Characteristics of prolactin-releasing response to salsolinol in vivo in cattle


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Abstract

The aims of the present study were to clarify the effect of salsolinol (SAL), a dopamine (DA)-derived endogenous compound, on the secretion of prolactin (PRL) in cattle. The experiments were performed from April to June using calves and cows. A single intravenous (i.v.) injection of SAL (5 mg/kg body weight [BW]) or sulpiride (a DA receptor antagonist, 0.1 mg/kg BW) significantly stimulated the release of PRL in male and female calves (P < 0.05), though the response to SAL was smaller than that to sulpiride. The secretory pattern of PRL in response to SAL or sulpiride in female calves resembled that in male calves. A single i.v. injection of SAL or sulpiride significantly stimulated the release of PRL in cows (P < 0.05). There was no significant difference in the PRL-releasing response between the SAL- and sulpiride-injected groups in cows. A single intracerebroventricular injection of SAL (10 mg/head) also significantly stimulated the release of PRL in castrated calves (P < 0.05). These results show that SAL is involved in the regulatory process for the secretion of PRL, not only in male and female calves, but also in cows. The results also suggest that the potency of the PRL-releasing response to SAL differs with the physiological status of cattle.

Keywords: Salsolinol; Prolactin; Cattle; Dopamine

1. Introduction

The secretion of prolactin (PRL) is under the dominant and tonic inhibitory control of dopamine (DA) [1,2], and there is no consensus about the nature and identity of physiologically relevant PRL-releasing factors (PRFs). However, it has been demonstrated that salsolinol (SAL), a DA-derived compound, is a putative endogenous PRF in rats [3–5]. Administration of SAL to freely moving rats dose-dependently increased plasma concentrations of PRL [3,5,6]; in addition, stress- as well as suckling-induced release of PRL in rats were blocked by an antagonist of SAL (1MeDIQ) [7]. Recently, our studies have shown that intravenous (i.v.) injection of SAL stimulates the release of PRL in adult female goats [8–10]. A study by another group has also demonstrated that SAL is present in the infundibular nucleus-median eminence in lactating sheep and that the extracellular concentration of this compound increases in response to a suckling stimulus, which is associated with an increase in plasma PRL concentrations [11]. Salsolinol may thus be a physiolog-
ically relevant endogenous PRF, not only in rats but also in domestic animals. In cattle, we have found that intracerebroventricular (i.c.v.) injection of SAL stimulates the release of PRL in castrated calves [8]; however, the precise effects of SAL on the secretion of PRL associated with sex or age in cattle have not yet been determined. In the present study, to clarify the effect of SAL on the secretion of PRL in cattle, the PRL-releasing response to a single i.c.v. injection of SAL was examined in male calves, female calves, and cows. The relation between the inhibitory effect of endogenous DA on the release of PRL and the effects of SAL administration was also investigated using a DA receptor antagonist, sulpiride.

In addition, to confirm a possible hypothalamic site of action of SAL in our previous study in cattle, PRL release in response to a single i.c.v. injection of SAL was also examined in calves.

2. Materials and Methods

All animal procedures undertaken were approved by the Animal Care and Use Committee of Iwate University and the National Institute of Agrobiological Sciences.

2.1. Intravenous injection of SAL or sulpiride in male and female calves

Five male Japanese Black calves (age: 4-6 mo; mean body weight [BW]: 144 ± 16 kg [mean ± SEM]) and 5 female Japanese Black calves (age: 5-6 mo; BW: 144 ± 9 kg) were used. The calves were housed in pens, and natural light was allowed to enter through windows. The calves were fed hay and concentrate at 9:30 AM and 4:00 PM daily. Water was available continuously. On experimental days, the calves were not fed before or during the treatment; they were fed only after the treatment. The experiments were performed from May to June in Morioka, Japan. Male and female calves were given a single i.v. injection of SAL (5 μg/kg BW; SAL-hydrobromide, synthesized by Professor F. Fülöp, Institute of Pharmaceutical Chemistry, Szent-Györgyi Albert University, Szeged, Hungary) or sulpiride (0.1 mg/kg BW; Sigma, St. Louis, MO, USA), or 2 mL of saline as a control. The doses of SAL and sulpiride were chosen according to published results of previous experiments [8–10]. All calves received all the treatments, and the experiments were carried out at 2- or 3-d intervals. The order in which each calf received the treatments was determined at random. The substances were injected into freely moving animals via an indwelling catheter previously inserted into one of the external jugular veins. Blood was sampled from the indwelling catheter, and the blood samples (2.5 mL each) were drawn at 10-min intervals (for 60 min after the SAL, sulpiride, or saline injection) and 20-min intervals (for 60 min before the injection, and for 60 min starting 60 min after the injection). The blood samples were collected into centrifuge tubes containing heparin and were immediately chilled on ice. Individual plasma samples were obtained after centrifugation and stored at –30 °C until assayed for PRL.

2.2. Intravenous injection of SAL or sulpiride in cows

Three Japanese Black cows (age: 2 y; BW: 418 ± 15 kg) were used. The cows were between 86 and 116 d postpartum. Their calves were weaned 14-42 d before the experiment. Except for the animals used, the experiment was the same as that described above. The experiment was performed in June in Morioka, Japan.

2.3. Intracerebroventricular injection of SAL in castrated calves

Three castrated Holstein calves (age: 6 mo; BW: 204 ± 15 kg) were used. The experiments were performed in an environment-controlled room. The conditions were set as follows: room temperature 20 °C, humidity 60%, light-dark 500-10 lux (12 h/12 h). The calves were fed hay and concentrate at 8:30 AM and 3:30 PM daily. Water was available continuously. A guide cannula was stereotactically implanted into the third ventricle of each calf at least 3 wk before the experiment [12]. The experiments were carried out in April in Tsukuba, Japan. Catheters for collecting blood were inserted into a jugular vein at least 1 d before the experiment. On the day of the experiment, a microinfusion cannula was inserted into the third ventricle through the implanted guide cannula, and SAL (10 mg/calf) dissolved in 200 μL of saline, or 200 μL of saline vehicle alone (as a control), was infused over a period of 30 s. Blood was sampled from the indwelling catheter, and the blood samples (2.5 mL each) were drawn at 10-min intervals (for 60 min after the SAL or saline injection) and 20-min intervals (for 60 min before the injection, and for 120 min starting 60 min after the injection). The experiments were carried out at 2- or 3-d intervals.

2.4. Radioimmunoassay

Plasma PRL concentrations were measured by a double-antibody radioimmunoassay procedure with some modifications [13]. The hormone used for iod-
ination was NIDDK oPRL-1-3. The antiserum to ovine PRL, prepared in rabbits, was AFP-C358106. Anti-rabbit IgG was purchased from Phoenix pharmaceuticals, Inc. (Belmont, CA, USA). The intra- and interassay coefficients of variation were 8.8% and 6.0%, respectively. The least detectable concentration was 0.1 ng/mL. The displacement curves for increasing volumes of pooled bovine serum paralleled the PRL standard curves.

2.5. Statistical analysis

All data from the experiments are presented as the mean ± SEM. The area under the response curve (AUC) of PRL after the treatment of SAL, sulpiride, or saline was calculated in each experiment. The statistical significance of differences in the AUC among the groups was evaluated by 1-way analysis of variance (ANOVA), and the Newman-Keuls test was used as a post hoc test. The statistical significance of differences in the mean plasma concentration before treatments between male and female calves was evaluated by 2-way ANOVA with 2 factors (sex and treatment). The statistical significance of differences in the AUC between SAL- and saline-injected groups in the i.c.v. injection experiment was determined using the Student t test. All data were analyzed using GraphPad Prism (Version 3, GraphPad Software, San Diego, CA, USA). Results were considered significant at the P<0.05 level.

3. Results

3.1. PRL-releasing response to i.v. injection of SAL or sulpiride in male and female calves

The plasma PRL concentrations in response to a single i.v. injection of SAL or sulpiride in male and female calves are shown in Fig. 1. The injections of SAL or sulpiride significantly stimulated the release of PRL in male (Fig. 1a) and female (Fig. 1b) calves (P<0.05). There were no significant differences in the AUC of PRL for the 120-min period between the values produced after the injection of SAL and those produced by the controls; however, the AUC of PRL for the 60-min period (1198 ± 283 ng·min·mL⁻¹) was 3.6 times greater than the values for the controls (332 ± 35 ng·min·mL⁻¹) in male calves (P<0.05). The response to sulpiride was greater than that to SAL, and the AUC of PRL for the 120-min period after the injection of sulpiride (3376 ± 742 ng·min·mL⁻¹) was 5.2 and 2.1 times greater than the values for either the controls (648 ± 90 ng·min·mL⁻¹) or SAL-injected groups (1625 ± 401 ng·min·mL⁻¹) in male calves (P<0.05).

The secretory pattern of PRL in response to SAL or sulpiride in female calves resembled that in male calves, though the mean plasma concentration before treatments (-60 to 0 min) in the female calves was greater than that in the male calves (P<0.05). The AUC of PRL for the 120-min period after the injection of SAL (3854 ± 587 ng·min·mL⁻¹) was 3.0 times greater than the values for the controls (1280 ± 100 ng·min·mL⁻¹) in female calves (P<0.05). The AUC of PRL for the 60-min period after the injection of sulpiride (3854 ± 587 ng·min·mL⁻¹) was 3.0 times greater than the values for the controls (1280 ± 100 ng·min·mL⁻¹) in female calves (P<0.05). The AUC of PRL for the 120-min period after the injection of sulpiride (6856 ± 1121 ng·min·mL⁻¹) was 5.4 and 1.8 times greater than the values for either the controls or the SAL-injected groups in female calves (P<0.05).

3.2. PRL-releasing response to i.v. injection of SAL or sulpiride in cows

The plasma PRL concentrations in response to a single i.v. injection of SAL or sulpiride in cows are shown
in Fig. 2. The injections of SAL or sulpiride significantly stimulated the release of PRL \((P < 0.05)\). The secretory pattern of PRL in response to SAL or sulpiride was similar, and the AUC of PRL for the 120-min period after the injection of SAL \((6014 \pm 566 \text{ ng} \cdot \text{min} \cdot \text{mL}^{-1})\) or sulpiride \((6049 \pm 792 \text{ ng} \cdot \text{min} \cdot \text{mL}^{-1})\) was 9.8 and 9.9 times greater than the values for the controls \((614 \pm 158 \text{ ng} \cdot \text{min} \cdot \text{mL}^{-1})\) \((P < 0.05)\).

3.3. PRL-releasing response to i.c.v. injection of SAL in castrated calves

The plasma PRL concentrations in response to a single i.c.v. injection of SAL in castrated calves are shown in Fig. 3. The i.c.v. injection of 10 mg of SAL significantly stimulated the release of PRL \((P < 0.05)\). The AUC of PRL for the 180-min period after the injection of SAL \((14310 \pm 1724 \text{ ng} \cdot \text{min} \cdot \text{mL}^{-1})\) was 3.1 times greater than the values for the controls \((4596 \pm 1012 \text{ ng} \cdot \text{min} \cdot \text{mL}^{-1})\) \((P < 0.05)\).

4. Discussion

The present study provides the first data on the secretion of PRL in response to SAL in vivo in cattle. It was found that SAL is able to stimulate the release of PRL not only in male and female calves, but also in cows.

We used prepubertal male and female calves of similar ages. The body weights of the 2 groups were similar, and the experiments were performed at the same periods. In male and female Japanese Black cattle, puberty occurs around 14 and 8-12 mo of age, respectively [14]. In this study, the mean basal plasma PRL concentration in the female calves was greater than that in the male calves. This difference might be caused by physiological differences in the process of sexual development to puberty. The potency of SAL in inducing PRL release also tended to be greater in female than male calves. It is noted that the secretion of PRL is enhanced by estrogenic hormones acting on both the adenohypophysis and the hypothalamus [15].

The relation between SAL and sulpiride regarding the secretion of PRL in cows differed from that in calves; that is, the PRL-releasing response to SAL was similar to that to sulpiride in cows, and the PRL-releasing response to SAL was greater in cows than in either male or female calves. These differences might be associated with lactation in cows. The cows used were at 86 to 116 d postpartum and had nursed their calves until 14 to 42 d before the start of the experiment. A major function of PRL in cows is stimulation of milk production by the mammary gland for the support of neonatal offspring [15]. Therefore, the PRL-releasing response to SAL in cows might be greater than that in calves, even if the cows’ calves were weaned before the experiment. A recent study has shown that SAL acts as a neurotransmitter involved in regulating the secretion of PRL during lactation in sheep [11].

A single injection of 10 mg of SAL into the third ventricle strongly stimulated the release of PRL in calves. In our previous study, a single injection of 5 mg of SAL into the third ventricle in similar calves significantly increased plasma PRL concentrations at 30 and 40 min after the injection [8]. However, the PRL-releasing response to 10 mg of SAL in this study was obviously greater than that to 5 mg of SAL in the previous study. We could thus confirm that SAL exerts its effect, at least in part, on a hypothalamic site. Misztal et al
[11] have recently demonstrated that the concentrations of SAL in the infundibular nucleus-medium eminence in lactating sheep increased with the elevation of plasma PRL concentrations. High-affinity binding sites for SAL have been detected in the median eminence and hypothalamus in rats [4,16]. The precise site of SAL’s action on the hypothalamus is not yet known, however, it is most likely the hypothalamic neuroendocrine dopaminergic (NEDA) neurons or their terminals [4,5]. The results obtained when sulpiride was injected have confirmed that the secretion of PRL is under the dominant and tonic inhibitory control of DA in cattle. Given that it resembles DA in structure, SAL may be partially able to antagonize the effect of DA on its receptors to release PRL. However, SAL does not bind or has marginal affinity to DA receptors D1 and D2 [4,17,18], and DA receptor ligands did not compete with [3H]-SAL in binding with its hypothetical receptor [4,16]. In addition, SAL is synthesized in DA neurone [4,5]. Therefore, our results may suggest that the actual ratio at which PRL is secreted may be related to the ratio of DA to SAL in the hypothalamic NEDA system in cattle.

In conclusion, SAL is involved in the regulatory process for the secretion of PRL not only in male and female calves, but also in cows. Furthermore, the results suggest that the potency of the PRL-releasing response to SAL differs with the physiological status of cattle.

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