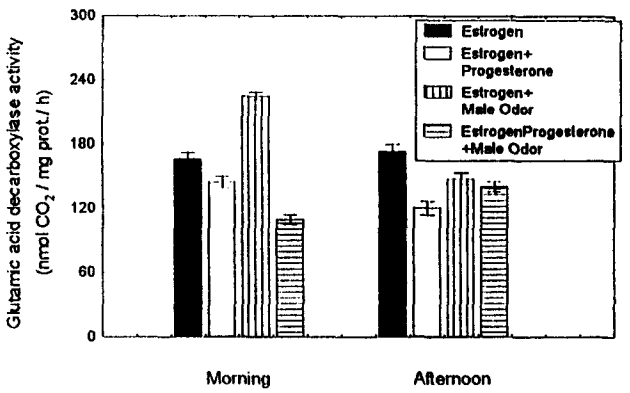

Fig. 1. Glutamic acid decarboxylase activity measured in the morning (11:00 h) and in the afternoon (17:00 h) in the accessory olfactory bulbs of ovariectomized rats. Each bar represents the mean ± S.E.M. for 5–10 rats. Morning experiments: estrogen vs. estrogen + male odor $P < 0.01$; estrogen-progesterone vs. estrogen-progesterone + male odor $P < 0.01$; estrogen + male odor vs. estrogen-progesterone + male odor $P < 0.01$. Afternoon experiments: estrogen vs. estrogen-progesterone $P < 0.01$; estrogen vs. estrogen + male odor $P < 0.01$; estrogen-progesterone vs. estrogen-progesterone + male odor $P < 0.05$. Morning vs. Afternoon: estrogen-progesterone vs. estrogen-progesterone $P < 0.01$; estrogen + male odor vs. estrogen + male odor $P < 0.01$; estrogen-progesterone + male odor vs. estrogen-progesterone + male odor $P < 0.01$. All other comparisons were not significant. Two-way ANOVA and a posteriori individual comparisons were made using Newman-Keuls test.

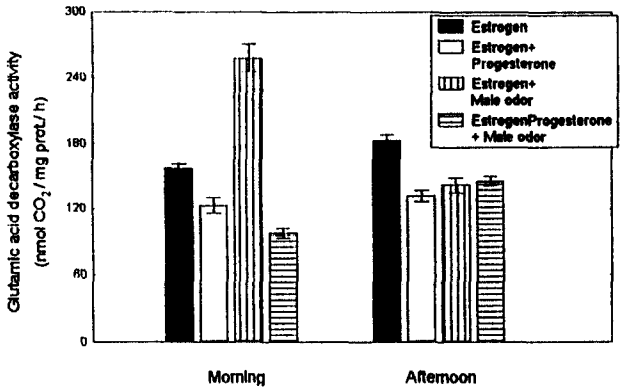

Fig. 2. Glutamic acid decarboxylase activity measured in the morning (11:00 h) and in the afternoon (17:00 h) in the main olfactory bulb of ovariectomized rats. Each bar represents the mean ± S.E.M. for 5–10 rats. Morning experiments: estrogen vs. estrogen-progesterone $P < 0.01$; estrogen vs. estrogen + male odor $P < 0.01$; estrogen + male odor vs. estrogen-progesterone + male odor $P < 0.01$; estrogen-progesterone vs. estrogen-progesterone + male odor $P < 0.01$. Afternoon experiments: estrogen vs. estrogen-progesterone $P < 0.01$; estrogen vs. estrogen-progesterone + male odor $P < 0.01$. Morning vs. afternoon: estrogen vs. estrogen $P < 0.01$; estrogen + male odor vs. estrogen + male odor $P < 0.01$; estrogen-progesterone + male odor vs. estrogen-progesterone + male odor $P < 0.01$. All other comparisons were not significant. Two-way ANOVA and Newman-Keuls post-hoc test.

4. Discussion

Experimental evidence indicates the existence of a circadian rhythm of GABA content and glutamic acid decarboxylase activity in the rat hypothalamus (Cattabeni et al., 1978; Munaro et al., 1991). The current studies demonstrated that a diurnal rhythm in glutamic acid decarboxylase activity was also present in the olfactory bulbs of ovariectomized rats. In the morning significantly higher levels of enzyme activity were found than in the afternoon (Table 1). These results contrast with the pattern of diurnal fluctuations in glutamic acid decarboxylase activity described in castrated male rats, where higher activity of the enzyme in olfactory bulbs was found in the afternoon (Navarro Becerra and Munaro, 1996). The exposure of ovariectomized rats to male odors did not change the diurnal pattern of enzyme activity in the accessory bulbs

whereas it decreased significantly the enzyme activity of the main bulbs in the morning. These findings underscore the importance of studying fluctuations in enzyme activity during the day. In agreement with previous reports (Wallis and Luttge, 1980; McGinnis et al., 1980), we found that an acute treatment with estrogen decreased the enzyme activity. This effect was observed both in the accessory and main olfactory bulbs in the morning but not in the afternoon. It is evident from the effects of pheromones (Table 1) and estrogen (Fig. 2) on the activity of glutamic acid decarboxylase in the main olfactory bulbs that the response was time-specific. As indicated by previous studies, the decrease in glutamic acid decarboxylase activity induced by estrogen administration may be due to a direct effect on the enzyme via neoformation, in view of the fact that GABAergic neurons are targets for estrogen (Flugge et al., 1986), or may be the result of a transsynaptic phenomenon mediated by other neurotransmitters.

Steroid treatment was shown to decrease glutamic acid decarboxylase concentration in the preoptic area (Leigh et al., 1990). This sort of response agrees with that found in olfactory bulbs of ovariectomized rats treated with estrogen-progesterone, since glutamic acid decarboxylase activity was reduced in the main olfactory bulbs at both times of the day. A more specific response to the estrogen-progesterone combination was found in the accessory olfactory bulbs since the decrease in enzyme activity was observed only in the afternoon. The acute administration of estrogen-progesterone induced gonadotropin release (Caligaris et al., 1971) in ovariectomized rats in the afternoon, while glutamic acid decarboxylase concentration was reduced in the hypothalamus (Leigh et al., 1990). We found a similar response only in the afternoon for the glutamic acid decarboxylase activity in the accessory olfactory bulbs of ovariectomized rats treated with estrogen-progesterone.

In ovariectomized rats treated with estrogen, male pheromone exposure is capable of modulating gonadotropin release in the afternoon (Beltramino and Taleisnik, 1983). Estrogen treatment followed by male pheromone exposure decreased enzyme activity in both bulbs in the afternoon (Fig. 1 and Fig. 2). The similar pattern of activity found in both bulbs suggests that the glutamic acid decarboxylase response in ovariectomized rats treated with estrogen plus male pheromone is not different in the two bulbs. However, a clear difference in the response of the enzyme between the accessory and the main olfactory bulbs was observed when the interaction estrogen-progesterone and male odors was studied. In the morning, the enzymatic response in both bulbs (Fig. 1 and Fig. 2) was prevented by steroid treatment, while in the afternoon, enzyme activity in the accessory olfactory bulbs was increased (Fig. 1). Gonadotropin secretion is inhibited in proestrous rats exposed to male pheromones (Dr Beltramino, personal communication). Also, glutamic acid decarboxylase activity in the accessory olfactory bulbs and the main olfactory bulbs increased in proestrous rats exposed to male odors in the afternoon (unpublished results). The increase in enzyme activity in the accessory bulbs induced by male odor in the afternoon could be related to the role that GABA plays in the release of gonadotropin (Morello et al., 1989). Our studies demonstrate that enzyme activity in the olfactory bulbs of ovariectomized rats changed under the influence of specific odors implicated in reproduction and that these changes were related to the hormonal status of the rat. These responses led us to presume that the GABAergic system of the olfactory bulbs may modulate reproductive processes.

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