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Inhibition of Prolactin Secretion by GABA in Female and Male Rats[1] (40482)

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Gamma aminobutyric acid (GABA) is found in large amounts in the brain and has been related to neuronal function, including synaptic transmission (1, 2). In recent publications, GABA has been reported both to inhibit (3–8) and to release (9, 10, 6, 7) prolactin from the pituitary. The aim of the present work was to investigate the effect of exogenous and endogenous GABA on prolactin secretion under physiological conditions in lactating rats and intact normal male rats.

Materials and Methods. Adult Wistar rats were maintained in an air conditioned room with controlled lighting (from 0700 to 1900) and given free access to laboratory chow and tap water.

a) In the first series of experiments, pregnant female rats (230–270 g bw) were used. The day after parturition was designated day 1 of lactation; dams and their litter were maintained in individual cages until experiments were performed on day 10–15 of lactation. The experimental procedures were performed as described previously (11). The day before the experiment, in some dams a silastic catheter was inserted into the jugular vein and a stainless steel cannula implanted into the third cerebral ventricle. The morning of the day of the experiment, the pups were separated from their dams for a period of 5–5½ hr. After an initial blood sample was withdrawn from the catheter, 2 μl of 0.9 NaCl solution as control or a solution of GABA (Sigma) was microinjected into the cerebral ventricle by the cannula. The doses of GABA used were 150 μg (1.46 μmol/rat) and 825 μg (8.00 μmol/rat) (I Vent route).

A second group of dams without ventricular cannula were injected ip with aminooxyacetic acid hemi-hydrochloride (AOAA; Sigma), 30 mg/kg bw, or an equal volume of saline solution. Since AOAA produced a sharp increase of GABA content in the CNS by 90 min (12); ip injections were done 1½ hr before zero time. Blood samples were withdrawn before AOAA injections (pre-basal sample) and at zero time (basal sample). After basal sample withdrawal and GABA or AOAA injections, pups were caged with their treated mothers. When they began to suckle actively, the time was recorded and subsequent samples were collected 10 and 30 min later (suckled mothers: SM).

One set of dams was similarly treated except that the pups were kept separated from them (non-suckled mothers: NSM). In these rats, the time of drug injection was designated as zero time.

b) In a second series of experiments, normal male rats (250–300 g bw) were used to determine if GABA has a physiological role in the prolactin increase that occurs during stress.

A group of males bearing chronic ventricular cannula were kept anesthetized with ether during the entire procedure. Three min after inducing anaesthesia, an initial blood sample was collected from the external jugular vein and 2 μl of saline solution or of a solution containing 8 μmol GABA were immediately microinjected into the ventricle. A subsequent blood sample was collected similarly 15 min after injection.

Another group of males were injected with saline solution or with 30 mg/kg of AOAA, 90 min before the experiment. Each group was divided into two subgroups: one was left undistributed and the other was subjected to

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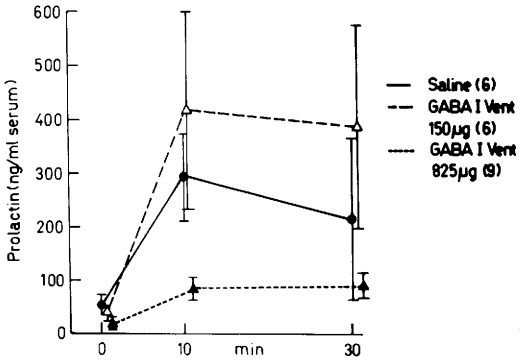

FIG. 1. Partial suppressive effect of GABA microinjected in the third ventricle on prolactin release in suckled mothers. After 10 min, serum prolactin in GABA (825 µg) treated rats was lower than that in controls (saline) ($P < 0.05$). In this and subsequent figures vertical lines above and below the means indicate 1 SEM. The number of animals per group is shown in parentheses.

stress until rats were decapitated and blood was collected from the trunk. Stress was induced by keeping the rats under ether fumes for 3 min before blood collection.

Serum prolactin was determined by RIA using kits provided by NIAMDD[5]; results were expressed in terms of RP-1 rat prolactin standard. A paired *t* test was used to calculate the significance of changes in prolactin titers produced by treatment. Student's *t* test was used to compare differences between the groups.

Results. Intraventricular GABA and prolactin secretion of suckled mothers. In control dams injected with saline into the ventricle, suckling sharply enhanced serum prolactin (Fig. 1). At the lower dose, GABA did not alter serum prolactin of SM, but at the higher dose it partially suppressed the serum prolactin rise due to suckling. After 10 min, serum prolactin in GABA treated rats was lower than that in controls ($P < 0.05$).

Intraperitoneal AOAA in suckled mothers. Injection of AOAA, i.p., significantly suppressed the prolactin increment during the suckling period of 10 and 30 min ($P < 0.05$) (Fig. 2). AOAA did not modify serum prolactin titers in SM before pups were caged with their treated mothers and rats began to suckle actively (between pre-basal and basal samples).

GABA and AOAA in non-suckled mothers. In NSM microinjected with saline into the ventricle prolactin remained low in all samples (Fig. 3). GABA (825 µg) did not modify prolactin levels in NSM compared with controls. In addition intraperitoneal AOAA was ineffective in altering serum prolactin in NSM during the entire experiment (not shown).

Intraventricular GABA in male rats under ether stress. After 3 min of ether stress and before any drug administration, the high level of serum prolactin was similar in both groups

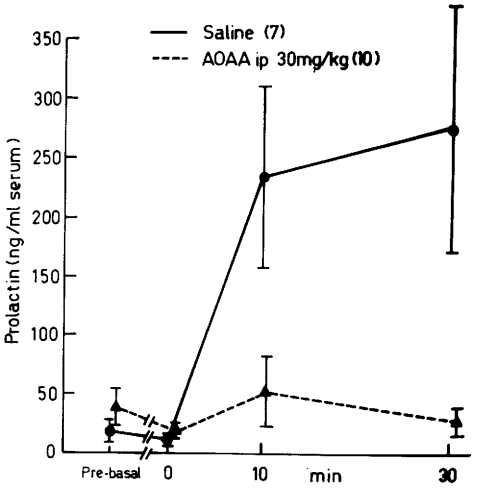

FIG. 2. Aminooxyacetic acid suppressed the prolactin increment during the suckling period of 10 and 30 min ($P < 0.05$). AOAA did not modify serum prolactin titers between prebasal and zero min samples.

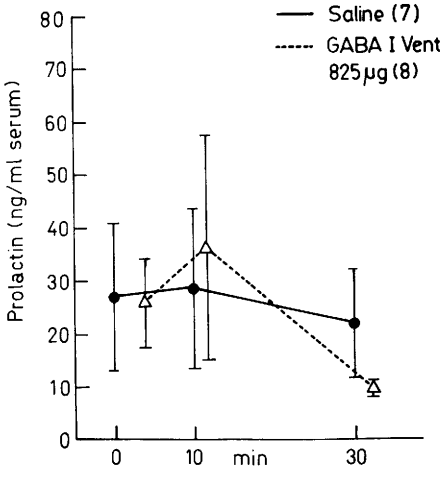

FIG. 3. In non suckled mothers microinjected with saline, prolactin remained low in all samples. Intraventricular GABA had no effect.

[5] The authors thank NIAMDD for the prolactin kits.

(Fig. 4); 18 min after administering ether (i.e. 15 min after injections) prolactin levels were lower in GABA injected rats than in controls ($P < 0.05$).

AOAA in normal male rats under ether stress. As expected, ether stress sharply enhanced serum prolactin in control adult male injected with saline ip ($P < 0.001$). In contrast, in rats injected with AOAA, a partial suppression in the hormone increase was observed; no significant difference was found between both AOAA injected groups (Fig. 5).

Discussion. Our present results indicate that microinjection of GABA into the third ventricle of suckled mothers partially prevented the rise in serum prolactin. AOAA, which produced a sharp increase in GABA content in the brain at the dose, route and time used (12), showed a more complete and lasting suppressive action on prolactin release in suckled dams. Possibly, AOAA had other effects apart from inhibiting GABA degradation, but there is close similarity in the results produced by GABA and AOAA in this and previous studies (2, 3, 13, 14). In non suckled mothers, GABA injected into the ventricle, or AOAA i.p., had no effect on prolactin release. In normal male rats, GABA or AOAA significantly suppressed the stress-induced elevation of serum prolactin.

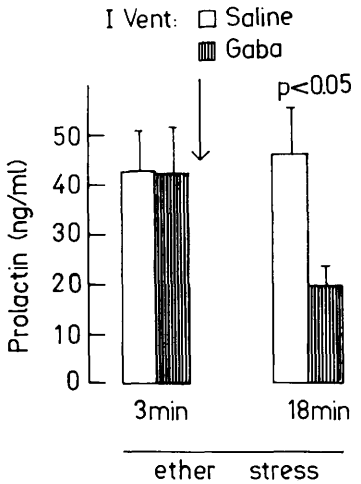

FIG. 4. After 3 min of ether stress and before saline or GABA injections, the high level of serum prolactin was similar in both groups. After 18 min of ether stress (i.e. 15 min after injections) serum prolactin in GABA treated rats was lower than controls ($P < 0.05$; 6–9 animals per group).

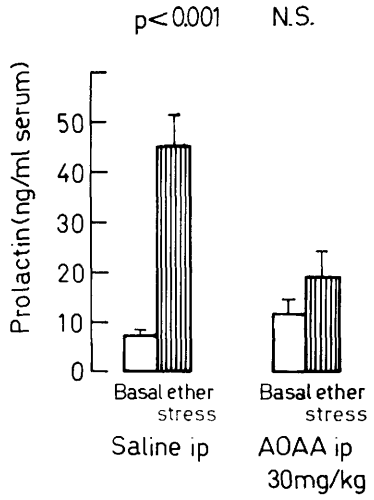

FIG. 5. Ether stress enhanced serum prolactin in saline injected rats ($P < 0.001$). In contrast, in rats injected with AOAA a suppression in the hormone increase was observed (6–9 animals per group).

A small decrease in serum prolactin after intraventricular injection of 150 μg of GABA was described in ovariectomized estrogen-primed rats, while AOAA was able to decrease the hormone release dramatically in those animals (3). In suckled mothers, the blocking effect of GABA was observed with 825 μg, but not with 150 μg. This difference in the amount of GABA needed to significantly decrease serum prolactin, could be due to the fact that suckling is one of the most powerful stimuli for the release of this hormone.

The inhibitory effect of GABA on prolactin secretion is in agreement with previous studies: Schally *et al.*, first reported that GABA inhibited prolactin release, both "in vitro" and "in vivo" (4). Hall *et al.* (5) also described an inhibitory effect of GABA on prolactin release by rat pituitaries incubated "in vitro", in which prolactin synthesis and release is enhanced (15). Fuxe *et al.* reported inhibition of prolactin release by ibotonic acid, a GABA agonist drug (16) and more recently, Rivier and Vale found a suppressive action of GABA "in vitro", while GABA "in vivo" was able to interfere with some drugs which are stimulators of prolactin secretion (6).

By contrast, a stimulatory effect of GABA on prolactin release was described by others. Mioduszewski *et al.* found that infusion of

GABA into the lateral ventricle of intact and ovariectomized rats significantly stimulated prolactin secretion (10). Ondo and Pass described the same releasing action of GABA in male rats (9); a slight stimulation of the hormone secretion was also observed in steroid primed male rats (6).

Thus, previous work has yielded some apparently controversial results, with some investigators reporting inhibition of prolactin release and others stimulations. These apparent discrepancies could have been due to differences in dose (7) or the experimental conditions used, or some other factor. The present results clearly show that an increase in GABA content in the brain, due to microinjection of exogenous GABA or by suppression of enzymatic inactivation of endogenous GABA, was able to inhibit prolactin release under physiological situations i.e., in lactating mothers and in normal male rats under stress. Furthermore in some circumstances, such as NSM, GABA was ineffective. According to these results, and taking into account publications reported in the literature, it seems that inhibitory effects of GABA are more easily demonstrable when levels of prolactin are high or when the prolactin release is stimulated. On the other hand, stimulatory effects of GABA on prolactin release seems to be obtained more easily in situations with low initial level of the hormone.

The present results have provided some further insight into the regulation of prolactin release but the relationship between GABA, peptides containing GABA and the proposed prolactin inhibitory and releasing factors is still not completely understood and warrants further investigation.

Summary. The effect of GABA on prolactin secretion from the pituitary under physiological conditions in lactating female rats and in normal male rats was investigated. Prolactin was measured by RIA in blood samples taken from lactating mothers by means of an intrajugular silastic catheter, 10–15 days after delivery. GABA in doses of 825 μg, but not 150 μg, microinjected into the third ventricle partially suppressed the serum prolactin rise due to suckling. Aminooxyacetic acid (AOAA), 30 mg/kg ip, which has been reported to produce a sharp increase in GABA content in the CNS, significantly suppressed the prolactin increment during the suckling period. By contrast, in non suckled mothers, GABA or AOAA was ineffective in altering serum prolactin. In normal male rats, GABA and AOAA were able to partially suppress prolactin release due to ether stress. These results clearly show that increments in GABA content in the brain after microinjection of exogenous GABA or following suppression of enzymatic inactivation of the endogenous amino acid were able to inhibit prolactin release under physiological conditions.

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