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Effect of 3-Aminopropanesulfonic Acid and Allylglycine on Prolactin Release in Male Rats (41262)

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Abstract. The effect of the administration of a γ-aminobutyric acid (GABA) receptor agonist, 3-aminopropanesulfonic acid (3-APSA), and an inhibitor of the enzyme glutamic acid decarboxylase, allylglycine, on prolactin secretion was studied in castrated male rats. Serum prolactin levels were determined in basal conditions, after the injection of the drugs, and after the administration of sulpiride. The injection of 3-APSA did not significantly alter serum prolactin levels, but did decrease the release of prolactin after the administration of sulpiride. The injection of allylglycine increased the prolactin response to sulpiride. These data are consistent with the possibility that GABA may have an inhibitory influence on prolactin secretion.

During the past few years considerable interest has been centered on the role of γ-aminobutyric acid (GABA) on prolactin secretion (1–7). Some authors have reported a prolactin-releasing effect of GABA (1–3), while, more recently, a number of authors reported that GABA might have an inhibitory role on prolactin secretion (4–7). A useful approach to study the role of GABA on prolactin secretion would be to decrease the synthesis of this amino acid in the brain by blocking glutamic acid decarboxylase (GAD), the enzyme that regulates the formation of GABA from glutamic acid (8). In the present investigation, allylglycine (AG), a GAD inhibitor (9), was given to castrated male rats, and serum prolactin levels were studied in basal conditions and after the injection of sulpiride, a drug that induces prolactin release (10). In addition, the effect of 3-aminopropanesulfonic acid (3-APSA), a GABA receptor agonist (11), was also studied, and serum prolactin levels were determined before and after stimulation of prolactin release with sulpiride.

Materials and Methods. Male rats of the Wistar strain were used throughout the experiments. The animals were fed laboratory chow and water *ad lib*. In the first part of the experiment, male rats aged 3 months were castrated and used 3 days later. On the day of the experiment the rats were anesthetized with urethane (0.6 ml/100 g body wt of a 25% solution). Blood was drawn through the jugular vein and immediately thereafter 3-APSA (Sigma Chemical Co.), dissolved in saline, at a dose of 40 mg/rat was injected iv. Control rats were injected with saline. Fourteen rats per group were used. Blood was again drawn at 30, 180, and 290 min after the injection. After the last blood sample was obtained, saline or sulpiride (100 μg/rat) was injected iv and 20 min later the rats were decapitated and blood was collected from the trunk. Sera were separated and kept frozen until assayed for prolactin. In the second part of the experiment, male rats aged 4 months were castrated and used 7 days later. On the day of the experiment the rats were anesthetized with urethane (at the same dose described before). Blood was drawn through the jugular vein and immediately thereafter allylglycine (Sigma Chemical Co.), dissolved in saline, was injected iv at a dose of 10 mg/100 g body wt. Control rats were injected with saline. Seventeen rats per group were used. Blood was again drawn at 3, 5, and 6 hr after the injection. After the last blood sample was obtained, saline or sulpiride (100 μg/rat) was injected iv. Twenty minutes later the rats were decapitated and blood was collected from the trunk. Sera were separated and kept frozen until assayed for prolactin. Prolactin was determined in each serum sample using the double-antibody radioimmunoassay de-

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scribed by Niswender *et al.* (12), with kits distributed by the NIAMDD, NIH, Bethesda, Maryland. The significance of the differences between groups was evaluated with Student's *t* test and Duncan's new multiple range test (13).

Results. In the first part of the investigation the iv injection of saline did not significantly modify serum prolactin levels from 0 to 290 min. Serum prolactin levels, in the same time period, in rats injected with 3-APSA did not differ from those of control rats. Sulpiride induced a highly significant release of prolactin in rats previously treated with saline or 3-APSA ($P < 0.01$) (Fig. 1). The magnitude of the increment of serum prolactin was significantly lower in the rats treated with 3-APSA than in the respective control rats ($P < 0.01$). In the second part of the investigation, serum prolactin levels in the group of rats treated with allylglycine were not significantly different from those of control rats between 0 and 6 hr, although at this last time period serum prolactin levels in allylglycine-treated rats showed a trend to increase (53.9 ± 12.1 in control group vs 104.6 ± 25.8 in the allylglycine group). The iv injection of sulpiride resulted in significant serum prolactin increases in both control and allylglycine-treated rats ($P < 0.01$). The level of prolactin after sulpiride was significantly greater in allylglycine-treated rats than in the control animals ($P < 0.05$) (Fig. 2).

Discussion. From the results of the present investigation it is evident that the rats injected with 3-APSA had a lower response to sulpiride whereas the rats treated with allylglycine had a higher response to this drug, in terms of prolactin release. The specificity of 3-APSA as a GABA receptor agonist was previously shown by the fact that 3-APSA inhibits [^{3}H]muscimol binding to rat brain membranes (14). The effect of 3-APSA was also shown to be blocked by bicuculline, a GABA receptor antagonist (15). Although allylglycine cannot be considered as absolutely specific, it has been reported that its administration to mice did not affect brain catecholamines or their metabolites (16). This finding suggests that the modifications of prolactin release seen in our experiments can be considered to be due to GABA alterations and not to modifications of catecholamine levels in the brain induced by this drug. The rather long delay in prolactin modifications after allylglycine is consistent with the report by Lores Arnaiz *et al.* (9), which showed that the effects of allylglycine are not demonstrated until 2 hr after the injection of the drug. This delay

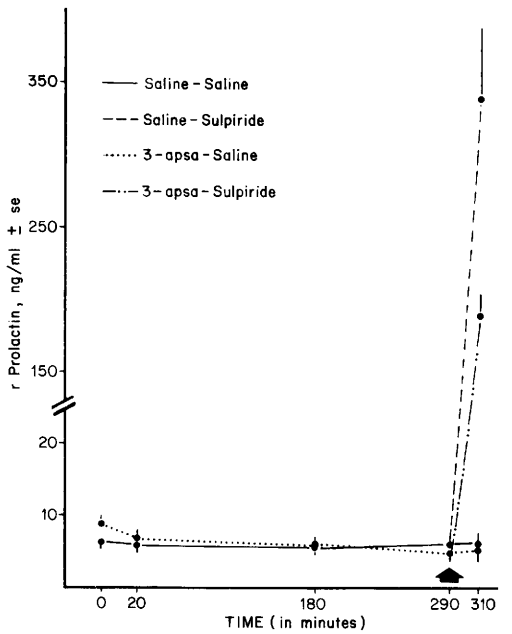

FIG. 1. Effect of 3-APSA on prolactin levels in serum before and after the acute injection of sulpiride. Values are means ± SE.

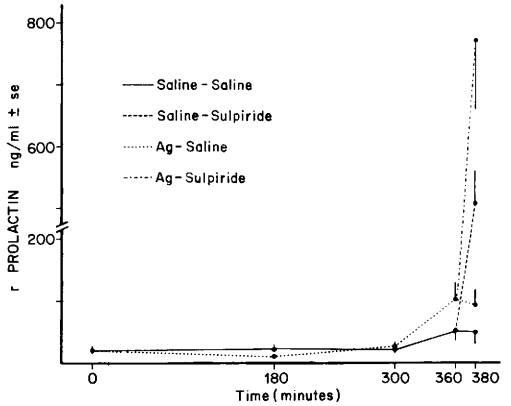

FIG. 2. Effect of allylglycine (AG) on serum prolactin levels before and after the acute injection of sulpiride. Values are means ± SE.

is due to the fact that the active substance does not seem to be allylglycine itself, but a metabolite, 2-keto-4-pentenoic acid, which is slowly formed after allylglycine administration (17). Allylglycine, being an inhibitor of GAD, decreases GABA levels in the brain (18). Allylglycine enters rapidly into the central nervous system (18), so its effects on prolactin secretion should be exerted on brain structures that normally influence the release of this pituitary hormone. It must be pointed out that the anterior pituitary gland completely lacks GAD activity (19); therefore a direct action of allylglycine on the adenohypophysis could be ruled out. The increase of prolactin in serum and a higher response to sulpiride after the administration of allylglycine suggest an inhibitory role of GABA on prolactin secretion. The injection of a GABA agonist acting at the receptor level, as 3-APSA, was followed by a decreased release of prolactin after sulpiride, which again suggests an inhibitory influence of GABA on prolactin secretion. Since it has been recently demonstrated that the anterior pituitary gland contains GABA receptors (7), it is possible that 3-APSA exerted at least part of its effects on prolactin release directly on the prolactin cells, in addition to a possible effect on brain tissue. These results are consistent with a recent report from our laboratory (20) and offer further evidence that GABA may be involved in the control of prolactin secretion as an inhibitory modulator, acting at the brain and also probably at the pituitary level. Other authors, however, found that the intraventricular injections of GABA induced a release of prolactin, rather than inhibiting it (1). McCann *et al.* (21) reported that according to the dose of GABA injected intraventricularly, inhibition or stimulation of prolactin release can be found. Libertun and McCann (22) reported that the intraventricular injection of aminooxyacetic acid, an inhibitor of the enzyme GABA-transaminase, was followed by decreased prolactin release. Locatelli *et al.* (23) tried to explain these conflicting results by stating that GABA may exert an inhibitory effect on prolactin release by acting directly at the level of the anterior pituitary gland, but being stimulatory by acting through central pathways ending in the hypothalamus.

Beyond the conflicting results reported by different groups of authors, it is becoming evident that GABA might have a role in the control of prolactin secretion as well as of other pituitary hormones, although the physiological characteristics of this role are still far from being completely understood.

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