Substance P stimulates gonadotropin-releasing hormone release from rat hypothalamus in vitro with involvement of oestrogen

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Abstract. The effects of substance P on the release of LH and GnRH were examined in a sequential double-chamber perfusion system by perifusing the medio-basal hypothalamus and/or pituitary excised from normal female rats in dioestrus or ovariectomized rats. When the medio-basal hypothalamus and pituitary from normal rats were perifused in series with substance P (10^{-6} mol/l), the concentration of LH in the efflux was significantly (P < 0.05) increased by 70-120% compared with that before the injection, but substance P had no effect on LH release from the pituitary perifused alone. This LH release by substance P increased in a dose-dependent manner and was blocked by substance P antagonist. Administration of 10^{-6} mol/l substance P induced a significant release (40-80% increase, P < 0.05) of GnRH from the medio-basal hypothalamus. Infusion of 10^{-6} mol/l substance P induced significant release (50-100% increase, P < 0.05) of LH and GnRH in ovariectomized rats with an implanted oestradiol capsule, but caused no significant increase in LH release in ovariectomized rats without an oestradiol capsule. Progesterone injection to both ovariectomized rats and ovariectomized rats with an implanted oestradiol capsule had no significant effect on the response of LH to substance P. These findings suggest that substance P induces GnRH release from the medio-basal hypothalamus, resulting in LH release from the pituitary, and that oestrogen may be involved in these processes.

Substance P (SP) was the first hormonal peptide discovered to have a dual brain-gut distribution. Subsequently, SP was found to be present not only in the brain, but also in the peripheral sensory and autonomic nervous system (Takahashi et al. 1974; Brownstein et al. 1976; Cuello et al. 1977). SP immunoreactive material is present in synaptic vesicles in nerve terminals of the rat spinal cord, and is concentrated in vesicular subcellular fractions of the rat brain (Cuello et al. 1977). These findings provide evidence for the role of SP as a neurotransmitter.

There are reports that SP is involved in the regulation of pituitary hormone secretion (Kato et al. 1976; Rivier et al. 1977; Vijayan & McCann 1979; Eckstein et al. 1980; Antonowicz et al. 1982; Coslovsky et al. 1984; DePalatis et al. 1985). Intravenous injection of SP significantly increases the release of growth hormone and prolactin (Kato et al. 1976; Vijayan & McCann 1979). The effect of SP, however, on gonadotropin secretion is uncertain. Vijayan & McCann (1979) reported that following intraventricular injection, SP induced a significant increase in the plasma LH level, but that it induced a slight decrease of the plasma LH level when administered iv. Fisher et al. (1974) observed in in vitro studies that SP induces release of LH and FSH from the rat pituitary.

The present study, using an in vitro double-chamber perfusion system, was designed to determine the effects of SP on the secretion of hypothalamic GnRH and pituitary LH and to assess the roles of the steroid hormones oestrogen and progesterone in these processes in rats.
Material and Methods

Animals
One hundred and thirty-six female Wistar-Imamichi rats, weighing 200–250 g, were divided into two groups. Vaginal smears were taken from the rats in group I every day for 1–2 weeks, and the rats were decapitated in dioestrus II of the oestrous cycle. The rats in group II were ovariectomized (OVX), and a capsule containing 1 mg of oestradiol (E₂) plus 9 mg of cholesterol was implanted into half of them 14 days later (OVX+E₂). Two days later, half of the rats in group II (OVX+P, OVX+E₂+P) received an ip injection of 5 mg of progesterone (P) at 08.00 h.

Perfusion procedure
Rats were decapitated at 12.30 h, and the medio-basal hypothalamus (MBH) and pituitaries were excised and perifused in the serial double-chamber perifusion system described previously (Miyake et al. 1982). The MBH tissue block was demarcated by the hypothalamic sulci laterally, the caudal aspect of the optic chiasma rostrally, the rostral aspect of the mammillary bodies caudally, and a plane 2 mm from the ventral surface of the median eminence dorsally. In this system, the effluent from the first chamber perifuses the second chamber and the effluent from the second chamber is collected in 0.5-ml fractions at 10-min intervals. One MBH was placed in the first 0.1 ml plastic chamber, and one pituitary in the second chamber. In some experiments, the two were perifused in series, whereas in others either the MBH or the pituitary alone was perifused. These sequential chambers were perifused with Medium 199 (Handai-Biken, Japan), Medium 199 containing various concentrations of SP or Medium 199 containing 10⁻⁷ mol/l SP plus 10⁻⁶ mol/l SP antagonist. The medium was saturated with 95% O₂-5% CO₂ at 37°C at a flow rate of 3 ml per h. The system was equilibrated for 2.5 h before collection of samples was started. Then six samples were collected over a period of 1 h, Medium 199 containing substances or Medium 199 alone as a control was perifused for the next 30 min, thereafter the samples were collected for the next 1.5 h. Fractions were totally collected over a 3-h period.

Hormone determination
LH and GnRH in the effluent fractions were measured by radioimmunoassay as described previously (Miyake et al. 1980, 1982). The sensitivities of these assays were 20 pg NIADDK (National Institute of Arthritis, Digestive and Kidney Diseases) rLH-RP-2/tube for LH and 1 pg/tube for GnRH, and the intra-assay coefficients of variation were 7.5% and 15.8%, respectively. Each group consisted of 8 experiments. The statistical significances of differences between hormone concentrations before and after each treatment were examined by two-way analysis of variance.

Results
The mean (± SEM) basal concentrations of LH in the effluent from the pituitary excised from normal, OVX and OVX + E₂ rats were 5.37 ± 0.29 µg/l, 41.65 ± 2.09 µg/l and 19.91 ± 1.08 µg/l, respectively, and the mean ± (SEM) basal concentrations of the efflux GnRH from the MBH of normal rats were 81.0 ± 6.0 ng/l. The mean concentrations of LH and GnRH in the 6 fractions collected during the first hour were taken as the basal levels, and values during experiments

Changes in LH in the perifusion efflux from the pituitary perifused alone or in series with the MBH. The mean LH concentration of 6 fractions collected in the first 1 h in each group was taken as the basal level. Values during the experiments were calculated as percent changes from the mean basal value in each group. Points are means (± SEM) for 8 experiments. Vertical shaded areas indicate times when medium with SP (10⁻⁶ mol/l) was perifused. LH increase by SP with hypothalamus is significant by two-way analysis of variance (P < 0.01). LH increase without hypothalamus is not significant.
were calculated as per cent changes from the mean basal values.

Fig. 1 shows the changes of LH in the perfusion efflux from the pituitary with or without MBH in group I. When the MBH and pituitary were perifused in sequence, $10^{-6}$ mol/l SP significantly ($P < 0.01$) raised the LH concentration in the effluent by 70–120% of the initial level for over 90 min. When the pituitary alone was perifused, SP caused no significant change in the LH level. As shown in Fig. 2, administration of $10^{-6}$ mol/l SP significantly ($P < 0.01$) increased the GnRH concentration in the effluent by 40–80% of the pre-infusion level for about 50 min.

Fig. 3 shows the net increase of LH during 2 h from the start of $10^{-6}$–$10^{-10}$ mol/l SP administration when MBH and pituitary were perifused in series. The net increase in LH after SP infusion was dose-dependent.

The effect of SP antagonist is shown in Fig. 4. When the medium containing SP antagonist ($10^{-6}$ mol/l) was perifused, the LH increase by SP ($10^{-7}$ mol/l) was significantly ($P < 0.05$) suppressed.

Fig. 5 shows the changes in LH secretion in the perfusion efflux from the pituitary excised from OVX, OVX + E$_2$ and OVX + E$_2$ + P rats. Infusion of $10^{-6}$ mol/l SP induced a significant increase in LH in the OVX + E$_2$ group (50–85%, $P < 0.05$) and in the OVX + E$_2$ + P group (50–80%, $P < 0.05$). However, $10^{-6}$ mol/l SP had no effect on LH secretion in the OVX group and the OVX + P group. A slight increase in LH was observed in the OVX + P group. However, this increase is not significant. The changes in GnRH secretion from the MBH in the perfusion efflux are shown in Fig. 6. Administration of $10^{-6}$ mol/l SP caused a significant ($P < 0.01$) increase in GnRH in both OVX + E$_2$ and OVX + E$_2$ + P group.

![Fig. 2.](image1)

GnRH changes in the perfusion efflux from the MBH by SP ($10^{-6}$ mol/l). The mean GnRH concentration of 6 fractions collected in the first 1 h was taken as the basal level. Other explanations are as for Fig. 1. GnRH increase by SP is significant by two-way analysis of variance ($P < 0.01$).

![Fig. 3.](image2)

Dose-effect relationship between net increase in LH and SP concentrations when MBH and pituitary were perifused in series. Significant differences between the mean of two groups are indicated by asterisks (Student's t-test).
Discussion

Previous studies have not shown the site or mechanism of action of SP in inducing gonadotropin secretion. The present study clearly demonstrated that SP increases GnRH release from the MBH, resulting in LH release from the pituitary. Vijayan & McCann (1979) observed that intraventricular injection of 0.5 μg of SP into ovariectomized rats induced significant increase in the plasma LH level, but that iv injection of 1 μg of SP caused no significant increase in the LH level. These findings suggest that SP acts at the hypothalamic level or at a higher level in the central nervous system, and the present data are consistent with their conclusion. Fisher et al. (1974), however, found that SP causes release of LH and FSH from the pituitary in vitro. Our findings are not consistent with those of Fisher et al. (1974). The discrepancy of the results as to whether SP has a direct effect on LH release from the pituitary or not may depend on the dose of SP used. Fisher et al. (1974) observed in in vitro studies that 10–100 mg/l (7.1 × 10^{-6}–7.1 × 10^{-5} mol/l) SP resulted in an increased LH release from the rat pituitary, but that 1 mg/l (7.1 × 10^{-7} mol/l) SP had no effect on the LH concentrations. Brownstein et al. (1976) reported that the highest concentration of SP found in the brain is 2 × 10^{-12} mol/10 mg wet weight in the pituitary, and suggested that the local concentration of SP is actually less than 2 × 10^{-7} mol/l. These findings taken together suggest that at lower concentrations (≤ 10^{-6} mol/l), SP may cause release of GnRH from the MBH, whereas at higher concentrations (≥ 10^{-5} mol/l) it may induce release of LH from the pituitary.

In the present study a significant release of LH from the pituitary in series with the MBH and GnRH release from the MBH was observed until 2 h after the start of SP administration. In the perifusion system used, the medium containing SP in the chamber does not change so rapidly when the medium is changed to the medium without SP. The half-time of SP disappearance in the sequential double-chamber system is about 20 min. This slow disappearance may have resulted in the continuation of the significant release of LH and GnRH after the cessation of SP infusion.

We furthermore found that E2, but not P, is

\[ \frac{\text{Medium or Substance P antagonist}}{\text{Percent changes in LH}} \]

\[ \frac{\text{Substance P (10}^{-7}\text{M)}}{\text{Substance P (10}^{-7}\text{M}) + \text{antagonist (10}^{-9}\text{M)}} \]

Fig. 4.
LH changes in the perifusion efflux from the pituitary by SP (10^{-7} mol/l) when Medium 199 alone or Medium 199 containing SP antagonist (10^{-6} mol/l) were perifused. LH increase by SP was significantly (P < 0.05) suppressed by SP antagonist (two-way analysis of variance).

250
involved in the release of gonadotropin by SP. There are reports that in rats the content of SP in the median eminence (Antonowicz et al. 1982) and the pituitary gland (DePalatis et al. 1985) changes during the oestrous cycle and that that in the pituitary changes on ovariectomy and/or E₂ administration (Coslovsky et al. 1984; DePalatis et al. 1985). These results suggest that the SP content depends on the level of gonadal hormone in the animal. Together with the present results, these findings indicate that oestrogen may be physiologically important for the regulation of the synthesis or release of SP and for the effect of SP on hypothalamic GnRH release. Further studies are necessary on the relation of SP and oestrogen in the regulation of GnRH and LH secretion.

Fig. 5.
LH changes in the efflux from the pituitary by SP (10⁻⁶ mol/l) perifused in series with the MBH. LH increase is significant (P < 0.05) in the OVX + E₂ and OVX + E₂ + P group, but not significant in the OVX and OVX + P group by two-way analysis of variance.

Fig. 6.
GnRH changes in the efflux from the MBH by SP (10⁻⁶ mol/l). GnRH increase is significant (P < 0.01) in the OVX + E₂ and OVX + E₂ + P group by two-way analysis of variance.
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References


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