Plasma prolactin concentrations and copulatory behavior after salsolinol injection in male rats

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Abstract

Purpose The dopamine-derived endogenous compound, R-salsolinol (SAL), was recently identified as a putative endogenous prolactin (PRL)-releasing factor. However, how SAL influences copulatory behavior is unknown. In this study, we examined the relationship between SAL and copulatory behavior in male rats.

Methods Male Sprague-Dawley rats administered SAL were exposed to female rats in estrus, the plasma PRL concentration was measured, and the behavioral frequency and time during copulatory behavior were noted.

Results In the control and SAL groups, plasma PRL concentrations at 15 min before exposure to the female were 7.3 ± 2.0 and 8.0 ± 1.5 ng/ml, respectively. Moreover, plasma PRL concentrations in males immediately after exposure to the female were 7.4 ± 1.2 and 68.0 ± 5.9 ng/ml, respectively (P < 0.05). All (8/8) of the control animals ejaculated in the presence of the female, whereas only 33% (2/6) of the SAL group ejaculated. An increasing tendency for mount latency and intromission latency and a decreasing tendency for intromission frequency were observed in the SAL group.

Conclusions Copulatory behavior was inhibited in male rats after SAL injection, suggesting that SAL is a copulatory behavior inhibiting factor.

Keywords Copulatory behavior · Hyperprolactinemia · Male rat · Prolactin-releasing factor · Salsolinol

Introduction

In developed countries, the older age population increases at an accelerated rate due to a decrease in the birth rate and the prolongation of life through medical development. Moreover, increases in the elderly population allow the prediction of an increase in hyperprolactinemia caused by aging. Hyperprolactinemia decreases libido and causes oligozoospermia [1]. Sexual ability is inhibited through a central mechanism by serotonin (5-hydroxytryptamine, 5HT) and stimulated by dopamine (DA). Prolactin (PRL) secretion is accelerated by salsolinol (SAL) and 5HT but inhibited by DA [2–4].

PRL is a polypeptide hormone that is synthesized and secreted from the mammotropes in the anterior lobe (AL) of the pituitary gland [3]. Many studies have documented...
a critical role for PRL in the initiation and maintenance of lactation in women and female animals [5, 6], but little is known about the physiological role of PRL in human men and other male animals. In particular, few reports associating PRL with sexual behavior exist. However, the previous study reported that copulatory behavior in male rats is inhibited by hyperprolactinemia induced by grafting pituitary glands under the kidney capsules [7].

R-salsolinol (R-1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline [SAL]) is a dopamine-derived endogenous compound recently identified as a putative endogenous PRL-releasing factor (PRF) that can be detected in high concentrations in the neurointermediate lobe and median eminence in male and female rats [4, 8, 9]. SAL seems to be a selective and potent stimulator of PRL secretion in vivo and in vitro without affecting the secretion of other pituitary hormones [4, 8, 9].

To investigate the effect of PRL on sexual behavior in male rats, plasma PRL concentrations and copulatory behavior were examined in response to a SAL injection.

Materials and methods

Animals

The experiments were conducted with 10-week-old male Sprague-Dawley rats weighing 411.5 ± 18.5 g (mean ± SE) and in 11–15-week-old female rats (Charles River, Sulzfeld, Germany and Budapest, Hungary). All animals were maintained under controlled conditions (12 h light/12 h dark cycle; temperature, 24°C) with free access to water and standard rat chow pellets.

Ovariectomy and induced estrus in copulatory-partner female rats

Two weeks before the experiment, female rats were anesthetized with pentobarbital sodium (30 mg/kg, i.p.), and their ovaries were excised. These female rats were made sexually receptive with a subcutaneous (s.c.) injection of estradiol benzoate (20 µg/0.1 ml/head in olive oil; Sigma, St. Louis, MO, USA), followed 48 h later with a s.c. injection of progesterone (0.5 mg/0.1 ml/head in olive oil; Sigma) at 5 h before the observations. The estrous-induced female rats were used for the experiment.

Cannulation

One day before the experiment, male rats were anesthetized with pentobarbital sodium (30 mg/kg, i.p.), and a permanent cannula (silicon tubing, inner diameter 0.5 mm, outer diameter 0.9 mm; Dow Corning, Midland, MI, USA; Becton-Dickinson, Parsippany, NJ, USA) was implanted into the right jugular vein. This cannulated vein was connected to a polyethylene extension, and the tube was flushed with heparinized saline (300 IU/0.5 ml).

Observation of the copulatory behavior and blood sampling

Copulatory behavior was observed at 1 h after lights-out under a red light. A male rat was placed in an observation cage (400 × 300 × 260 mm) 30 min before the estrous-induced female rat. Control group male rats were injected with saline (0.2 ml/head i.v.) 1 min before exposure to the female rat. SAL group male rats were injected with SAL (4 mg/kg body weight i.v.) 1 min before exposure to the estrous-induced female rat. Behavioral observations were conducted just after putting the female rat into the observation cage. After ejaculation or 15 min, the female rat was removed from the observation cage, and the behavioral observations continued until 5 min after the first ejaculation or for 20 min if the first ejaculation did not occur. Copulatory behavior was measured using the methods of Heimer and Larsson (mount frequency, MF; intromission frequency, IF; mount latency, ML; intromission latency, IL; ejaculation latency, EL) [10].

Blood (0.5 ml) was obtained at four different time points (15 min before exposure to the female rat, just after exposure, at ejaculation or 15 min after exposure to the female rat, and 5 min after ejaculation or 20 min after exposure to the female rat). The same volume of heparinized saline (50 IU/0.5 ml) was injected as a replacement for the lost volume. Blood plasma was separated and stored at −70°C until it was assayed for PRL.

Hormone analysis

Plasma PRL concentrations were measured by radioimmunoassay (RIA) (kits provided by the National Pituitary Agency, NIDDK, and Dr. A.F. Parlow). The RIA procedure has been described previously [11]. NIAMDD-Rat-RP-3 was used for the standard curve.

Statistical analyses

All data are presented as mean ± SEs. Statistical analysis was performed using Mann–Whitney U test and Chi-square test. Values were considered significantly different at P < 0.05.
Results

Appearance of ejaculation during copulatory behavior

Figure 1 shows the percentage of males that ejaculated during copulatory behavior in the control and SAL groups. All (100%) six rats in the control group ejaculated during copulatory behavior, whereas two (33.3%) of the six rats in the SAL group ejaculated during copulatory behavior. The incidence of ejaculation in the control group (100%) differed significantly from that in the SAL group (33.3%) ($P < 0.01$).

Plasma PRL concentration

Figure 2 shows the plasma PRL concentration during copulatory behavior in the control and SAL groups (non-ejaculation and ejaculation). Plasma PRL concentration at 15 min before exposure to the female rat (at rest time) in the control, SAL non-ejaculation group, and SAL ejaculation group were 7.6 $\pm$ 2.0, 9.5 $\pm$ 0.5, and 6.2 $\pm$ 0.8 ng/ml, respectively. The control group values did not differ significantly from those of the SAL non-ejaculation group. However, plasma PRL in the SAL non-ejaculation group was significantly greater upon exposure to the female rat (65.0 $\pm$ 7.3 ng/ml) compared to the control group (8.3 $\pm$ 1.9 ng/ml) ($P < 0.01$). Plasma PRL concentration in the control group peaked at ejaculation (26.1 $\pm$ 7.4 ng/ml) and declined to 23.5 $\pm$ 6.6 ng/ml 5 min after ejaculation. Similarly, the SAL ejaculation group peaked at ejaculation (88.5 $\pm$ 41.1 ng/ml) and declined to 57.1 $\pm$ 26.8 ng/ml 5 min after ejaculation. In contrast, plasma PRL in the SAL non-ejaculation group peaked at 15 min after exposure to the female rat (76.0 $\pm$ 14.7 ng/ml) and declined to 57.1 $\pm$ 15.2 ng/ml 20 min after exposure to the female rat.

Analysis of copulatory behavior

The behavioral frequency and timing of the control and SAL groups (non-ejaculation and ejaculation) during copulatory behavior are shown in Table 1. For most behaviors, the control group did not differ significantly from the SAL group (except for the SAL ejaculation group). However, the ML and IL of the SAL group (92.3 $\pm$ 63.3 and 163.3 $\pm$ 65.5 s) (except for the SAL ejaculation group) were significantly higher than those in the control group (7.5 $\pm$ 1.4 and 17.0 $\pm$ 5.9 s).

Discussion

This study demonstrated that PRL increases following a SAL injection and that copulatory behavior is inhibited in male rats by hyperprolactinemia. The dosage of this SAL was an adequate amount, as has been reported before [12]. Similar to a previous report [7], this suggests PRL and SAL, which is a PRF, are copulatory behavior inhibiting factors. Furthermore, the results suggest that PRL and SAL inhibit libido during copulatory behavior.

Numerous attempts have been made to demonstrate that PRL contributes to sexual behavior. A study by Drago and Lissandrello [13] using male rats suggested that acute and chronic central PRL treatments have both stimulatory and inhibitory effects on copulatory behavior. However, two points must be made. The first concerns the hyperprolactinemic model animal following PRL administration. The second pertains to the result that copulatory behavior increases in acute hyperprolactinemic animals following PRL administration. Hyperprolactinemia is believed to
Data are mean ± SEM. *P < 0.05 versus control.

**Table 1** Comparison of behavioral frequencies and latencies in male rats after treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Mount frequency (range)</th>
<th>Intromission frequency (range)*</th>
<th>Mount latency (range)*</th>
<th>Intromission latency (range)*</th>
<th>Ejaculation latency (range)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAL (ejaculation)</td>
<td>2</td>
<td>6.5 ± 1.5 (5–8)</td>
<td>6.5 ± 0.5 (6–7)</td>
<td>7.0 ± 1.0 (6–8)</td>
<td>70.0 ± 55.0 (15–125)</td>
<td>592.5 ± 67.5 (525–660)</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>7.3 ± 0.7 (5–9)</td>
<td>6.5 ± 1.0 (3–9)</td>
<td>7.5 ± 1.4 (5–13)</td>
<td>17.0 ± 5.9 (7–40)</td>
<td>466.0 ± 80.5 (225–725)</td>
</tr>
</tbody>
</table>

Data are mean ± SEM
Mann–Whitney U test (except for SAL ejaculation group); * P < 0.05 versus control

Acknowledgments
The author thanks Dr. G.M. Nagy, Neuroendocrine Research Laboratory, Department of Human Morphology, Hungarian Academy of Science and Semmelweis University, for inviting me to spend 6 months as a researcher in his laboratory. I also thank all the staff in his laboratory, Dr. V. Hricisák, and Dr. A. Horvath.

References


influence the PRF as well as PRL during copulatory behavior. Therefore, it is not necessary in a copulatory behavior experiment to administer PRL in inducing hyperprolactinemia. A case of hyperprolactinemia is classified roughly into physiological, pharmaceutical, and etiological cases. In the etiology of hyperprolactinemia, subthalamic disorders exist such as a craniopharyngioma, histiocytosis X, sarcoidosis, germinoma, and pituitary adenoma [2]. In addition, not only high plasma PRL concentrations but increases in PRF are believed to contribute to a portion of the hyperprolactinemia in these etiologies. That is, studying the copulatory behavior in a hyperprolactinemic model animal using SAL, which is a PRF, is important. Second, the behavior of the acute hyperprolactinemic model animal must change during copulation. A change in copulatory behavior in an acute hyperprolactinemic model animal using SAL administration was examined in this study. The copulatory behavior increased in the study by Drago and Lissandrello [13], but decreased in this study (Fig. 1; Table 1). The difference is the PRF, which suggests that copulatory behavior changes with PRF participation.

In this study, PRL was secreted significantly in SAL-administered male rats (Fig. 1), which also inhibited copulatory behavior (Fig. 2; Table 1). In this way, SAL induced PRL secretion as reported, suggesting that SAL acts as a PRF in the hypothalamo–hypophysial system [14]. This result suggests that copulatory behavior is inhibited similar to the results of an increase in PRL following inoculation with PRL-secreting tumor cells [15, 16], the administration of a dopamine antagonist [17], the chronic administration of estrogen [18], or grafted pituitary glands under the kidney capsule [7].

This study provides evidence that copulatory behavior in male rats is inhibited by hyperprolactinemia induced by SAL. In other words, SAL is important for inhibiting copulatory behavior. Further studies are needed to evaluate the mechanisms and functions of SAL during copulatory behavior.


