PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 225-229 (1976)

The Effects of Neurotransmitter Receptor Antagonists on Ether-Induced Prolactin Release in Ovariectomized, Estrogen-Treated Rats[1] (39515)

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The determination of the neurotransmitters that regulate ether-induced prolactin release is under current investigation in our laboratories. Numerous reports have indicated that ether increases plasma prolactin levels (1–10), but little is known about the neurotransmitter systems in the central nervous system that are involved in this response. The administration of nicotine has been shown not to affect the ether-induced increase in prolactin (9), whereas blockage of serotonergic receptors with methysergide (11) and stimulation of dopaminergic receptors with apomorphine (3) block this response. Evidence exists that ether influences noradrenergic systems in the rat brain (12); however, reserpine treatment failed to alter ether-induced prolactin release in male rats (11, 13). The purpose of this study was to determine if the antagonism of several putative neurotransmitter systems plays any role in the secretion of prolactin in ether-stressed, ovariectomized, estrogen-treated rats.

Materials and methods. Sixty mature, female Sprague–Dawley rats (Spartan Research Animals, Inc., Haslett, Mich.) weighing 180 to 260 g were randomly divided into 10 groups. After 6 to 7 days of acclimation to lighting conditions (lights on from 0600 to 2000 hr) the rats were bilaterally ovariectomized. Seven to 14 days after ovariectomy 0.5 mg of polyestradiol phosphate (PEP; 1 mg of Estradurin, Ayerst Laboratories, Inc.) was administered to each animal subcutaneously and 7 days later beginning at 0900 the following experimental protocol was initiated. All animals except one group (uninjected controls) were weighed and injected intraperitoneally with vehicle (saline or 95% EtOH) or drug. The dosage of each drug except the histaminergic antagonists was based on previous studies (18, 19). The dosage of each of the histaminergic antagonists was based on information supplied by the manufacturers. Twenty-five minutes after injection all animals were anesthesized by an initial exposure to ether vapor in a large container followed by maintenance with a nose cone. Ether anesthesia once initiated was maintained throughout the duration of the experiment. Blood samples (0.5 ml) were taken 5, 15, 25, and 35 min after ether exposure (30, 40, 50, and 60 min after injection) via orbital sinus puncture and transferred to tubes containing 0.5 ml of heparinized phosphate-buffered saline (50 units of heparin/ml). The diluted plasma was collected and stored at −20°C until assayed.

The drugs employed in this study were: phenoxybenzamine-HCl (a gift from Smith, Kline and French Laboratories, Philadelphia, Pa.), phentolamine-HCl (Regitine, a gift from Ciba Pharmaceutical Co., Summit, N.J.), propranolol-HCl (Inderal, a gift Ayerst Laboratories, Inc., New York, N.Y.), 2-chloro-2′-[3-dimethylamino)propyl]thio-cinnaminilide-HCl (SQ 10,631, a gift from E. R. Squibb and Sons, Inc., Princeton, N. J.), metiamide-HCl (a gift from Smith, Kline and French Laboratories, Philadelphia, Pa.), pyrilamine maleate (generously provided by Merck, Sharp and Dohme, Rahway, N.J.), and methysergide maleate (a gift from Sandoz Pharmaceutical Division, Hanover, N.J.). All drugs were given intraperitoneally at a dose of 10 mg/kg, except SQ 10,631 and methysergide, which were given at 2.5 mg/kg.

Plasma samples were assayed by double antibody radioimmunoassy (14) in duplicate, each at two dilutions. Rat prolactin NIAMDD-RP-1 (11 IU/mg), supplied through the Rat Pituitary Hormone Distri-

[1] Supported by N.I.H. General Research Support Grant No. RR05384-14 to Wayne State University School of Medicine (D.M.L.) and N.I.H. Grant HD 07722 (R.R.G.).

bution Program of the National Institute of Arthritis, Metabolism and Digestive Diseases, was used as the standard.

One-way analysis of variance (15) was used to assess statistical significance between the vehicle-injected control and experimental groups at each time period.

Results. The response of prolactin to ether anesthesia was significantly ($P < 0.025$) elevated by handling and/or injection of vehicles compared to non-handled uninjected controls. Plasma prolactin levels in saline- and ethanol-injected animals, however, were not different from each other at any of the time periods examined (Fig. 1A). The administration of α-adrenergic blocking drugs, phenoxybenzamine and phentolamine, significantly enhanced the ether-induced elevation in prolactin at 5 and/or 15 min but not at 25 or 35 min of ether anesthesia (Fig. 1B). In contrast, β-adrenergic blockade with propranolol had little to no effect on ether-induced prolactin release (Fig. 1C).

The administration of H_1 and H_2 histaminergic blockers, pyrilamine and metiamide,

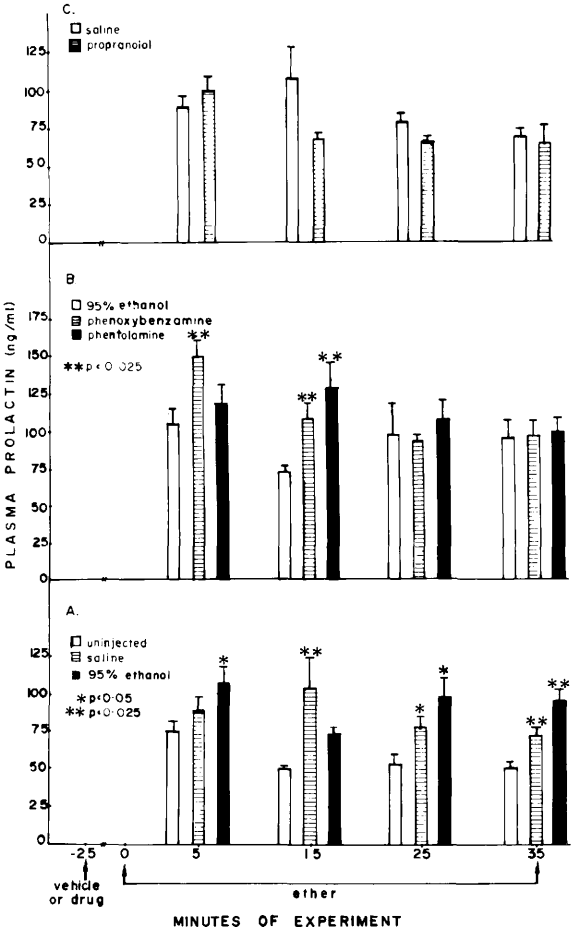

FIG. 1. (A) Comparison of plasma prolactin levels of uninjected and vehicle-injected ovariectomized, estrogen-treated rats during ether anesthesia. Values represent the mean ± SEM of six animals in each group. (B) Effect of α-adrenergic antagonists, phenoxybenzamine and phentolamine, at 10 mg/kg ip on plasma prolactin levels in ovariectomized, estrogen-treated rats during ether anesthesia. Values represent the mean ± SEM for six animals in each group. (C) Effect of a β-adrenergic antagonist, propranolol, at 10 mg/kg ip on plasma prolactin levels in ovariectomized, estrogen-treated rats during ether anesthesia. Values represented the mean ± SEM for six animals in each group.

tended to blunt the ether-induced release of prolactin but only pyrilamine showed a significant ($P < 0.025$) suppression at 25 min of ether anesthesia when compared to saline-injected controls (Fig. 2A).

Methysergide and SQ 10,631, two serotonergic antagonists, had different effects on the ether-induced prolactin response. Methysergide significantly ($P < 0.025$) increased prolactin secretion initially (5 and 15 min) whereas SQ 10,631 significantly ($P < 0.05$) depressed the prolactin response at 35 min of ether anesthesia (Fig. 2B).

Discussion. The data presented in this report indicate that the ether-induced increase in plasma prolactin in ovariectomized, estrogen-treated rats can be altered by nonspecific factors such as handling and intraperitoneal injection of vehicles and by the intraperitoneal administration of α-adrenergic, histaminergic, and serotonergic antagonists.

Previous data from our laboratories have shown that the ether-induced increase in prolactin may involve antagonism of an inhibitory dopaminergic influence on pituitary prolactin release (3). It is possible that ether may directly inhibit dopamine neurotransmission in the hypothalamo–hypophyseal axis or indirectly affect dopamine neurotransmission by stimulating other systems that have inhibitory effects. The report that ether enhances [^{3}H]norepinephrine disappearance from the brain (12) may support an indirect mode of action. Whether or not ether has such an action in selective areas of the hypothalamus which control prolactin secretion is unknown. The present data show that the ether response is significantly enhanced by α-adrenergic blockade with phenoxybenzamine and phentolamine (Fig. 1B), indicating that noradrenergic influences, like dopaminergic systems, may be antagonistic to ether-induced prolactin release. However, these agents may have some overlapping blockade of dopaminergic receptors. Dopamine antagonism has been demonstrated for phentolamine by MacLeod and Lehmeyer (16) at the level of the pituitary.

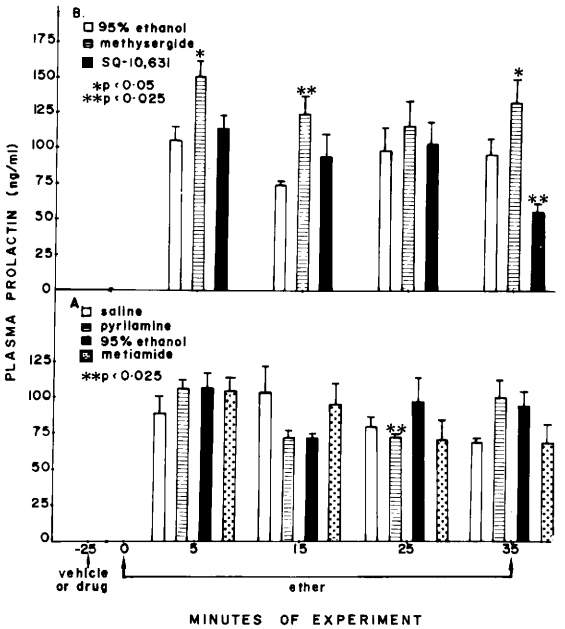

FIG. 2. (A) Effect of H_1- and H_2-histaminergic antagonists, pyrilamine and metiamide, respectively, at 10 mg/kg ip on plasma prolactin levels in ovariectomized estrogen-treated rats. Comparisons were made between pyrilamine and saline and between metiamide and 95% EtOH. Values represent the mean ± SEM for six animals in each group. (B) Effect of serotonergic antagonists, methysergide and SQ 10,631, at 2.5 mg/kg ip on plasma prolactin levels in ovariectomized, estrogen-treated rats. The values represent the mean ± SEM for six animals in each group.

To the authors' knowledge this is the first report to show a role of histaminergic neurotransmission in ether-induced prolactin release. Libertun and McCann (17) have shown that intraventricular histamine elevates basal levels of prolactin and that diphenylhydramine, an H_1-receptor antagonist, blocks this rise and also the rise induced by restraint. In the present investigation pyrilamine maleate, a fairly selective H_1-histaminergic receptor blocker, significantly blunted the ether-induced rise in prolactin whereas metiamide, a selective H_2-histaminergic blocker, had little to no effect on the ether-induced response.

The decrease in ether-induced prolactin release produced by serotonergic receptor blockade in females supports in general the observations of Marchlewska-Koj and Krulich (11) in males. The delayed inhibitory effect of SQ 10,631 on the ether-induced response was similar to that recently reported by us for pimozide-induced prolactin release (18). The stimulatory effect of methysergide observed here is in contradiction to a recent report that methysergide blocked the ether-induced increase in prolactin (11). This apparent conflict may be accounted for by a sex difference or by a difference in drug dosage (2.5 vs 50 mg/kg). The importance of a sexual difference in the prolactin response to methysergide is supported by the observations that methysergide induced no change in baseline values in males (11) but caused increased baseline values in females (19, 20). Indeed, this prolactin-releasing effect of methysergide in the female may be responsible for the elevated ether-induced response noted in the early time periods in the present study. These comments on sex and dose differences notwithstanding, SQ 10,631 and methysergide may produce different effects on prolactin secretion because of different mechanisms of blockade at the serotonin receptor. It is also possible that multiple types of serotonin receptors may exist in the CNS.

These pharmacologic studies by no means demonstrate unequivocally that the ether-induced prolactin response involves inhibition of hypothalamic α-adrenergic neurons or stimulation of H_1-histaminergic and serotonergic neurons, but do warrant more extensive studies along these lines. The additional possiblity that other nonspecific stresses such as handling and/or intraperitoneal injections can enhance the ether-induced response indicates the need for future work on the nature of the pathways over which different types of stresses increase prolactin release. The nonspecific effects of the drugs over and above the effects of handling and injection also cannot be completely discounted. In other studies, however, changes in blood pressure as a result of the injection of several drugs were not correlated with changes in prolactin release (unpublished observations). This suggests that the alterations in prolactin release observed in this study were probably not due to nonspecific stresses induced by the drugs.

Summary. The role of various neurotransmitter receptor antagonists in the ether-induced release of prolactin in estrogen-treated ovariectomized rats was investigated. Serial blood samples were obtained by orbital sinus puncture at 5, 15, 25, and 35 min of continuous ether anesthesia. Animals were pretreated 25 min before the onset of anesthesia with either α- or β-adrenergic, H_1- or H_2-histaminergic, or serotonergic antagonists. α-Adrenergic blockade increased the ether-induced release of prolactin during the early phase of ether anesthesia as did treatment with methysergide, a 5-HT antagonist. Blockade of H_1-histaminergic receptors and treatment with another 5-HT antagonist, SQ 10,631, decreased the ether-induced response in the later phase of anesthesia. β-Adrenergic and H_2-histaminergic blockade were without effect on the ether-induced release of prolactin. These studies indicate the possible involvement of noradrenergic, histaminergic, and serotonergic systems in the release of prolactin following ether anesthesia in the female rat.

The authors wish to express their appreciation to Mrs. C. Van De Walle for her expert technical assistance and to the Rat Pituitary Hormone Distribution Program of NIAMDD for the generous gift of rat prolactin.

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Received March 25, 1976. P.S.E.B.M., 1976, Vol. 153.