Role of histamine in the increased secretion of prolactin induced by ether in adult male rats

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SUMMARY

The purpose of the present work was to determine the possible role of the histamine receptors located in the rostral zone of the hypothalamus in the control of the prolactin surge induced by ether stress. Cannulae were implanted into the preoptic anterior hypothalamic area or the third ventricle of several groups of adult male rats under ether anaesthesia. On the following day the rats were cannulated in the jugular vein so that they could be bled frequently. Twenty-four hours later saline, metiamide (an antagonist of H₂ histamine) or pyrilamine (an antagonist of H₁ histamine) were injected into the brain. Fifteen minutes after the injection all rats were subjected to an ether stress. Blood samples were taken at regular times after the stress and prolactin levels determined by radioimmunoassay. A prolactin surge was observed in rats injected with saline which extended up to 15–30 min after the stress. When the histamine antagonists were administered directly in the rostral hypothalamus both pyrilamine and metiamide inhibited the prolactin surge. When the histamine antagonists were administered into the third ventricle only metiamide was able to block the prolactin response completely.

The present results suggest that histamine receptors in the rostral hypothalamus of the rat are involved in the control of prolactin secretion induced by stress.

INTRODUCTION

There is strong evidence supporting the possibility that histamine controls the secretion of prolactin in the rat. Histamine, when administered into the brain, is able to increase the basal secretion of prolactin (Libertun & McCann, 1976; Donoso, 1978; Gibbs, Plotsky, Greef & Neill, 1979). This effect seems to be mediated by specific receptors, since selective antagonists block the prolactin response (Donoso & Banzán, 1980) and, as expected, appropriate histamine agonists produce the response (Donoso, 1981). It is known that the preoptic area of the hypothalamus of the rat is important for the control of the secretion of prolactin (Velasco, Castro-Vasquez & Rothchild, 1974; Halász, Gerendai, Köves, Lukáts, Marton, Molnár & Nagy, 1978; Kawakami & Yokohama, 1981) and recently histamine-responsive sites influencing the secretion of this hormone were described in this rostral hypothalamic zone (Alvarez & Donoso, 1981). One important question raised by these findings is whether or not these histamine receptors play a physiological role in the regulation of prolactin secretion. Prolactin is under complex control in the rat and its basal rate of secretion can be modified by several exteroceptive stimuli. Although, at present, the exact mechanism by which some exteroceptive stimuli are able to induce an increased secretion of prolactin is not clear, it appears that the afferent connections from the rostral hypothalamus are involved in stress. Exposure to ether vapour induces stress, resulting in increased circulating levels of prolactin (Wakabayashi, Arimura & Schally, 1971; Krulich, Hefco, Illner & Read, 1974; Chi & Shin, 1978; Mattheij & Swartz, 1980). A
cut caudal to the preoptic anterior hypothalamic area (POA–AHA) interferes with this release of prolactin (Kruilch, Hefco & Aschenbrenner, 1975). All these observations led to this study of the participation of the rostral hypothalamic histamine receptors in the increased secretion of prolactin induced by ether in adult male rats. A preliminary account of this study has been published elsewhere (Alvarez, 1982).

MATERIALS AND METHODS

Animals

Adult male rats (250–300 g) from a Wistar–Holtzman-derived colony were used. The animals were housed in a constant-temperature room at 22 ± 2 °C with a controlled light cycle (lights on 05.00–19.00 h). Rat chow and water were available ad libitum.

Implantation of cannulae

A unilateral double-barrelled stainless-steel cannula was placed stereotaxically in either the POA–AHA or the third ventricle of rats under ether anaesthesia. The outer guide of the cannula (23-gauge, 14 mm long) was implanted using the co-ordinates of de Groot (1959), with some modifications according to the size of the animal (described in detail in Alvarez & Donoso, 1981). Once the experiments were completed the rats were decapitated and the brain dissected out and stored in 10% formaldehyde. The positions of the cannulae were microscopically verified in coronal sections in all rats. The only rats included in the present results were those in which cannulae were located in the sites specified.

Blood sampling and experimental protocol

Twenty-four hours after the implantation of the guide cannulae, the rats were anaesthetized with ether and the right external jugular vein was exposed. A small Silastic catheter (Dow Corning, Midland, Michigan, U.S.A.; 0·51 mm internal diameter, 0·94 mm outside diameter, 102 mm long) was introduced into the jugular vein as far as the right atrium, as described previously (Alvarez & Donoso, 1981). The free end of the catheter was directed through the back of the animal and connected to the plastic hub of a disposable needle which was closed by a rubber plug. On the day of the experiments, 24 h after the jugular cannulation, a 150 mm long polyethylene tube filled with heparin in 0·9% NaCl (w/v) solution was attached to the catheter. Animals were left undisturbed for 2 h. A blood sample was taken at the beginning of the experiment (time – 15 min) and, with the cannula in the brain connected to a Hamilton microsyringe, injections were made into the POA–AHA or the third ventricle in less than 1 min. Fifteen minutes later (time zero) all animals were subjected to an ether stress of 2 min duration. At 5, 10, 15, 30, 60 and 120 min after the ether stress blood samples (0·6 ml) were collected from the jugular catheter. An equal amount of saline solution was reinjected into the vein in order to maintain the blood volume of the animals. Plasma prolactin levels were measured by radioimmunoassay.

Experiment 1

In this experiment, injections were made into the POA–AHA of three separate groups of rats. Animals were injected with 35 nmol of the H1 histamine antagonist pyrilamine maleate (PYR; about 9·55 µg, calculated as free base; Sigma, St Louis, Missouri, U.S.A.), 35 nmol of the H2 histamine antagonist metiamide (MET; about 8·54 µg; Smith, Kline and French Research Ltd, Welwyn, Herts) in 1 µl saline solution, or 1 µl saline as control. Fifteen minutes later (time zero) all rats were subjected to an ether stress.

Experiment 2

In this experiment, injections were made into the third ventricle of three separate groups of rats. Animals were injected with 410 nmol PYR (112 µg), 410 nmol MET (100 µg) in 4 µl
saline solution, or 4µl saline as control. Fifteen minutes later (time zero) all rats were subjected to the ether stress. The dose of the antagonists administered intraventricularly was similar to that previously shown to be effective in suppressing the stimulatory action of histamine on the secretion of prolactin (Donoso & Banzán, 1980).

Radioimmunoassays

The level of prolactin in samples of plasma was measured by the double-antibody rat radioimmunoassay as described by Alvarez & Donoso (1981). Values are expressed as ng NIAMDD-rat PRL-RP-2/ml plasma. Prolactin antiserum bound 33–44% of radiiodinated hormone. The second antibody was purchased from Antibodies Inc., Davis, California, U.S.A. and was used at a dilution of 1:4.

Statistics

The significance of the difference of the means in the three groups of both experiments was analysed by one-way analysis of variance and Duncan’s new multiple range test. Calculation of the significance of the means between the groups was performed by two-way analysis of variance and the ‘T’ test (Scheffé, 1959) for multiple comparisons. Values of P<0.05 were considered to be significant.

RESULTS

Experiment 1

The permanent implantation of cannulae in both the brain and jugular vein does not seem to interfere with the known increase of prolactin levels after a stimulus of acute ether stress (Wakabayashi et al. 1971; Krulich et al. 1974; Chi & Shin, 1978; Mattheij & Swartz, 1980), since the prolactin response was present in the animals injected with saline. The plasma concentrations of prolactin at 5, 10, 15 and 30 min after the stress were significantly different from the corresponding initial basal levels (–15 min; Fig. 1).

Fig. 1. Effect of administration of histamine antagonists into the preoptic anterior hypothalamic area on the release of prolactin induced by ether stress in rats. All injections (1µl) were given at –15 min and the ether stress was given at zero time. Results are expressed as means ± S.E.M. Saline-treated control rats (O: n = 4), rats treated with pyrilamine maleate (●: 35nmol = 9.55µg; n = 6) and rats treated with metiamide (•: 35nmol = 8.54µg; n = 11) are shown. *P<0.01 compared with controls (two-way analysis of variance, ‘T’ test); †P<0.01 compared with the value at –15 min (one-way analysis of variance, Duncan’s new multiple range test).
The administration of 35 nmol PYR in 1 µl saline solution into the POA–AHA interfered with the prolactin response to ether. Although 5 min after the stress the prolactin concentrations were not different from those found in stressed rats injected with saline, a significant decrease in hormone plasma levels was detected at 15 and 30 min (Fig. 1).

In the group of rats injected with 35 nmol MET into the POA–AHA the prolactin response was not present. Up to 30 min after the ether stress plasma levels of prolactin in the rats injected with MET were significantly lower than the corresponding values in the saline-injected group (Fig. 1).

**Experiment 2**

The intraventricular administration of 4 µl saline did not interfere with the prolactin response induced by ether stress. Thus 5 and 10 min after the application of the stimulus, the plasma prolactin levels were significantly higher than initial control values (Fig. 2).

![Fig. 2. Effect of administration of histamine antagonists into the third ventricle on the release of prolactin induced by ether stress in rats. All injections (4 µl) were given at −15 min and the ether stress was given at zero time. Results are expressed as means ± s.e.m. Saline-treated control rats (○, n = 9), rats treated with pyrilamine maleate (▲, 410 nmol = 112 µg; n = 12) and rats treated with metiamide (●, 410 nmol = 100 µg; n = 12) are shown. *P < 0.05, **P < 0.005, ***P < 0.001 compared with controls (two-way analysis of variance, T test); †P < 0.01, compared with the value at −15 min (one-way analysis of variance, Duncan’s new multiple range test).](image)

The intraventricular administration of 410 nmol PYR interfered partially with the prolactin response (Fig. 2). At 5 and 10 min after the stimulus the hormone plasma levels increased when compared with the initial control values but did not reach the levels found in the saline-treated rats. When the prolactin concentrations of the stressed rats injected with PYR were compared with the corresponding concentrations of the saline-treated group, significantly lower levels were observed at 5 and 10 min. After this time, although there was a tendency for plasma prolactin concentrations to be higher than before treatment in the PYR-treated group, they were not significantly different from the corresponding levels in the saline-treated group (Fig. 2).

In the group of rats injected intraventricularly with MET the prolactin response to ether was completely blocked (Fig. 2). The hormone levels in the group injected with MET were statistically lower than in the group injected with saline at 5 and 10 min.

In one small group of rats (four in the experiment with intraventricular MET and four in the PYR-treated group), very high initial plasma prolactin levels were detected. Conse-
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quently they were grouped separately and the corresponding hormone patterns after the ether stress are shown in Fig. 3. In those rats injected intraventricularly with MET, decreased hormone levels after the stress were observed at 5, 10, 15 and 30 min. Although a decrease in prolactin levels was found 15 min after PYR treatment the statistical analysis showed that such variation was not significant.

![Graph showing plasma prolactin levels](image)

**Fig. 3.** Effect of administration of histamine antagonists into the third ventricle on the release of prolactin induced by ether stress in rats with high initial prolactin levels. Injections of pyrilamine (▲; n = 4) and metiamide (●; n = 4) were given at −15 min. The ether stress was given at zero time. Results are expressed as means ± s.E.M. *P < 0.05, **P < 0.01 compared with the value at −15 min (one-way analysis of variance, Duncan's new multiple range test). The decrease in average prolactin concentration seen in pyrilamine-treated rats at 15 min was not significant. The dotted line represents prolactin levels in the saline-treated group, inserted from the previous figures to aid comparison.

**DISCUSSION**

In complete agreement with previous studies (Wakabayashi et al. 1971; Krulich et al. 1974; Chi & Shin, 1978; Mattheij & Swartz, 1980) an increased secretion of prolactin was found in control rats after ether stress.

Since the route of administration of drugs which act on histamine receptors has been shown to be important (Green, Cox & Lomax, 1976), both local injection into POA–AHA and intraventricular administration of histamine antagonists were used in the present study. No substantial differences were found (Figs 1 and 2). In both MET- and PYR-treated groups, the H₂ and H₁ histamine antagonists interfered with the prolactin response to stress (Figs 1–3). This indicates that the histamine receptors in the POA–AHA are involved in the mechanism responsible for the prolactin surge after ether stress, and that these histamine receptors are of H₁ and H₂ type. However, it must be borne in mind that PYR has some local anaesthetic and anticholinergic activity (Douglas, 1975) which MET does not share (Hind & Sutton, 1977). Therefore, it seems possible that the local anaesthetic effect of PYR could affect the central afferents to the POA–AHA and in this way reduce the magnitude of the prolactin response induced by ether. If this were the case, the inhibition of...
H₁ receptors would not be the only explanation of the blockade of the prolactin response by PYR. The present results suggest that H₂-histamine receptors facilitate the increase of prolactin secretion after ether stimulus. This suggestion is further supported by the findings of other authors who have used the same antagonists and selective histamine agonists (Donoso & Banzán, 1980; Donoso, 1981) but do not agree with some reports (Arakelian & Libertun, 1977; Falaschi, D'Urso, Frajese, Ruggieri, Scarnati, Forchetti & Agnoli, 1979). This discrepancy regarding the involvement of the H₂-histamine receptors in prolactin secretion may be partially explained by the fact that different experimental approaches have been applied. The inhibitory effect of the H₂ receptors was described in female pregnant rats and suckling was used as the stimulus for the induction of release of prolactin (Arakelian & Libertun, 1977). It is possible that the regulatory mechanisms of prolactin secretion by histaminergic receptors in the female rat are different with respect to those operating in males. There is evidence supporting this possibility. Only in female rats was 2-2-pyridil-ethyl-amine, an H₁ histamine agonist, able to induce a hypersecretion of prolactin (Donoso, 1981). This observation is in agreement with the stimulatory role of H₁ receptors in the secretion of prolactin in female rats suggested by Arakelian & Libertun (1977). Under the conditions used in our studies, MET always inhibited the release of prolactin in response to the stimulus used. This effect was evident even in those rats which had high initial plasma levels of prolactin before they were subjected to the ether stress (Fig. 3).

The present data therefore support a physiological role for histamine receptors in the POA-AHA. Together with previous findings regarding the distribution of histamine receptors in the brain (Alvarez & Donoso, 1981), the postulated role of histamine as an additional factor for the control of release of prolactin is further validated.

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REFERENCES

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