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Cimetidine Inhibits the Histamine-Induced Prolactin Release in Male Rats (40770)[1]

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The participation of hypothalamic monoamines in the regulation of anterior pituitary function is now clearly established. Dopamine, norepinephrine, and serotonin have all been extensively studied and are considered proven central neurotransmitters which modulate prolactin (PRL) release (1). Although the presence of histamine (HA) in pituitary and hypothalamic tissues has been established for decades, only recently has evidence accumulated suggesting that endogenous HA may be a central neurotransmitter (2, 3, 4). Intraventricular HA has been demonstrated to cause a release of PRL and LH, but the specificity and mechanisms of action of these effects are still unclear (5, 6).

Since the effects of HA are mediated through an interaction with specific H_1 and H_2 cellular receptors (7), the antagonists to these receptors were used in the present study in order to determine which group of receptors is involved in mediating the effects of PRL and LH release. In addition, cholinergic and serotoninergic antagonists were also utilized to evaluate whether the HA effect is direct or the result of an interaction with another monoaminergic system.

Materials and methods. Approximately 200 male Wistar rats (Harlan Labs) weighing 500–600 g and 16–18 weeks of age were utilized in these experiments. On the day prior to the experiment, groups of six rats were subjected to atrial catheterization according to the procedure described by Terkel (8). On the following morning, each animal was successively subjected to ether anesthesia, surgery, intraventricular (icv) infusion, and bleeding. An initial blood sample of 0.5 ml was taken just prior to infusion (0 time) with subsequent samples taken at 15, 30, 45 and 60 min after initiation of icv infusion. In the case of two infusions, sequential blood samples were taken following the second infusion. The volume of blood removed at each sampling was replaced with an equal volume of normal saline. Spontaneous outflow of cerebrospinal fluid from the infusion cannula confirmed proper placement within the third ventricle. Anesthesia was maintained until termination of infusion.

The infusion solutions of histamine, saline, and drugs were delivered at a rate of 1 μl/min. Histamine dihydrochloride was administered in concentrations of 25 or 90 μg (free base)/5 μl normal saline. Control animals were given 5 μl normal saline or saline acidified to pH 4.1 (pH of the histamine solution). For purposes of representation these saline control groups were combined, as the results were identical. Other groups of six animals each were given icv infusions of one of the following drugs: diphenhydramine 10 or 35 μg/2 μl saline; cimetidine 300 μg/2μl aqueous diluent; atropine sulfate 250 μg/2μl saline; methysergide maleate 12 μg/μl saline. Half of these groups received icv drugs alone, the remainder received icv infusions of drugs followed in 10–15 min by icv HA.

Serum PRL and LH were determined by radioimmunoassay using kits supplied by NIAMDD (NIH). Results were expressed as nanogram/milliter in terms of the reference preparations Rat-PRL-RP-1 and Rat-LH-RP-1. The data was divided into two separate groups according to the hormone assayed. A two-way analysis of variance (SAS, GLM Procedure) was performed on each group to ascertain the significance of the main effects of treatment and time, and their interaction. Since a major goal of this study involved comparisons of treatments at given times, statistically significant dif-

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ferences of these main effects were further defined by one-way analyses of variance. Subsequent specific differences between treatment and control values were established with Duncan's multiple range test.

Results. The infusion of 90 μg HA resulted in a dramatic rise in PRL to 266 ± 13 ng/ml at 15 min; LH levels, however were not altered (Fig. 1). The slight rise from basal levels in PRL seen following 25 μg HA was not statistically significant but was significant when compared to the saline control curve. In animals receiving icv saline or acidified saline the serum PRL level fell to below baseline values at 15 min and remained low, possibly due to termination of the stimulatory influences of ether and surgical manipulation on PRL release.

Diphenhydramine (DPH), an H_1 receptor antagonist, given icv prior to HA, failed to modify significantly the rise in PRL induced by HA as shown in Fig. 2 ($P > 0.05$). A larger dose of DPH (35 μg) was also given prior to icv HA (25 μg) in an attempt to equalize the concentrations of agonist and

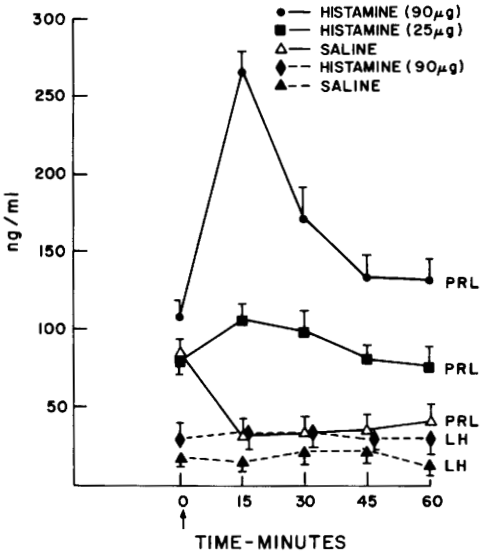

FIG. 1. Levels of serum PRL and LH in normal male rats subjected to icv infusions of HA or saline. In this and subsequent figures the infused substances and concentrations are indicated in upper right area of figure. Vertical arrow (↑) indicates onset of infusion of either HA or other substances when given alone. Shown are the mean ± SEM. $n = 6$. See text for details.

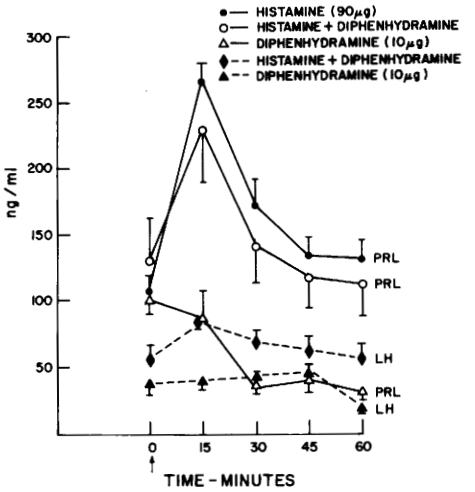

FIG. 2. Levels of serum PRL and LH in normal male rats subjected to icv infusions of HA (90 μg) and/or diphendydramine (10 μg). Shown are the mean ± SEM. $n = 6$.

antagonist. Again DPH did not alter the HA-induced rise in PRL (data not presented). Animals given DPH alone demonstrated a gradual decline in PRL values. Serum LH was unaltered by DPH, and the administration of DPH (10 μg) and HA (90 μg) was associated with an insignificant rise in LH levels at 15 min.

The results obtained with the use of cimetidine, an H_2 receptor antagonist, are shown in Fig. 3. Cimetidine given prior to HA significantly blunted the rise in PRL induced by HA at 15 min post infusion ($P < 0.01$). Animals given cimetidine alone demonstrated a rise in PRL similar to that induced by HA, but of lesser magnitude. Serum LH remained unchanged following cimetidine, and the slight rise following cimetidine and HA administration was not significant ($P > 0.05$).

Methysergide, a serotonin antagonist, had no effect on the PRl rise induced by HA when given icv prior to HA. Similar to cimetidine, methysergide also elicited a rise in PRL values at 15 min with return to baseline at 45 min. Serum LH was unaltered by methysergide administration either alone or prior to HA.

Intraventricular atropine sulfate, a muscarinic anticholinergic agent, also failed to

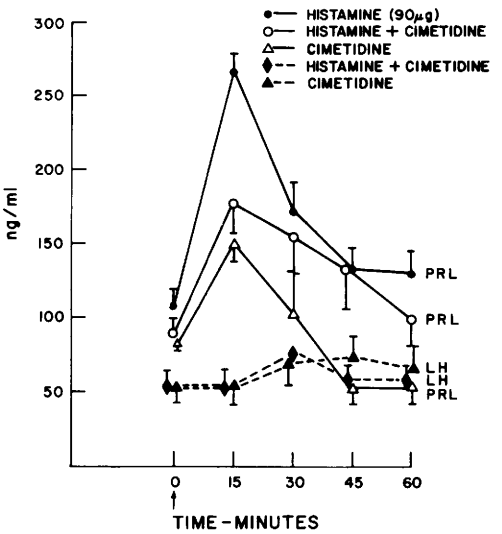

FIG. 3. Levels of serum PRL and LH in normal male rats subjected to icv infusions of HA (90 μg) and/or cimetidine (300 μg). Shown are the mean ± SEM. $n = 6$.

modify the rise in PRL induced by HA. The dose employed (250 μg) has previously been demonstrated to block the release of gonadotropins and PRL during proestrus in the rat (9). Atropine alone did not cause any change in PRL or LH levels. (Figures for the methysergide and atropine data not presented).

Discussion. These experiments clearly demonstrate that icv HA has a marked stimulatory effect on PRL release, as has been previously shown (5, 6). The major thrust of this study, however, was to examine the mechanisms and specificity of action of HA on PRL release. This was investigated by use of specific antagonists administered into the third ventricle to avoid difficulties associated with systemic administration, including drug distribution across the blood–brain barrier.

Pretreatment with the H_1 and H_2 receptor antagonists, diphenhydramine and cimetidine, demonstrated that only cimetidine blocked the stimulatory effect of HA on PRL release. This suggests that H_2 receptors are involved in mediating the HA effect on PRL release. In addition to this inhibitory effect of cimetidine, the drug alone elicited an increase in PRL levels. This effect of cimetidine may have been due to an initial stimulatory interaction with H_2 receptors, or due to the slight dopamine antagonist properties of cimetidine.

While this work was in progress, two studies evaluating histaminergic blockers were published (10, 11). Rivier and Vale (11) reported that iv administration of DPH prior to HA exerted an inhibitory effect on PRL release. However, systemically administered HA penetrates the blood–brain barrier poorly (12) and is not the appropriate method of administration for evaluating the central effects of HA. Additionally, the dose used (1 mg) may have profound stressful effects, causing a nonspecific release in PRL. Because of this questionable specificity of systemic HA, it is difficult to interpret this inhibitory effect of DPH. Arakelian and Libertun (10) examined a different animal model, the lactating female rat, in the suckled and nonsuckled state. Intraventricular DPH suppressed the suckling-induced rise in PRL levels but was not tested directly with the agonist HA. This latter study also demonstrated that 4-methylhistamine, an H_2 agonist, suppressed the PRL rise in suckled animals, suggesting an inhibitory role for H_2 receptors in lactating rats. Our data suggests that in the male rat, H_2 receptors are the dominant facilitatory histaminergic mechanism. The contrasting results suggest that the histaminergic mechanism of release of PRL differs in the male and lactating female rat.

Although PRL release is dominated by dopaminergic inhibitory tone, studies have suggested that cholinergic and serotoninergic influences are important. To reexamine these findings and to evaluate further the mechanism of HA-induced PRL release, antagonists of these monoamines were utilized. Neither of these agents modified the effect of HA, suggesting that these monoaminergic inputs are not involved in mediating the effect of HA. Perhaps histaminergic neurons terminate more distal to serotoninergic and cholinergic inputs in the basal hypothalamus, thus bypassing these monoaminergic afferents.

When given alone, methysergide had a marked stimulatory effect on PRL release.

This agent may act by inhibiting dopaminergic tone (1) or by stimulating serotoninergic receptors. The lack of effect of atropine on PRL levels is in agreement with the work of Lawson and Gala (13) and suggests that cholinergic muscarinic receptors are not involved in maintenance of basal PRL secretion.

In contrast to earlier studies (5, 6), LH levels in our study were not significantly altered. This difference in LH responsiveness may have been due to our use of the male rat not subjected to steroid priming.

An analysis of these results and the reports of other investigators suggests that HA acts at the level of the hypothalamus. The studies of Snyder *et al.* (12) indicate that icv HA localizes in the synaptosomes within hypothalmic tissue. Previous reports have not demonstrated an effect of HA on PRL release when infused directly into the anterior pituitary (6) or when tested *in vitro* in a pituitary cell culture assay (1). These data appear to support a hypothalamic site of action of HA, but they do not delineate the specific site of action of HA within the hypothalamus. It is possible that histaminergic neurons either stimulate those neurons which produce the PRL-releasing factor or inhibit those neurons responsible for the production of the PRL-inhibiting factor. In either case, the result would be an increase in PRL secretion.

Summary. The specificity and mechanisms through which HA alters basal PRl release were evaluated. Rats bearing atrial catheters were given icv infusions of histamine dihydrochloride or saline. Blood samples for PRL and LH were drawn before and at 15, 30, 45, and 60 min after infusion. Similar groups of animals were pretreated with icv infusions of diphenhydramine, cimetidine, atropine, or methysergide prior to icv HA. Animals given icv HA (90 μg) demonstrated at least a twofold rise in PRL at 15 min which fell to baseline levels at 45 min. Pretreatment with cimetidine, but not other agents, significantly inhibited the HA-induced rise in PRL. Serum LH was basically unaltered by these treatments. Histamine appears to be a specific stimulus to PRL release. This stimulatory effect of HA is inhibited by cimetidine, an H_2 receptor blocker, but not by H_1 receptor, cholinergic or serotoninergic antagonists.

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