Effect of Substance P on Basal and Thyrotropin-Releasing Hormone–Stimulated Thyrotropin Release in Humans

V. Coiro, R. Volpi, L. Capretti, D. Gramellini, C. Cigarini, C. Papadia, A. Caiazza, G. Caffarri, P. Caffarra, and P. Chiodera

To test the possible effects of intravenous administration of substance P (SP) on basal and thyrotropin-releasing hormone (TRH)-stimulated thyrotropin (TSH) release, SP was infused alone (0.5 or 1.5 pmol/kg^{-1}/min^{-1} for 60 minutes) or after TRH (20 or 400 μg in an intravenous bolus) in 21 normal male subjects (aged 26 to 36 years) and in 18 normal women (aged 25 to 32 years). Women were studied during follicular (day 6 to 8) and luteal (day 21 to 23) phases of following regular menstrual cycles. In addition, plasma cortisol levels during SP infusion were measured. In agreement with previous findings, significant increments in plasma cortisol levels were observed in men and women when the higher (1.5 pmol/kg^{-1}/min^{-1}) but not the lower (0.5 pmol/kg^{-1}/min^{-1}) amount of SP was administered. In contrast, in both men and women basal and TRH (20 or 400 mg)-induced TSH releases were not modified by SP at any tested amount. Results in the follicular and luteal phase were similar. These data suggest that in normal men and women plasma SP is not involved in the control of TSH release, at least not outside the blood-brain barrier.

SUBSTANCE P (SP) has been found in the pituitary gland of several animal species. Furthermore, this neuropeptide has been found capable of modifying pituitary hormonal secretions in various experimental conditions in vivo and/or in vitro.[1,2] These observations have led to hypotheses of a role for SP as a neurotransmitter or neuromodulator in the hypothalamus and/or as a local or paracrine agent at the pituitary level.[1,2]

In recent years, we have examined the effects of intravenously infused SP on the secretion of arginine vasopressin and oxytocin from the posterior pituitary and growth hormone, corticotropin, luteinizing hormone, and prolactin from the anterior pituitary.[3-7] At present, studies on a possible role of SP in the control of thyrotropin (TSH) secretion in humans are scant, even though such a possibility is suggested by the finding of SP in a subset of thyrotrophs in humans and by several animal studies.[8-11]

The present study was undertaken to evaluate the effects of SP on the TSH secretory pattern in normal human subjects. For this purpose, SP was infused intravenously in basal conditions and after activation with a minimal effective dose (20 μg) of thyrotropin-releasing hormone (TRH).

In addition, we evaluated whether an infusion of SP modifies the TSH-secreting cell capacity to respond to a maximal stimulation with a saturating dose (400 μg) of TRH.

Since previous studies in rats have shown different TSH responses in male and female rats to the intravenous injection of SP,[12,13] experiments were performed in men and women. Women were tested during the follicular and luteal phases.

In addition, plasma cortisol levels were measured during tests in order to obtain an independent parameter of SP activity.

SUBJECTS AND METHODS

Twenty-one men (aged 26 to 36 years) and 18 normally menstruating women (aged 25 to 32 years) participated in this study after giving informed consent. The study was performed in accordance with the Helsinki II declaration. All subjects were within 10% of their ideal body weight. They were fully ambulatory, well nourished, and without clinical or laboratory evidence of endocrine, metabolic, renal, hepatic, or neoplastic diseases. None of them were taking drugs before and during the period of the study or engaged in excessive alcohol consumption (<300 g ethanol/wk). Basal triiodothyronine and thyroxine levels and the presence of antithyroid-globulin and antithyroid microsomal antibodies were evaluated in all subjects. Circulating levels of 17β-estradiol (E2) and progesterone (P) were measured at weekly intervals in all women and served for determination of the various phases of the menstrual cycles.

Subjects were randomly divided into three groups of seven men and three groups of six women. Each group participated in a different study.

SP Infusion Study

At 8:30 AM of the experimental day, synthetic SP (Clinalfa, Laufelfingen, Switzerland) was infused intravenously over a period of 60 minutes at a dose of 0 (vehicle), 0.5, or 1.5 pmol/kg^{-1}/min^{-1}. The calculated dose (total amount, 12 mg/100 g body weight) was dissolved in 50 mL isotonic saline containing 87 mmol/L human serum albumin (Istituto Sierovaccinogeno Italiano, Santantimo, Italy). The blood samples were collected 10 minutes before (time −10) and just before (time 0) SP infusion and at 10, 20, 30, 40, 50, 60, 70, 80, and 90 minutes after SP administration. The tests were performed in random order in men and women. Men were tested three times with an interval of at least 10 days. Women were tested three times in the follicular phase (day 6 to 8) and three times in the luteal phase (day 21 to 23) of three following regular menstrual cycles.

In a previous study, we determined that the dose 0.5 pmol/kg^{-1}/min^{-1} SP does not modify cortisol secretion in humans.[5] In the present study, tests with 0.5 pmol/kg^{-1}/min^{-1} SP were performed

From the Departments of Internal Medicine, Obstetrics and Gynecology, and Neurology, School of Medicine, University of Parma, Parma; the Endocrine Unit, Hospital of Codogno, Codogno; the Division of Obstetrics and Gynecology, Hospital of Reggio Emilia, Reggio Emilia; the Division of Internal Medicine, Hospital of Fidenza, Fidenza; and the RIA Laboratory, Hospital of Guastalla, Guastalla, Italy.

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Address reprint requests to V. Coiro, MD, Department of Internal Medicine, Via Gramsci 14, 43100 Parma, Italy.

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to evaluate the effects of SP on TSH secretion without the possible interference of increased plasma cortisol levels on TSH secretion.

TRH (20 μg) Plus SP Infusion Study

The tests were performed to establish whether SP modifies TSH sensitivity to TRH.

For this purpose, 20 μg TRH (minimal effective dose) was used. In previous studies,[14] this amount has been found to elicit a slight but significant increase in serum TSH secretion.

All tests started at 8:30 AM. Men and women were infused with 0 (vehicle), 0.5, or 1.5 pmol/kg^{-1}/min^{-1} SP for 60 minutes. After 30 minutes of SP infusion (time 0), TRH was administered in an intravenous bolus as in the control test. Blood samples were taken just before and every 10 minutes for the next 1.5 hours after TRH injection.

Men were tested three times with an interval of at least 10 days between one test and the following. Women were tested three times in the follicular phase (day 6 to 8) and three times in the luteal phase (day 21 to 23) of three following normal menstrual cycles. In men and women, tests were performed in random order.

TRH (400 μg) Plus SP Infusion Study

These tests were performed in the remaining seven men and six women with the purpose being to clarify whether SP treatment modifies the TSH-secreting cell capacity to respond to a maximal stimulation with TRH. The tests were performed as described above, except for the injection of 400 instead of 20 μg TRH.

At each sampling time during all tests, blood pressure and heart rate were monitored.

Serum TSH levels were measured in all samples with an immunoradiometric method. All samples from a single subject were run in duplicate and in the same assay. The limit of detection of the TSH assay was 0.02 mU/L. The intraassay and interassay coefficients of variation were 4.8% and 6.7%, respectively. Plasma cortisol levels were measured in all samples by radioimmunoassay.

Serum triiodothyronine, thyroxine, E2, and P levels were measured with radioimmunoassay methods in samples taken at time 0 of all tests. Commercial kits were used for all hormonal measurements. The presence of antithyroid antibodies was detected in samples taken at time 0 of the SP infusion and TRH studies, using a hemoagglutination technique (Wellcome reagents Thymuse-T and -M, Pomezia, Rome, Italy).

Results were analyzed by ANOVA with repeated measures and Wilcoxon's matched-pair rank sum test, as appropriate. Values are expressed as the mean ± SE.

RESULTS

SP Infusion Study

The infusion either of saline, the lower dose (0.5 pmol/kg^{-1}/min^{-1}), or the higher dose (1.5 pmol/kg^{-1}/min^{-1}) of SP did not change the secretory pattern of TSH at any time during tests in men and in women during follicular and luteal phases (Fig 1).

The mean plasma levels of E2 were 65.3 ± 2.8 pg/mL in the follicular phase and 81.4 ± 4.2 in the luteal phase. P concentrations in the plasma were 0.9 ± 0.2 ng/mL in the follicular phase and 10.3 ± 0.6 in the luteal phase. E2 and P values reported above are the mean ± SE of levels found at time 0 of saline and 0.5- and 1.5-pmol/kg^{-1}/min^{-1} × 60 min SP tests. Similar values of E2 and P were found in women tested with TRH (20 or 400 μg).

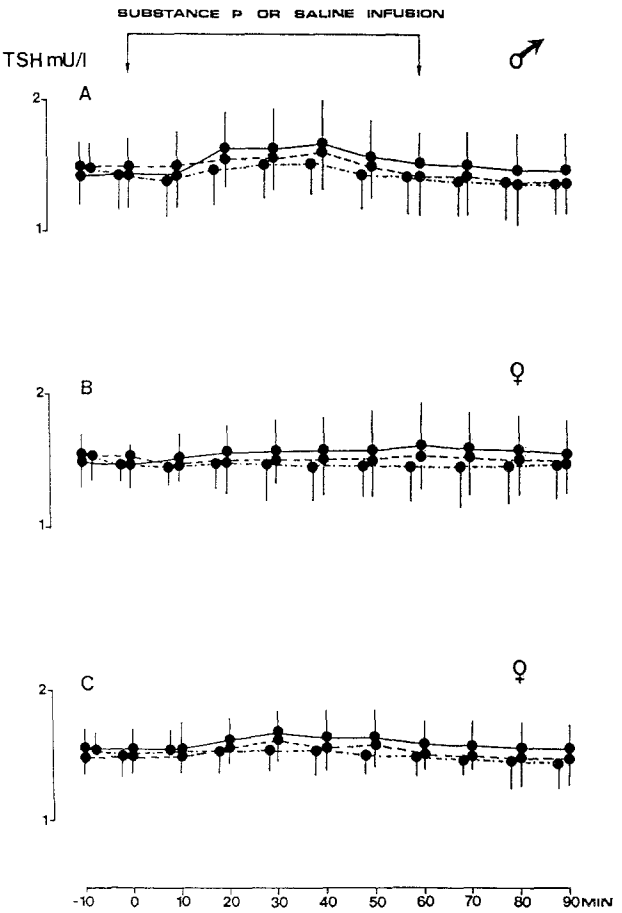

Fig 1. Serum TSH levels after SP or saline infusion (A) in men (n = 7) and women in (B) follicular phase (n = 6) and (C) luteal phase (n = 6). Each point represents the mean ± SE of the observations.

TRH (20 μg) Plus SP Infusion Study

Administration of 20 μg TRH induced a significant increase in serum TSH levels. The mean peak response of TSH was observed 30 minutes after TRH injection ($P < .01$ v time 0). The infusion of either the lower (0.5 pmol/kg^{-1}/min^{-1}) or the higher (1.5 pmol/kg^{-1}/min^{-1}) dose of SP did not change the pattern or magnitude of the TSH response to TRH. Similar results were found in men (Fig 2) and in women during the follicular and luteal phases (Figs 3 and 4).

TRH (400 μg) Plus SP Infusion Study

Administration of 400 μg TRH induced a high TSH response with a mean peak 30 minutes later ($P < .01$ v time 0). The TSH response to TRH did not change when either the lower (0.5 pmol/kg^{-1}/min^{-1}) or the higher (1.5 pmol/kg^{-1}/min^{-1}) dose of SP was infused. Similar results were found in men (Fig 2) and in women during follicular and luteal phases (Figs 3 and 4).

In all studies, plasma cortisol levels showed a slight physiological decline during the infusion of saline or 0.5 pmol/kg^{-1}/min^{-1} SP, regardless of TRH administration. In contrast, plasma cortisol concentrations increased significantly during 1.5-pmol/kg^{-1}/min^{-1} SP infusions. The mean

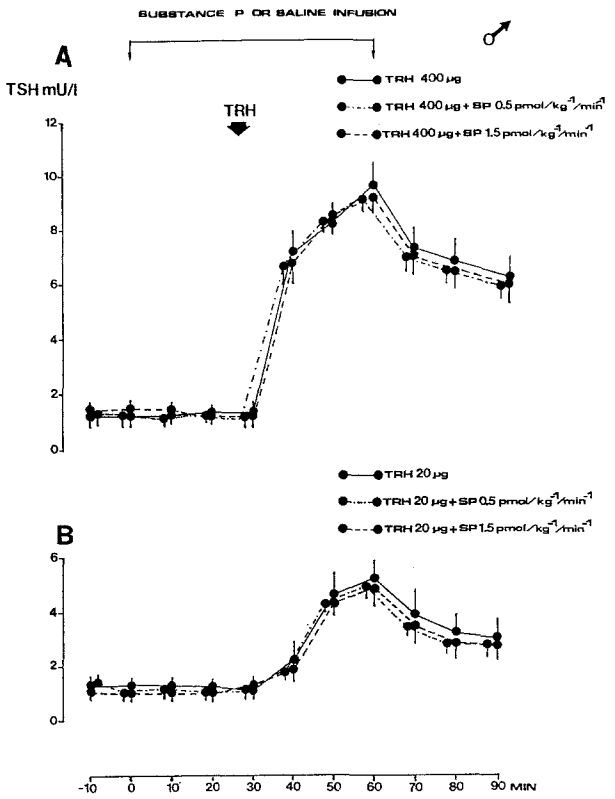

Fig 2. Effect of (A) 400 µg or (B) 20 µg of TRH with or without SP on TSH secretion in seven normal men. Each point represents the mean ± SE of the observations.

peak response was observed at 40 minutes (SP infusion study: men at baseline, 289.7 ± 24.1 pmol/L [mean ± SE]; 40 minutes, 507.7 ± 41.4, $P < .01$; women in follicular phase at baseline, 287.4 ± 23.0; 40 minutes, 511.4 ± 38.5, $P < .01$; women in luteal phase at baseline, 290.4 ± 22.7; 40 minutes, 513.8 ± 40.6, $P < .01$). No significant differences in cortisol secretory patterns were observed between men and women tested during follicular or luteal phases. The cortisol responses to SP were similar in both the presence and absence of TRH (200 or 400 µg, data not shown).

Blood pressure and heart rate remained constant in all subjects during all tests.

DISCUSSION

A physiological involvement of SP in the regulation of the hypothalamic-pituitary-thyroid axis has been suggested by studies in rats showing that the thyroid status exerts a strong influence on pituitary SP concentrations,[12,13] synthesis, and/or release.[15-17] The finding of a close relationship between SP nerve fibers and TSH-secreting cells in both rats and humans[9-11,18] suggested possible effects of SP in the control of TSH secretion. However, further in vivo studies failed to show significant stimulatory effects of SP on TSH secretion at the pituitary level.[19,20] In concurrence with these latter findings in rats, our present results exclude that in humans SP influences TSH secretion at the pituitary level. In fact, infusion of SP did not change the TSH response to 20 µg TRH, demonstrating that the sensitivity

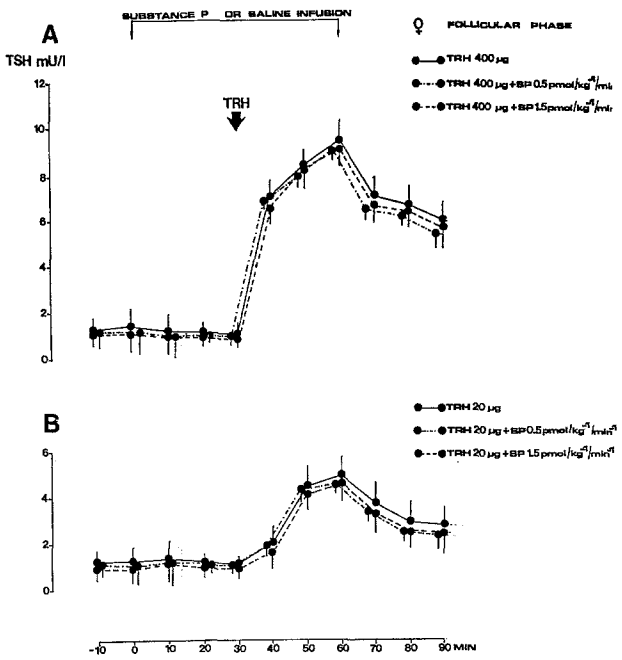

Fig 3. Effect of (A) 400 µg or (B) 20 µg of TRH with or without SP on TSH secretion in six normal women during the follicular phase. Each point represents the mean ± SE of the observations.

of TSH-secreting cells to TRH is not modified by SP. Furthermore, the maximal cell capacity to release TSH remained unchanged when 400 µg TRH were administered in the presence of SP. It is unlikely that SP failure was because of the use of an inadequate dose (1.5 pmol/kg^{-1}/min^{-1}) or the route of administration of SP, because similar

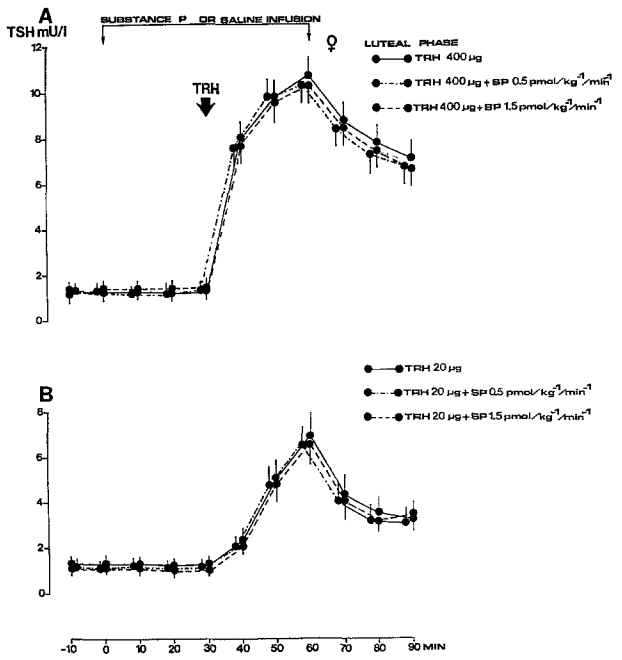

Fig 4. Effect of (A) 400 µg or (B) 20 µg of TRH with or without SP on TSH secretion in six normal women during the luteal phase. Each point represents the mean ± SE of the observations.

protocols have been used successfully to produce a significant increment in basal cortisol, luteinizing hormone, and growth hormone levels and in the growth hormone response to growth hormone–releasing hormone.[4-6]

Previous studies have shown that after estrogen priming, male or ovariectomized rats show significant TSH responses to intravenous administration of SP alone.[13,21] The lack of direct effects of SP in vitro on pituitary cells from estrogen-treated rats, and the observations that in these animals both intravenous and intraventricular administrations of SP are capable of stimulating TSH secretion, led these investigators to hypothesize that in estrogen-primed rats SP stimulates TSH release at the hypothalamic level.[13,20] We did not find any significant effects of an intravenous infusion of SP alone on TSH secretion either in men or in women. Women were studied during both the follicular and luteal phase, at two different but physiological estrogen levels. In both conditions, no significant effects of circulating SP on TSH secretion were found. Differences between species might be responsible for the discrepant findings in humans and rats. More likely, these depended on the different experimental conditions. In fact, the dose of 1 μg SP injected as an intravenous bolus in 300-g rats (ie, 333 ng/100 g body weight)[13] far exceeded the amount per 400 g body weight of SP (12 ng/100 g body weight) that we infused in our subjects over 60 minutes. The dose that we used is known to produce plasma SP levels in the upper part of the range found in normal subjects.[22] Higher doses produce untoward side effects[22] and possible stress-related hormonal responses. On the other hand, the effects of SP on TSH secretion in rats were observed after a pharmacological treatment with estrogen, whereas it is unknown whether similar findings might be reproduced in normal female rats.[13,21]

In conclusion, the findings of the present study show that in humans plasma SP does not modify the basal release of TSH and the maximal TRH-stimulated TSH secretion. Furthermore, our results indicate that SP does not act as a primer for the release of TSH induced by stimulation of the pituitary gland with a minimal dose of TRH.

SP presumably does not cross the blood-brain barrier because of its proteic nature; therefore, further studies are needed to establish whether in humans SP plays a role in regulation of TSH secretion inside the blood-brain barrier.

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