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Direct Action of Ethanol on Pituitary Prolactin Secretion *in vitro**

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The effect of ethanol on prolactin release *in vitro* has been studied in order to investigate the direct action of ethanol on pituitary gland of the female rats. Animals were sacrificed in diestrus 2 and pituitary glands were incubated in TC-199 medium containing dopamine, noradrenaline, serotonin, TRH or cycloheximide with or without ethanol. The total amount of prolactin after the incubation period was calculated. Alcohol significantly increased the prolactin release in all groups. Cycloheximide and dopamine decreased the prolactin synthesis, but ethanol reduced the effect of dopamine. It is concluded that part of ethanol-induced hiperprolactinaemia, is due to a direct action of the alcohol on pituitary, affecting release and/or synthesis of prolactin.

Key words: Ethanol, Prolactin, Pituitary incubations.

Ethanol produces a large number of physiological disturbances and an important action on endocrine system. There are evidences (5, 6, 16) that acute or chronic alcohol administration, markedly disrupts the secretion of most hormones (LH, FSH, estradiol, prolactin, testosterone ACTH, TSH). The effect of ethanol on prolactin secretion are confused and complex due to existence of discordant findings. There are studies indicating that the ethanol increases, inhibits or does not affect the prolactin release (8, 15, 22, 27). Previous studies performed in our laboratory (1, 2, 17) have demonstrated that the ethanol administration in a preovulatory period caused a strong increase in both release and synthesis prolactin during estrous cycle.

Prolactin secretion is under a tonic inhibitory influence through hypothalamic

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prolactin inhibitory factors (PIF). There are direct and pharmacological evidences that dopamine (DA) is involved in the inhibitory regulation of the prolactin release (3, 18, 19). Other neurotransmitters like noradrenaline (NA), serotonin (5-HT) or hypothalamic releasing factors as tyrotrophin-releasing hormone (TRH) can also affect the prolactin release (12, 21, 24).

Ethanol administration has been reported to affect the concentration, turnover or synthesis of some hypothalamic neurotransmitters (13, 20). HOFFMAN and TABACOFF (10) remarked that ethanol could originate a hypoactivity on dopaminergic system. The DA inhibition could explain the stimulating effect of ethanol on prolactin secretion, although a direct action of ethanol on pituitary cells could also be responsible. In order to evaluate whether the hyperprolactinaemia caused by ethanol *in vivo* could be due to a direct action of alcohol on pituitary gland, it became the purpose of this work to determine *in vitro* the ability of ethanol to modify the prolactin release induced by neurotransmitters (DA, NA, 5-HT) or TRH.

Materials and Methods

Adult female Wistar rats weighing 180-220 g were maintained under constant temperature (22 ± 2 °C) and controlled lighting (14:10 light:dark). Animals received food and water *ad libitum*. Estrous cycle was checked daily by vaginal smears and rats exhibiting regular 4-day estrous cycle were sacrificed at 16.00 h of diestrus 2.

Incubations. — The pituitary was rapidly removed, the posterior lobe was isolated from the anterior pituitary and it was hemisected. Each hemipituitary was weighed (4.13 ± 0.34 mg) and placed into different incubation flasks containing 2 ml of TC-199 medium (Difco). The incubations were carried out in a thermostatic and shaking-bath at 37 °C and gassing with 95 % O_2 and 5 % CO_2. The glands were preincubated during 30 minutes, the medium being removed and replaced by 1.8 ml of fresh medium. The final volume of incubation was completed up to 2 ml of different experimental substances. The incubations were performed during 4 hours and 100 µl of the medium were collected at one hour interval. At the end of incubation period, hemipituitaries were blotted, weighed, homogenized and centrifuged in phosphate-buffered saline containing 1 % of bovine serum albumin (PBS-BSA 1 %). The samples (medium and homogenates) were stored at −20 °C until assay.

During the experimental incubations ethanol 100 mM, dopamine 10^{-2} M (3-hydroxytyramine), noradrenaline 10^{-2} M (Arterenol), serotonin 10^{-2} M, tyrotrophin releasing-hormone 10^{-2} M and cycloheximide 50 µg/ml, all of them from Sigma, were added to the medium. The pituitaries were incubated in free medium (Control group) or in medium with the different substances, in presence or absence of ethanol.

Prolactin assay. — Samples of medium and pituitary homogenates were diluted with PBS-BSA 1 % and prolactin concentrations were measured by radioimmunoassay using NIDDK kits. The rat prolactin antigen was labelled in our laboratory with ^{125}I by the chloramine-T method (11). The results are expressed in ng of prolactin RP-3 per mg of tissue. The amount of prolactin released during the incubation period were corrected for the loss of volume produced by sampling. The total prolactin content was calculated as prolactin release into the medium plus the prolactin remaining on pituitary gland at the end of the incubation period. This value can yield information about the prolactin synthesis, provided that no degradation of the hormone takes place.

ANOVA and Student t-test were used to examine statistically significant differences.

Results

Incubation in free medium. — When hemipituitaries were incubated in free medium (in absence of experimental substances), prolactin secretion was elevated during the incubation period. During the first hour prolactin release was low, but later, between the second and fourth hour, the secretion was greatly increased (fig. 1). The prolactin remaining in the gland after the incubation was low, due to the large hormone release during the incubation period (table I).

Incubation with ethanol. — No significant differences were observed during the first and second hour respect to the control group (fig. 1). In the third and fourth hour, ethanol increased the prolactin secretion 49 % and 21 % respectively, ($p \leq 0.01$). Prolactin remaining in the gland is lower in the ethanol group than the control group, whereas the total prolactin content is not different between either group (table I).

Incubation with DA. — Figure 2 shows the inhibitory pattern of the prolactin release during incubation with DA. Prolactin release decreased 70 % at the second hour and 90 % both at the third and fourth hour of incubation, with respect to the control group ($p \leq 0.01$). The prolac-

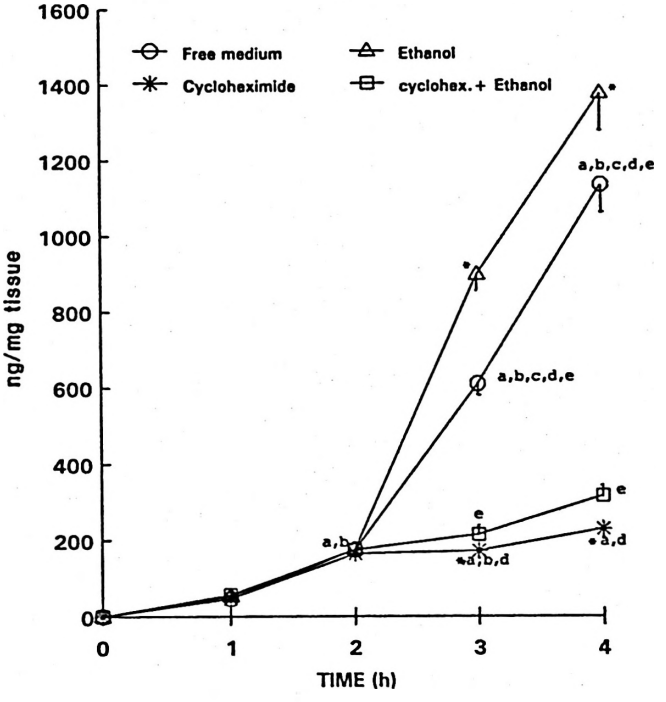

Fig. 1. *Effects of ethanol on prolactin release from pituitaries of rats sacrificed at the 18.00 h of diestrous 2 and incubated during 4 hours in free medium or medium with cycloheximide.*
Results are expressed in ng of prolactin RP-3 per mg of tissue (mean ± S.E.M.). N = 5. *, a, b, c, d, e significant differences with respect to the control (free medium), DA, NA, 5.HT, TRH or cycloheximide groups, respectively.

Table I. *Total content of prolactin (prolactin released into the medium plus pituitary content) after incubation of pituitary glands from female rats during 4 hours.*
Results are expressed in ng of prolactin RP-3 per mg of tissue (mean ± S.E.M., n = 5).

Addition to the medium	Pituitary content	Total content
None	524.0 ± 42.5	1658.0 ± 105.3
Ethanol	378.0 ± 19.7*	1754.0 ± 70.4
Dopamine	896.7 ± 61.4*,b	1009.0 ± 61.2*,b
Dopamine + ethanol	961.0 ± 26.0	1597.0 ± 42.9a
Noradrenaline	1340.2 ± 180.1*,a	1540.7 ± 190.3a
Noradrenaline + ethanol	1198.2 ± 119.2	1517.0 ± 170.0
Serotonin	1396.0 ± 173.6*,a	1691.0 ± 198.2a
Serotonin + ethanol	1224.5 ± 84.0	1607.5 ± 85.8
TRH	574.0 ± 55.2a,b,c	1431.0 ± 139.1a
TRH + ethanol	374.7 ± 31.2d	1408.0 ± 142.6
Cycloheximide	347.0 ± 18.7*	575.0 ± 21.5*,a-d
Cycloheximide + ethanol	249.0 ± 14.5e	564.0 ± 31.0

*,a,b,c,d,e Significant differences with respect to the control, DA, NA, 5-HT, TRH or cycloheximide groups, respectively.

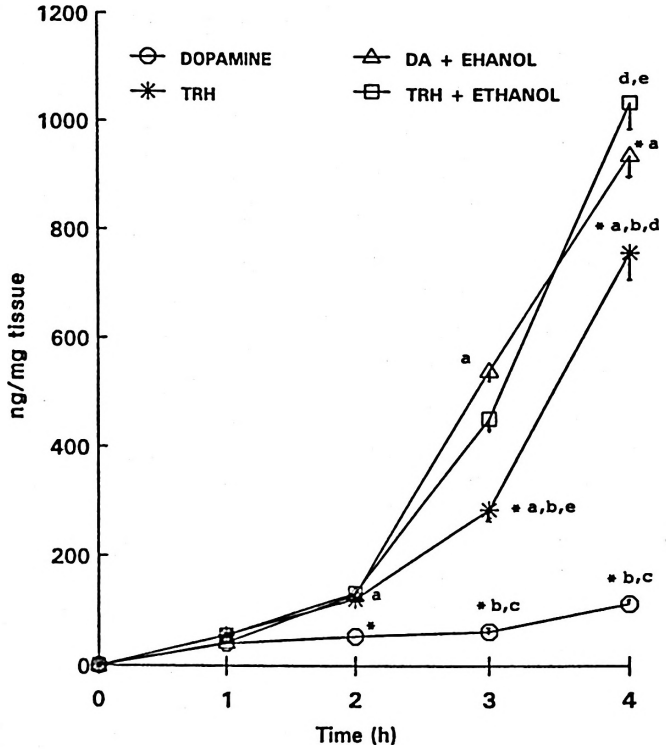

Fig. 2. *Effects of ethanol on prolactin release from pituitaries of rats sacrificed at 18.00 h of diestrous 2 and incubated during 4 hours in medium with DA or TRH.*
Results are expressed in ng of prolactin RP-3 per mg of tissue (mean ± S.E.M.). N = 5. Statistical significance as in fig. 1.

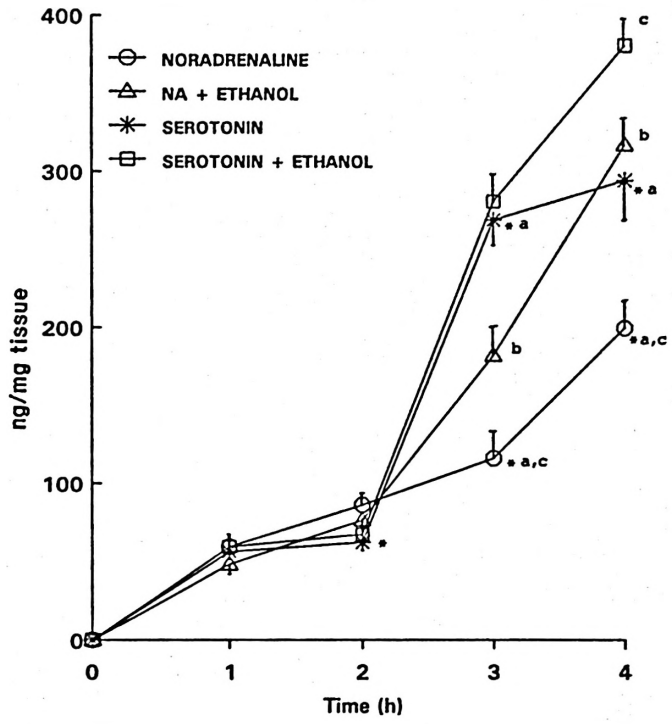

Fig. 3. *Effects of ethanol on prolactin release from pituitaries of rats sacrificed at 18.00 h of diestrous 2 and incubated during 4 hours in medium with NA or 5-HT.*
Results are expressed in ng of prolactin RP-3 per mg of tissue (mean ± S.E.M.). N = 5. Statiscal significance as in fig. 1.

tin remaining in the gland was significantly higher ($p \leq 0.01$) in the DA-incubated group than in the control group (table I). However, the total prolactin content after incubation was lower in the DA-group ($p \leq 0.01$).

Ethanol added to the medium with DA decreased the prolactin inhibition induced by DA. Prolactin release increased after two hours of incubation ($p \leq 0.01$) and the total prolactin content was higher than in the DA-group ($p \leq 0.01$).

Incubation with noradrenaline. — NA was able to inhibit the prolactin release from the second hour of incubation. However, the NA-induced prolactin inhibition was lower than the DA-induced prolactin inhibition ($p \leq 0.01$). The total content was higher in the NA-group than in the DA-group ($p \leq 0.01$), but there are no significant differences with respect to the control group, which would appear to indicate that NA affects the prolactin release *in vitro*, but not the synthesis (fig. 3, table I).

Prolactin inhibition induced by NA was decreased by ethanol only at the third and fourth hour of incubation ($p \leq 0.05$).

Incubation with serotonin. — 5-HT added to the incubation medium, inhibited the prolactin release at the third and fourth hour of incubation with respect to the control ($p \leq 0.01$). The 5-HT-induced prolactin inhibition was lower than

the prolactin inhibition induced by DA or NA (fig. 3).

Ethanol affected the 5-HT-induced prolactin inhibition only at the fourth hour of incubation, but no changes in the pituitary content were observed.

Incubation with TRH. — Prolactin release with TRH was lower than prolactin release during the incubation in free medium ($p \leq 0.01$ at the third and fourth hour), but higher than prolactin release produced by DA, NA or cycloheximide (fig. 2, table I).

Ethanol increased the TRH-induced prolactin release only at the fourth hour of incubation, with respect to the TRH-group ($p \leq 0.05$).

Incubation with cycloheximide. — Cycloheximide decreased prolactin release after three hours of incubation with respect to the control of TRH-groups ($p \leq 0.01$). However, prolactin release was significantly higher with respect to the DA ($p \leq 0.01$) or NA-groups ($p \leq 0.05$ at the second and third hour). The total prolactin content was significantly lower than other groups (fig. 1, table I).

Ethanol added to the medium with cycloheximide, produced a light increase in prolactin release at the third and fourth hour with respect to the cycloheximide-group ($p \leq 0.05$ and $p \leq 0.01$ respectively). However, ethanol did not alter the prolactin synthesis inhibited by cycloheximide.

Discussion

Most investigators have found increases in prolactin levels after ethanol administration (7, 16, 28). Previous studies performed in our laboratory have reported that the acute administration of ethanol in female rats at the 18.00 h of diestrus 2, elevated prolactin release during the estrous cycle and it also affected the pituitary prolactin concentration (1, 2). The ethanol-induced prolactin release could be due to the action of ethanol on hypothalamic neuronal system and/or ethanol could affect directly the pituitary responses to hypothalamic factors. The results obtained in this work indicate that the incubation of pituitaries in free medium produces a great secretion of prolactin. When ethanol was added to the medium this secretion was still greater, indicating that ethanol acts directly on the pituitary. These results agree with other studies performed *in vitro*. THORNER *et al.* (25) reported that alcohol stimulates prolactin release from perfused rat pituitary cells. SEILICOVICH *et al.* (23) demonstrated that ethanol was able to stimulate both prolactin secretion and synthesis from male pituitaries.

When the protein synthesis was inhibited by cycloheximide, the total prolactin content was lower than in the other groups, indicating that the synthesis of prolactin during the incubation period, contributes to the great prolactin secretion in absence of inhibitory factors. However, with cycloheximide ethanol does not increase the prolactin synthesis as it was observed *in vivo* and *in vitro* studies (1).

DA inhibited the synthesis and release of prolactin, confirming previous reports (18, 19). Ethanol decreased prolactin inhibition induced by DA. Some authors have reported that ethanol alters the properties of the lipidic membrane (9, 14), and consequently the capacity of ethanol to modify the pituitary response to DA may be due to either the action of ethanol on dopaminergic receptors, inhibition on membrane proteins or modification of the ionic flow. NA decreased the prolactin release but did not affect the synthesis. Ethanol altered the pituitary response to NA, increasing prolactin release to the medium; however, this increase was lower than the rise produced by ethanol in presence of DA.

Some authors have found variations of prolactin release with 5-HT in the incu-

bation medium (4). We have observed that this neurotransmitter does not affect the prolactin synthesis, but decreases the release of this hormone, although this effect is lower that the inhibition produced by DA or NA. The prolactin secretion with TRH was lower than the incubations in free medium, but the synthesis was similar. Ethanol only affects the prolactin secretion in response to 5-HT and TRH after three hours of incubation. VALIMAKI and YLIKAHRI (26), observed that TRH-induced prolactin release increased during alcohol intoxication.

It can be concluded that ethanol acts directly on pituitary, stimulating the prolactin release and modifying the pituitary response to DA, NA, 5-HT and TRH. Nevertheless, further studies are necessary for a greater understanding of the mechanism of the ethanol effects on prolactin.

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Resumen

Se estudia el efecto del etanol sobre la secreción de prolactina *in vitro*, a fin de determinar las acciones directas del etanol sobre la adenohipófisis de la rata hembra. Los animales se sacrifican en diestro 2 y las glándulas se incuban en medio TC-199 con dopamina, noradrenalina, serotonina, TRH o cicloheximida en presencia o ausencia de etanol. El alcohol aumenta la secreción de prolactina en todos los grupos. La dopamina y la cicloheximida disminuyen la secreción y la síntesis de prolactina, mientras que el alcohol reduce el efecto de la dopamina. De estos resultados se concluye que la hiperprolactinemia inducida por el alcohol es debida, al menos en parte, a una acción directa del etanol sobre la hipófisis.

Palabras clave: Etanol, Prolactina, Incubación de hipófisis.

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