Effect of serotonin on basal and TRH-induced release of prolactin from rat pituitary glands in vitro

Marta E. Apfelbaum

Instituto de Investigacion Medica Mercedes y Martin Ferreyra, Cordoba, Argentina

Abstract. The effect of serotonin on the release of prolactin (PRL) was studied in vitro. Anterior hemipituitary glands from ovariectomized rats were incubated for 1 h in the presence of different doses of serotonin. Serotonin added into the culture medium caused a significant increase in basal PRL release. The effect was dose-related between 10 and 30 nmol/l serotonin, but responsiveness declined towards basal levels with higher concentrations. When studied as a function of incubation time, basal release of PRL was significantly increased up to 1 h but decreased thereafter. Serotonin also enhanced the release of prolactin induced by 30 nmol/l thyrotropin-releasing hormone (TRH), at all doses tested. A serotonin concentration of as little as 30 nmol/l was already effective. A significant response was seen at 15 min and further increases occurred during the following incubation periods. Serotonin (approximately EC₅₀ 4.6 × 10⁻⁸ mol/l) was less potent than TRH (EC₅₀ about 1.2 × 10⁻⁸ mol/l) to increase basal PRL release. On the other hand, the indole amine appeared to act with similar potency in stimulating PRL release both basal and TRH-induced. In addition, the combined effect of the releasing agents was found to be additive. These results suggest that serotonin and TRH could act through separate mechanisms. Methysergide, a serotoninergic blocking agent, had no effect on the in vitro PRL release either basal or TRH-induced, but it completely blocked that evoked by serotonin suggesting that serotonin may interact with specific receptors on the lactotropes. These findings clearly demonstrate that serotonin may stimulate the release of PRL by acting directly at the pituitary gland level.

There is extensive evidence supporting the involvement of serotonin (5-hydroxytryptamine; 5-HT) in the regulation of prolactin secretion. Central serotoninergic pathways have been shown to participate in the oestrogen-induced release of prolactin (Caligaris & Taleisnik 1974), in the suckling-induced rise of plasma prolactin in lactating rats (Kordon et al. 1973/74), and in stress (Marchlewiska-Koj & Krulich 1975).

Although there appear to be a general agreement that 5-HT stimulates the release of prolactin (Clemens et al. 1977; Kamberi et al. 1971; Lawson & Gala 1978), the mechanism by which this action is exerted is unknown. For the most part, 5-HT is thought to influence the release of prolactin at the hypothalamic level by stimulating the secretion of prolactin-releasing factors or inhibiting the secretion of prolactin-inhibiting factors. However, some in vivo studies suggest that 5-HT may release prolactin by a direct action on the anterior pituitary gland (Stobie & Shin 1983; Wehrenberg et al. 1980). Therefore, 5-HT may well be exerting its endocrine effect on the hypothalamus as well as on the pituitary gland itself.

Since in vivo experiments have not allowed a definitive separation between hypothalamic and pituitary serotonin influences on prolactin regulation, the present communication, using an in vitro system, was conducted to determine whether the hypophysis is a site of action of this indole amine. Previous studies (Apfelbaum & Taleisnik 1976, 1977) have established that the rat anterior pituitary gland incubated in vitro is an appropriate experimental model for the study of substances which may exert direct hypophysyal actions.

In this report, the effect of 5-HT in vitro on the basal and thyrotropin-releasing hormone (TRH)-stimulated release of prolactin from incubated pituitary glands was studied.
Material and Methods

Adult female rats (250–300 g body weight), ovariectomized 30 days before being used, were maintained in a lighting schedule of 14 h light: 10 h darkness, with food and water available ad libitum.

The animals were killed by decapitation. The anterior lobe of the pituitary gland was cut into halves along the mid-line, and then quartered. Each half (two quarters from either the right or left) of the gland was placed in 1 ml of Eagle’s Minimum Essential Medium (Grand Island Biological Co, Grand Island, NY, USA), containing 10^{-4} mol/l ascorbic acid in order to prevent oxidation. One hemipituitary was used as the experimental gland and the other as the control. The incubation procedure was as described in a previous report (Apfelbaum & Taleisnik 1976). Six animals were used per group.

Serotonin (5-hydroxytryptamine creatinine sulphate) and TRH were obtained from Sigma Chemical Co (St. Louis, MO, USA), and methysergide bimaleate was a gift from Sandoz AG (Basel, Switzerland). Stock solutions (10^{-2} mol/l) were made in 10^{-2} mol/l ascorbic acid and serially diluted with incubation medium.

Three experimental designs were employed to study the in vitro prolactin (PRL) release in response to 5-HT. In the first, the dose-response relationship for 5-HT and TRH was studied. Different doses of 5-HT (3 \times 10^{-9} to 10^{-5} mol/l) and TRH (10^{-9} to 10^{-6} mol/l) were added to the experimental medium after a 30-min pre-incubation period. Control hemiglands were incubated with medium alone. For the study of the 5-HT effect on TRH-induced release of PRL, 3 \times 10^{-8} mol/l TRH and different doses of 5-HT were added simultaneously to the experimental medium, the respective control hemiglands being incubated in medium containing 3 \times 10^{-8} mol/l TRH. Both experimental and control hemiglands were incubated for 1 h.

In the second approach, the time-course of the 5-HT and TRH action was examined. After a pre-incubation period (considered as time 0), experimental hemiglands were incubated in the presence of: a) 10^{-7} mol/l 5-HT; b) 3 \times 10^{-8} mol/l TRH or c) 10^{-7} mol/l 5-HT plus 3 \times 10^{-8} mol/l TRH coadministered. The respective control halves were incubated with: a) and b) non-treated medium; or c) medium containing 3 \times 10^{-8} mol/l TRH. Both hemiglands from groups a and b were incubated for 7\% 15, 30, 60 and 120 min, those of group c for 15, 30 and 60 min.

In the third set of experiments, the effect of methysergide in vitro on the pituitary response to 5-HT was studied. Four groups of pituitary glands incubated under the following conditions were used: a) non-treated controls incubated in medium alone; b) hemipituitary glands incubated with 10^{-7} mol/l 5-HT; c) hemiglands incubated with 3 \times 10^{-8} mol/l TRH; d) hemiglands incubated with 10^{-7} mol/l 5-HT plus 3 \times 10^{-8} mol/l TRH coadministered. In addition, experimental hemiglands from each group were incubated in the presence of 3 \times 10^{-6} mol/l methysergide during the 1-h incubation as well as during the 30-min pre-incubation period.

After the incubation period, the media were decanted and stored at −20°C until hormone determination. PRL was measured in media samples at two dose levels by double-antibody radioimmunoassay. The amount of PRL was calculated in terms of NIADDK rat standard P-RP-3 (30 IU/mg). The results were expressed as ng of PRL per mg of pituitary gland.

Procedures employed for statistical analysis of the data from this study included Student’s t-test or analysis of variance and Duncan’s multiple range test. The level of significance for differences between groups was taken as P < 0.05.

Results

Effect of 5-HT on the basal release of prolactin in vitro

To study the in vitro effect of 5-HT on the release of prolactin, anterior hemipituitary glands from ovariectomized rats were incubated for 1 h in the presence of different concentrations of serotonin creatinine sulphate (Fig. 1). Addition of 5-HT to the incubation medium stimulated basal prolactin release. The effect was directly related to the dose with up to 300 nmol/l 5-HT, but a lower response was obtained with 1 µmol/l 5-HT. Responsiveness declined to basal levels with 10 µmol/l 5-HT (data not shown). Increments in medium prolactin concentration induced by 5-HT were highly significant (P < 0.01) when doses between 30 and 1000 nmol/l were tested. Maximal stimulation to 155% of that in controls was obtained with 300 nmol/l 5-HT, the EC50 being approximately 4.6 \times 10^{-8} mol/l.

When studied as a function of time (Fig. 2), incubation of hemiglands with 100 nmol/l 5-HT resulted in an enhanced release of prolactin at all times studied, with consecutive increases (P < 0.01) by 1.23-, 1.43-, 1.56- and 1.30-fold over control values after 15, 30, 60 and 120 min, respectively. The rate of 5-HT-stimulated prolactin release declined during the second hour of incubation to 67% of that obtained after 1 h (approximately 544.9 and 795.3 ± 27.6 ng/mg of pituitary per hour, respectively). Basal release of prolactin from control hemiglands rose linearly from 0 to 2 h, as previously observed (Apfelbaum 1983).
Release of PRL from incubated hemipituitary glands of ovariectomized rats in response to increasing doses of 5-HT. Concentration of PRL released into the medium during a 1-h incubation. Serotonin was added to the medium of experimental hemiglands incubated with (●) or without (▲) 30 nmol/l TRH. The values in 36 non-treated (Δ) or 36 TRH-treated (○) control hemiglands (six per group) were combined to illustrate PRL concentration at 0 nmol/l 5-HT, since the means of each separate group were not statistically different. Each point is the mean value of six hemiglands and the vertical lines indicate ± SEM.

Effect of 5-HT on the TRH-induced release of prolactin in vitro
A preliminary experiment to establish the dose-response relationship for TRH was done using pituitary glands incubated for 1 h. Release of prolactin was greatly stimulated by the addition of TRH to the medium at doses between 1 and 1000 nmol/l (Fig. 3). The effect was dose-related. The magnitude of hormone release induced by TRH was significantly higher (P < 0.01) at all concentrations tested, except with 1 nmol/l TRH. Maximum increase to 200% of control values was reached with 100 nmol/l TRH, the approximate EC50 being 1.2 × 10−8 mol/l. Hemiglands incubated for different periods in the presence of 30 nmol/l TRH (Fig. 2) showed a marked stimulation of the prolactin release at each time studied. Except at 7½ min, the differences to the respective controls were highly significant (P < 0.01). The TRH-induced release of prolactin increased linearly with time. On the basis of the above findings, a dose of 30 nmol/l TRH was used to test the in vitro responsiveness to different doses of 5-HT during a 1-h incubation. Serotonin increased the releasing effect of TRH at all concentrations tested (Fig. 1). Increments to almost 120% (P < 0.01) were obtained by the simultaneous treatment of hemiglands with 30 nmol/l TRH and 30 nmol/l 5-HT. A higher concentration of 5-HT (300 nmol/l) increased the prolactin release...
by more than 140% ($P < 0.01$) of the response obtained with TRH alone. The $EC_{50}$ value was estimated to be about $4.3 \times 10^{-8}$ mol/l.

The time-course of the prolactin response to 30 nmol/l TRH was also stimulated by 100 nmol/l 5-HT treatment (Fig. 3), the effect being already evident at 15 min of incubation ($P < 0.05$). As shown in Table 1, the response produced by the combined treatment (5-HT plus TRH) was almost equal to the sum of net effects obtained independently from each treatment.

Effect of methysergide on 5-HT-stimulated release of prolactin

In order to obtain some information on the mechanism through which 5-HT stimulates the prolactin release, the effect of a serotonin receptor-blocking agent, methysergide, was studied. Methysergide, at a single concentration of 3 µmol/l, was added to the media of experimental nontreated hemiglands, and to experimental hemiglands treated with either 30 nmol/l TRH, 100 nmol/l 5-HT or 30 nmol/l TRH plus 100 nmol/l 5-HT coadministered.

The data in Table 2 show that methysergide exerted no effect on either basal release of prolactin or the release of prolactin stimulated by TRH. In contrast, the release of prolactin induced by 5-HT, either alone or in combination with TRH, was completely blocked by methysergide.

**Discussion**

The present findings support previous reports (Clemens et al. 1977; Kordon et al. 1973/74; Pilote & Porter 1981) of a stimulatory role of 5-HT on prolactin secretion. In all those studies, it was assumed that the 5-HT action was mediated via an effect on hypothalamic inhibiting or releasing factors. However, some in vivo studies, involving pituitary glands disconnected from the hypothalamus, suggest that 5-HT may release prolactin by a direct action on the anterior pituitary gland. Thus, intravenous injection of 5-HT into stalk-sectioned female rhesus monkeys caused significant increases in serum prolactin (Wehrenberg et al. 1980) and stimulated prolactin secretion from ectopic pituitaries in hypophysectomized male rats (Stobie & Shin 1983).

The results presented in this study clearly demonstrate that 5-HT stimulates the release of prolactin by acting directly at the pituitary gland level. Serotonin added into the culture medium of adenohypophyses from ovariectomized rats caused a significant rise in basal prolactin release. The effect was dose-related between 10 and 300 nmol/l 5-HT, the approximate $EC_{50}$ being $4.6 \times 10^{-8}$ mol/l. This direct pituitary action is consistent with the work by Fang (1976) showing that 100 nmol/l 5-HT can elevate prolactin release from a clonal strain of rat pituitary cells in culture. However, several reports (Birge et al. 1970; Lamberts & McLeod 1978) failed to demonstrate any action of 5-HT on pituitary lactotropes in vitro. The discrepancy with the results presented here may be due to the short period of incubation (1 h) with
Table 1.
The release of PRL induced by TRH or 5-HT and the effect of their combined treatment, as a function of incubation time.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ng PRL/mg of pituitary gland</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>15 min</td>
</tr>
<tr>
<td>Control</td>
<td>124.5 ± 5.6</td>
</tr>
<tr>
<td>(a) TRH (Δ)</td>
<td>50.7 ± 4.9</td>
</tr>
<tr>
<td>(b) 5-HT (Δ)</td>
<td>29.0 ± 5.7</td>
</tr>
<tr>
<td>(c) TRH + 5-HT</td>
<td>202.4 ± 12.2</td>
</tr>
<tr>
<td>(d) Sum of TRH and 5-HT effects (a + b control)</td>
<td>204.2</td>
</tr>
</tbody>
</table>

(a) Δ: TRH-treated (30 nmol/l) hemiglands minus non-treated hemiglands.
(b) Δ: 5-HT-treated (100 nmol/l) hemiglands minus non-treated hemiglands.
(c) Hemiglands treated with TRH (30 nmol/l) plus 5-HT (100 nmol/l).
(d) Sum of the net effects (Δ) of TRH (a) and 5-HT (b) plus control non-treated hemiglands.

Control vs (c): P < 0.001; (a) vs (b): P < 0.01. Values are mean ± SEM. Values (a), (b), (c) and control values were taken from Fig. 2.

Table 2.
Effect of methysergide on 5-HT-stimulated release of prolactin.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ng PRL/mg of pituitary</th>
</tr>
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<tbody>
<tr>
<td>(a)</td>
<td>C –</td>
<td>536.6 ± 27.6</td>
</tr>
<tr>
<td></td>
<td>E: METH</td>
<td>584.8 ± 33.0</td>
</tr>
<tr>
<td></td>
<td>E: N.S.</td>
<td></td>
</tr>
<tr>
<td>(b)</td>
<td>C: TRH</td>
<td>883.8 ± 38.8</td>
</tr>
<tr>
<td></td>
<td>E: TRH + METH</td>
<td>867.9 ± 51.1</td>
</tr>
<tr>
<td></td>
<td>E: N.S.</td>
<td></td>
</tr>
<tr>
<td>(c)</td>
<td>C: 5-HT</td>
<td>764.8 ± 37.1</td>
</tr>
<tr>
<td></td>
<td>E: 5-HT + METH</td>
<td>507.0 ± 39.7</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>(d)</td>
<td>C: TRH + 5-HT</td>
<td>1128.2 ± 53.5</td>
</tr>
<tr>
<td></td>
<td>E: TRH + 5-HT + METH</td>
<td>858.9 ± 42.5</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Paired hemiglands, (a) non-treated group, treated with (b) 30 nmol/l thyrotropin-releasing hormone (TRH), (c) 100 nmol/l serotonin (5-HT), and (d) 30 nmol/l TRH plus 100 nmol/l 5-HT (TRH + 5-HT), were incubated in the presence (experimental: E) or absence (control: C) of 3 μmol/l methysergide (METH). (c) E vs (a) E: N.S.; (d) E vs (b) E: N.S.

P: Comparison between experimental and control hemiglands. N.S.: not significant.

Values are means ± SEM; N = 6 glands per group.

The indole amine used in the present work, compared with the 4–5 h of incubation used by these authors. Serotoninergic response may be brief and therefore difficult to detect during prolonged incubations. This possibility is supported by the data presented in Fig. 2 showing that stimulation by 5-HT increased with time up to 1 h, but decreased thereafter.

As previously demonstrated (Tashjian et al. 1971; Drouin et al. 1976), TRH increased the basal release of prolactin in a dose- and time-dependent manner. The addition of 5-HT to the incubated pituitaries also increased the release of prolactin induced by TRH and the effect was directly related to the dose. Serotonin already stimulated the release of TRH-induced prolactin at 15 min and further increases occurred thereafter.

When the responsiveness of hemiglands to either TRH or 5-HT alone is compared in terms of their EC50, 5-HT shows to be less potent than TRH in stimulating basal release of prolactin. On the other hand, the profiles of prolactin release, either basal or TRH-induced, showed comparable patterns when stimulated with different doses of 5-HT. The indole amine appeared to exert its stimulating action with similar potency in both situations. In addition, the combined effect of the releasing agents was found to be additive. These
results, taken together, suggest that 5-HT and TRH may act by separate regulatory mechanisms.

Several authors have reported a blockade of 5-HT action by methysergide administration (Caligaris & Taleisnik 1974; Clemens et al. 1977; Lawson & Gala 1978). Beside this effect, methysergide was claimed to possess a mixed dopamine receptor agonist-antagonist activity (Krulich et al. 1981; Lamberts & McLeod 1978). No evidence for such an action has been observed in this study, since methysergide alone failed to exhibit any effect on the in vitro prolactin release, either basal or TRH-stimulated. In contrast, it was effective in reversing the stimulatory effect of 5-HT. These results suggest that 5-HT may act on the lactotropes by binding to receptor sites. Although serotonergic receptors have not yet been characterized, recent evidence has shown the existence of a high affinity in vitro uptake mechanism for 5-HT in cells of rat anterior pituitary (Johns et al. 1982). Furthermore, the presence of 5-HT in rat anterior pituitary has been reported using a radioenzymatic assay (Saavedra et al. 1975).

Even though 5-HT has the capacity to release prolactin in vitro, this fact alone does not indicate that it plays a physiologic role. In order to accept such an effect, it will be necessary to demonstrate that 5-HT rises in hypothalamus-hypophysial portal blood under conditions where prolactin is released. Serotonin is present in the hypothalamus (Saavedra et al. 1974) and serotonin containing axons are found within the perivascular region of the median eminence (Calas & Alonso 1974). In addition, serotonin neurons have been described in the medio-basal hypothalamus, which could account for the persistence of relatively high concentrations of endogenous 5-HT and its biosynthetic enzyme, tryptophan hydroxylase, in several hypothalamic nuclei, after complete deafferentation of the hypothalamus (Brownstein et al. 1976). This anatomical distribution gives the possibility for 5-HT to be released directly into the portal vessels thus reaching the anterior pituitary gland. These observations suggest that 5-HT may regulate prolactin release by acting not only at the hypothalamic but also at the pituitary level.

Acknowledgments

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Dr Marta E. Apfelbaum,
Instituto de Investigacion Medica,
Mercedes y Martin Ferreyra,
Casilla de Correo 389,
RA-5000 Cordoba, Argentine.