THE EFFECT OF PROLACTIN ON GLUTAMATE DECARBOXYLASE ACTIVITY AND GABA CONCENTRATION IN HYPOTHALAMIC SLICES

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SUMMARY

The effect of prolactin on the activity of GABA-related enzymes and GABA concentration were studied in hypothalamic slices incubated in vitro. After short periods of incubation (up to 40 min), prolactin (0.25 µg/ml) added to the incubation medium produced a significant increase (21% at 20 min of incubation) in glutamic acid decarboxylase (GAD) activity in the hypothalamic slices. A higher concentration of prolactin (1.0 µg/ml) produced a slight but significant decrease (8% at 20 min of incubation) in hypothalamic GAD activity. However, after longer periods of incubation (over 8 hr), both doses of prolactin induced a sustained increase in hypothalamic GAD activity, a response which depends upon protein synthesis. No changes were observed in GABA-transaminase (GABA-T) activity of hypothalamic slices incubated in the presence of prolactin. Prolactin decreased GABA concentration in the hypothalami incubated for 10 hr and, at the same time, increased GABA release into the medium. These results indicate that prolactin modifies the synthesis and release of hypothalamic GABA and suggest the existence of a feedback mechanism that prolactin may exert directly at the hypothalamic level.

INTRODUCTION

The hypothalamus contains high concentrations of GABA in specific neuronal pathways which are associated with pituitary function. Tuberoinfundibular neurons have GABA and glutamic acid decarboxylase (GAD EC 4.1.1.15) in their cell bodies at the arcuate and paraventricular nuclei and in their terminals at the external layer of the median eminence (Vincent et al., 1982; Tappaz et al., 1983). Hypothalamic GABA has been postulated to mediate the secretion of anterior pituitary hormones. Both inhibitory (Libertun & McCann, 1976; Schally et al., 1977; Debeljuk et al., 1980; Debeljuk et al., 1981; McCann et al., 1981) and stimulatory (Miodszewski et al., 1976; Pass & Ondo, 1977; Vijayan & McCann, 1978) effects of GABA on prolactin secretion have been observed. This control, however, seems to be very complex, with actions at both hypothalamic and pituitary levels (Enjalbert et al., 1979; Grandison & Guidotti, 1979; Racagni et al., 1982). If GABAergic cells within the hypothalamus participate in the regulation of pituitary function, they may be expected to be target cells for circulating pituitary hormones. Administration of high concentrations of prolactin (Duvilanski et al., 1983a; Apud et al., 1984) or substances that induce hyperprolactinemia (Prato et al.,...
1981; Nicoletti et al., 1983) produce an increase of hypothalamic GAD activity, the key enzyme of GABA synthesis.

In this study we have used an in vitro system to examine the direct effect of prolactin on GAD activity and GABA concentration in hypothalamic slices from male rats.

**MATERIALS AND METHODS**

Adult male rats of the Wistar strain, weighing approximately 200 g, were used. The animals had free access to food and water.

Unanesthetized rats were decapitated and the hypothalamus was exposed. The hypothalamus was dissected using the optic chiasma as the rostral limit, the mammillary bodies as the caudal border, and hypothalamic fissures as the lateral limits. Approximately 1.0 - 1.5 mm of the hypothalamic tissue was included dorsally (18 - 20 mg wet wt). Preincubations and incubations were carried out in 1 ml of Krebs-Ringer bicarbonate buffer (KRB) containing 10 mM glucose and 25 mM Hepes, gassed with 95% O₂ - 5% CO₂, at 37°C, under constant oscillation in a Dubnoff metabolic shaker. In every case, individual hypothalamic slices were preincubated for 10 min. At the end of preincubation the medium was replaced by a fresh one containing ovine prolactin (NIADDK, NIH, Bethesda, Maryland) at 0.1 - 1.0 µg/ml dissolved in KRB. Some incubations were performed in the presence of 0.05 mM cycloheximide. At the end of the incubation time the tissue was rapidly washed with KRB and stored at −20°C until GAD activity was measured. Hypothalamic slices incubated for 10 hr were fixed in 2.5% glutaraldehyde and processed for electron microscopy.

GAD activity was assayed by measuring the production of ¹⁴CO₂ from [1-¹⁴C]-glutamic acid (New England Nuclear; sp. act. 47.5 mCi/nmol) under anaerobic conditions. The tissue was homogenized (2.5% w/v) with 50 mM KPO₄ buffer (pH 6.5) containing 0.5 mM pyridoxal-5'-phosphate, 1 mM 2-aminoethylisothiouronium bromide hydrobromide (AET), and 0.2% Triton X-100 (standard buffer).

GAD activity was assayed in 0.1 ml of homogenate. The reaction was started immediately after the addition of 25 mM glutamic acid - 0.1 µCi DL[1-¹⁴C]-glutamic acid (final concentration) in standard buffer. The samples were incubated for 30 min at 37°C in tubes capped with rubber stoppers in a Dubnoff metabolic incubator. The reaction was stopped by the injection of 0.25 ml 4 M H₂SO₄ into the reaction mixture. The ¹⁴CO₂ evolved from the labelled glutamate was trapped in 0.1 ml Protosol (New England Nuclear) contained in a plastic cup suspended from the rubber stopper. To ensure complete release of CO₂ and absorption in Protosol, the tubes were incubated for an additional 60 min. The ¹⁴CO₂ absorbed in Protosol was counted in a liquid scintillation counter. GAD activity was expressed as nmol ¹⁴CO₂/mg protein/hr.

GABA transaminase (GABA-T, EC 2.6.1.19) was determined using the method described by Sterry & Fonnum (1978), as modified by Tunicliff & Smith (1981), and expressed as nmol glutamate/mg protein/hr.

In the experiment in which GABA concentration was determined, the hypothalami were homogenized in water. Medium and tissue homogenates were heated for 10 min in a 100°C water bath (Enna et al., 1977) and centrifuged at 10,000 g for 10 min. The supernatants were stored at −70°C until GABA was determined by the [H]-muscimol radioreceptor assay (Bernasconi et al., 1980). Data were expressed as nmol GABA/mg protein.

The protein concentration was determined by the method of Lowry et al. (1951), using bovine albumin as standard.

Data were analyzed by analysis of variance and Duncan's multiple range test (Steel & Torrie, 1960). Differences between means were considered significant if p < 0.05.

**RESULTS**

The effect of different concentrations of prolactin on GAD activity in hypothalami incubated for 15 min is shown in Fig. 1. Prolactin at 0.1 and 0.25 µg/ml produced a significant increase in GAD activity, as compared with the control group. GAD activity was not significantly different from the control value when 0.5 µg/ml of prolactin was added to the incubation medium, while 0.75 µg/ml and 1.0 µg/ml produced a significant decrease in GAD activity.

Under our experimental conditions, hypothalamic GAD activity slightly decreased over the incubation time up to 2 hr (Fig. 2). After 4 hr of incubation, the GAD activity was about 65 - 70% of that obtained after 20 min of incubation, and it then remained stable (until 10 hr). Hypothalamic GAD activity at 20 min of incubation was not significantly
The effect of increasing concentrations of prolactin on GAD activity in hypothalamic slices. Each point represents the mean ± S.E.M. of five hypothalami. ★ p < 0.05; ★★ p < 0.01.

different from that obtained in hypothalami dissected immediately after death and stored overnight at -20°C (274.56 ± 9.69 nmol ¹⁴CO₂/mg prot./hr). GAD activity was significantly stimulated by prolactin (0.25 µg/ml) after 20 and 40 min of incubation. At this concentration, prolactin did not modify GAD activity between 60 min and 6 hr as compared with control values. However, this hormone significantly increased GAD activity after 8 hr of incubation. Prolactin (1.0 µg/ml) produced a slight but significant decrease in hypothalamic GAD activity after 20 min of incubation (Fig. 2). It then remained unchanged until 6 hr of incubation. After longer periods of incubation, prolactin significantly stimulated GAD activity.

In order to investigate the nature of the enhanced GAD activity induced by prolactin, cycloheximide, an inhibitor of protein synthesis, was used. Cycloheximide did not modify basal GAD activity, but it significantly inhibited the increase of GAD activity observed when the hypothalami were incubated with prolactin for 10 hr (Fig. 3).

When hypothalamic slices were incubated for 1 hr with prolactin and thereafter 9 hr without the hormone, both doses of prolactin significantly stimulated GAD activity, as compared with the control group (Fig. 4).

After 10 hr of incubation, hypothalamic GABA-T activity was about 60% of that obtained in hypothalamic slices without incubation (485.19 ± 16.24 nmol glutamate/mg prot./hr). Prolactin did not affect hypothalamic GABA-T activity after 10 hr of incubation (control: 283.79 ± 18.73, n = 5; 0.25 µg/ml prolactin: 315.79 ± 24.93, n = 5;
1.0 μg/ml prolactin: 286.46 ± 23.88, n = 5; 1.0 μg/ml prolactin 1 hr: 259.26 ± 20.78, n = 5).

The effect of prolactin on GABA concentration in the hypothalamic slices and in the medium is shown in Figs 5 and 6. Hypothalamic slices incubated for 10 hr in the presence of prolactin at 0.25 or 1.0 μg/ml showed a significantly lower GABA concentration than that of the control group (Fig. 5). At the same time, GABA concentration in the medium was significantly higher than that of the control group (Fig. 6). When prolactin (1.0 μg/ml) was present only during the first hour of a 10 hr incubation, the GABA
FIG. 3. The effect of cycloheximide on the prolactin-induced increase of GAD activity in hypothalamic slices. Each column represents the mean ± S.E.M. of five to six hypothalami. * p < 0.01: prolactin vs control without prolactin. ☆ p < 0.05: cycloheximide vs respective controls without cycloheximide.

FIG. 4. Ability of prolactin to induce a delayed increase of hypothalamic GAD activity. Hypothalamic slices were incubated for 1 hr in the presence of prolactin and thereafter for 9 hr without the hormone. Each column represents the mean ± S.E.M. of five to six hypothalami. ☆ p < 0.01.
Fig. 5. The effect of prolactin on hypothalamic GABA concentration. Hypothalamic slices were incubated for 10 hr without prolactin (open bars), 10 hr with 0.25 μg/ml prolactin (hatched bars), 10 hr with 1.0 μg/ml prolactin (dotted bars), or 1 hr with 1.0 μg/ml and thereafter 9 hr without the hormone (squared bars). Each bar represents the mean ± S.E.M. of five hypothalami. * p < 0.05.

concentration in the hypothalamic slices and in the medium was similar to, or slightly higher than, that of the control group (Figs 5 and 6).

DISCUSSION

Prolactin secretion is regulated by several neurotransmitters, among which dopamine (DA) is considered the major prolactin-inhibiting factor (PIF). However, a great deal of evidence indicates that GABA may account for at least part of the non-catecholaminergic PIF activity (Schally et al., 1977; Grandison & Guidotti, 1979; Racagni et al., 1982). DA and GABA-synthesizing enzymes coexist in neurons of the arcuate nucleus and in their terminals in the external layer of the median eminence (Tappaz et al., 1983; Everitt et al., 1984). Both transmitters are released into hypophysial portal blood (Gudelsky & Porter, 1980; Mitchell et al., 1983; Gudelsky et al., 1983), reach the anterior pituitary gland, and interact independently with their specific receptors to inhibit prolactin release. Moreover, lesions of the median eminence result in an increase of DA and GABA binding to their specific anterior pituitary receptors (Fiszer de Plazas et al., 1982; Creese et al., 1983). Systemic or intracerebroventricular (ICV) administration of prolactin increases the synthesis and turnover of DA in the median eminence and hypothalamus (Gudelsky et al., 1976; Demarest et al., 1984), pointing to the existence of a feedback mechanism of prolactin on tuberoinfundibular dopaminergic neurons. Since GABA participates in the regulation of prolactin secretion, a feedback interaction between prolactin and the
hypothalamic GABAergic system is to be expected. Lending support to this hypothesis, it has been demonstrated that systemic or ICV administration of prolactin increases hypothalamic GAD activity (Duvilanski et al., 1983) and GABA turnover (Apud et al., 1984). Furthermore, pharmacological procedures influencing serum prolactin levels modulate hypothalamic GAD activity (Duvilanski et al., 1983b; Nicoletti et al., 1983). However, these methods do not allow one to determine whether the substance under study acts directly at the hypothalamic level or on other regions of the central nervous system. Brain slice preparations have been employed to resolve questions of a biochemical or physiological nature. Many of the advantages of this technique have been described by Dunwiddie et al. (1983). One of them is its utility in differentiating between direct and indirect effects of neurotransmitters and drugs. Nishihara et al. (1983) have obtained stable extracellular recordings in hypothalamic slices incubated for up to 12 hr. In our hypothalamic slice preparations, GAD and GABA-T activities partially decreased during the incubation time. The analysis at the ultrastructural level of sections obtained from hypothalamus incubated for 10 hr showed that the cellular structure remained unchanged, although signs of swelling of the tissue were present (data not shown).

In the present study, evidence is furnished indicating a direct in vitro effect of prolactin on hypothalamic GAD activity and on GABA concentration in tissue and medium. After short periods of incubation, prolactin produced a stimulation of hypothalamic GAD
activity. This rapid response to prolactin may occur by activation of the enzyme already present in the tissue. When prolactin was present at a very high concentration in the incubation medium, a transitory decrease in hypothalamic GAD activity was observed. These results are in agreement with previous observations after acute intraperitoneal administration of prolactin (Duvilanski et al., 1983a). A sustained increase in hypothalamic GAD activity was induced by prolactin after long incubation times. This effect seems to be due to an increase in the synthesis of the enzyme, since it involves a process depending upon protein synthesis. These results confirm those reported by Apud et al. (1984), who observed an enhancement of hypothalamic GAD activity after 16 hr of ICV administration of prolactin.

Prolactin induced an increase in hypothalamic GAD activity, but it did not affect GABA-T activity. At the same time, a decrease in hypothalamic GABA concentration and an increase in GABA concentration in the medium were observed, suggesting that prolactin stimulated GABA release. Apud et al. (1984) reported that prolactin increased GABA release into hypophysial portal blood. Moreover, we have demonstrated that prolactin increased GABA release in vitro from small hypothalamic fragments containing median eminence (Duvilanski et al., submitted).

The delayed enhancement of hypothalamic GAD activity also was observed when prolactin was present in the medium only during the first hour of a 10 hr incubation. However, in this condition, GABA concentrations in the hypothalamus and in the medium were similar to those of the control group. These results suggest that prolactin need not necessarily be present throughout the incubation time in order to induce an increase in enzyme activity. However, it may be necessary that prolactin be present for a longer period to induce GABA release.

The results of the present study suggest the existence of a feedback regulation by which prolactin interacts with the hypothalamic GABAergic system, thus showing a direct effect of this hormone at the hypothalamic level.

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REFERENCES


