Changes in Substance P Content at the Hypothalamic-Pituitary Axis during the Wallerian Degeneration of Peripheral Sympathetic Neurons after Superior Cervical Ganglionectomy in Male Rats: Effect of Hyperprolactinemia

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The effects of Wallerian degeneration of the peripheral sympathetic neurons projecting to the hypothalamus on the mechanism of interaction between prolactin and substance P (SP) were examined. The effects of superior cervical ganglionectomy (SCGx) on SP content in various hypothalamic regions and in the hypophysis were evaluated in control and hyperprolactinemic rats. Male rats that received pituitary transplants at the age of 5 days and age-matched sham-operated controls were used. Pituitary grafting significantly increased circulating values of prolactin, as did SCGx. In hyperprolactinemic rats, SCGx partially decreased plasma prolactin levels. Neonatal hyperprolactinemia decreased SP content in the anterior (AH) and posterior (PH) hypothalamus and in the median eminence (ME), but increased it in the mediobasal hypothalamus (MBH). Acute SCGx significantly increased SP in the MBH, PH, and ME. SCGx in hyperprolactinemic animals further increased SP content in MBH. In the ME and Ah, SCGx in pituitary grafted rats decreased SP content as compared with the controls. In the pituitary gland (PG), SCGx only decreased SP content in hyperprolactinemic, but not in control rats. An interaction between peripheral noradrenergic neurons and prolactin to regulate SP within the hypothalamus was positive in the MBH, AH, ME, and PG, but not in the PH. These data indicate the existence of interactive mechanisms between prolactin and the peripheral sympathetic neurons to regulate SP content at the hypothalamic-pituitary axis. Interrelationships between prolactin and SP were also observed.

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Axons leaving the superior cervical ganglia (SCG) provide sympathetic innervation to facial structures, the skull, and the neck. The internal carotid nerve pathway is the pathway followed by postganglionic fibers from the superior cervical ganglia, innervating intracranial structures such as the pineal gland, median eminence, the neurohypophysis, or the choroid plexus (1, 2). After bilateral ablation of the SCG (SCGx), two periods have been characterized: an acute period characterized by an increase in the release of norepinephrine during the wallerian degeneration of the sympathetic terminals in the hypothalamus (3), and a chronic period defined by the marked decrease of median eminence norepinephrine of up to 40%, starting around 24 hr after surgery; this is the beginning of the chronic depletion of the neurotransmitter in the sympathetic terminals (3). These data indicate that a significant number of peripheral noradrenergic nerve terminals come from the SCG, in addition to others originating in anatomical structures outside the ventral bundle (4–7).

The increases in norepinephrine release immediately after SCGx were accompanied by temporal modifications in hypothalamic and hypophyseal hormone release, which indicated a neuroendocrine function of the peripheral sympathetic neurons (1, 2, 8).

In previous studies, the acute and chronic effects of SCGx on circulating levels of several hormones, including prolactin and growth hormone (GH) (9), luteinizing hormone releasing hormone (LHRH) and luteinizing hormone (LH) (10), adrenocorticotropin (ACTH) (11), and on the hypothalamic content of their respective regulatory neuropeptides were examined (9–11). Additionally, the chronic effects of this surgery on mediobasal hypothalamic dopamine and serotonin metabolism were examined (12). The changes found indicate a neuroendocrine modulatory role of

the SCG (13). However, the physiological significance of these projections is not fully understood.

Substance P (SP) is involved in the modulation of a great variety of physiological functions (14). It is well established that SP is one of the hypothalamic neuropeptides that regulates prolactin secretion (14–16). SP exerts a stimulatory role on prolactin secretion at the pituitary level (17, 18), whereas an inhibitory effect mediated by dopamine takes place at the hypothalamic level (19). While hypoprolactinemia increased SP at the pituitary level (20, 21), hyperprolactinemia induced less pronounced changes in SP (22). Although several neuropeptides involved in the regulatory mechanism of prolactin secretion have been examined in the hypothalamus after SCGx (9), both acutely and chronically, the possible changes in SP following sympathetic denervation are unknown.

This work was undertaken to obtain information on the changes in SP concentration in the hypothalamic-pituitary axis during the Wallerian degeneration of the sympathetic nerve terminals after SCGx in pituitary-grafted and control rats. The following questions were addressed: Does acute SCGx affect SP content in the anterior (AH), mediobasal (MBH), and posterior hypothalamus (PH), median eminence (ME), and pituitary gland (PG)? Does hyperprolactinemia affect the content of SP in these tissues? Are ganglionectomy-induced changes in SP content modified by hyperprolactinemia?

Materials and Methods

Adult male rats of the Wistar strain, bred in our animal facilities, were used in all experiments. Animals were kept under standard conditions of light (12:12-hr, lights on at 08:00 hr) and temperature (22° ± 2°C) and with access to food and water *ad libitum*. The studies were conducted in accordance with the principles and procedures outlined in the National Institutes of Health guide for the Care and Use of Laboratory Animals. Surgery was performed under tribromoethanol anesthesia (20 mg/kg body wt, intraperitoneally).

Anterior pituitary glands from littermate female donors were grafted within the breast muscles of male recipients on Day 5 of life, as described previously (23). Sham-operated rats of the same age were used as controls. On the 60th day of life, both (pituitary-grafted and sham-operated rats) were subjected to a bilateral SCGx or sham operation, as described previously (24). Twenty hours after SCGx or sham surgery, animals were sacrificed by decapitation.

Immediately after sacrificing the animals, the AH, MBH, PH, ME, and the PG were dissected as described previously (25). Tissues were homogenized in 2 N acetic acid and were boiled for 7 min at 100°C. They were centrifuged at 22,000*g* for 30 min, and the supernatants were kept frozen at −80°C until further analysis. Protein content in all studied areas was determined by the Bradford method (26).

Prolactin levels were measured by a homologous specific double antibody system using materials kindly supplied by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) National Hormone and Pituitary Program and by Professor A.F. Parlow (Harbor, UCLA Medical Center, Torrance, CA). The intra- and interassay coefficients of variation were 7% and 8%, respectively. Sensitivity was 45 pg/ml using the r-prolactin RP-3 reference preparation. All samples were measured in the same assay to avoid interassay variations.

SP contents were determined by a specific double antibody radioimmunoassay (RIA) system using a specific antibody developed and generously provided by Professor L. Debeljuk (Southern Illinois University, Carbondale, IL). Specifications of this RIA were given previously (16). The intraassay coefficient of variation was 7%. All samples of this study were run in one assay to avoid interassay variations.

Statistical analysis of the data was performed through a factorial ANOVA followed by a Fisher multiple comparison test (Statwiev 512 for Apple). Differences among groups were considered significant at the level of $P < 0.05$.

Results

Pituitary grafting increased circulating levels of prolactin 2 months after surgery (Fig. 1) as compared with age-matched sham-operated control rats. Acute SCGx increased ($P < 0.05$) plasma prolactin levels as compared with the values found in the control group. However, this surgery in hyperprolactinemic animals decreased prolactin values as compared with sham-SCGx hyperprolactinemic animals (Fig. 1).

Hyperprolactinemia from Day 5 of life decreased SP contents in the AH as compared with controls, whereas

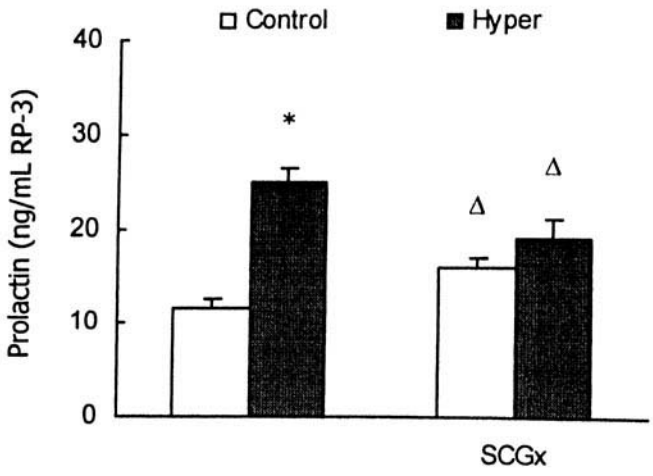

Figure 1. Effects the Wallerian degeneration of peripheral sympathetic neurons after SCGx on plasma prolactin levels in control and pituitary-grafted rats. Groups of 8 to 10 rats were sacrificed between 08:30 and 09:30 hr, 12 days after SCGx or its sham operation, to avoid circadian variations of the hormone. Prolactin values were measured by radioimmunoassay (RIA) and were expressed as nanograms per milliliter of r-PRL-RP3 ± SEM as described in "Material and Methods." $^{*}P < 0.05$ versus controls; $^{\Delta}P < 0.05$ versus respective groups without SCGx.

acute SCGx did not modify the neuropeptide content in this hypothalamic area. However, acute SCGx in pituitary grafted animals significantly reduced SP content ($P < 0.05$) as compared with hyperprolactinemic or respective control rats. An interaction between the SCGx and hyperprolactinemia was detected ($P < 0.05$) (Fig. 2).

A significant increase in SP content in the MBH ($P < 0.05$) was observed in neonatally pituitary-grafted as compared with sham-operated rats. Acute SCGx also significantly increased the content of this neuropeptide ($P < 0.05$) in this hypothalamic area compared with the values found in the control group. Wallerian degeneration of the sympathetic terminals in hyperprolactinemic rats further increased SP in the MBH. This last effect was significant as compared with either control ($P < 0.05$) or hyperprolactinemic animals ($P < 0.05$). An interaction between the SCGx and hyperprolactinemia was observed ($P < 0.05$) Fig. 3).

Hyperprolactinemia tended to decrease SP content in the PH, but this apparent difference was not statistically significant. However, acute SCGx significantly increased SP content in this hypothalamic area in both hyperprolactinemic and control animals ($P < 0.05$). No interaction between the SCGx and hyperprolactinemia was detected in this region of the hypothalamus (Fig. 4).

Neonatal pituitary grafting decreased SP content in the ME ($P < 0.05$), whereas acute SCGx significantly increased it ($P < 0.05$) as compared with controls. The effect of acute SCGx on SP content was abolished by hyperprolactinemia, thus the values obtained were similar to those found in the control group without SCGx, but significantly higher than those measured in hyperprolactinemic animals. An interaction between the SCGx and hyperprolactinemia was observed ($P < 0.05$) (Fig. 5).

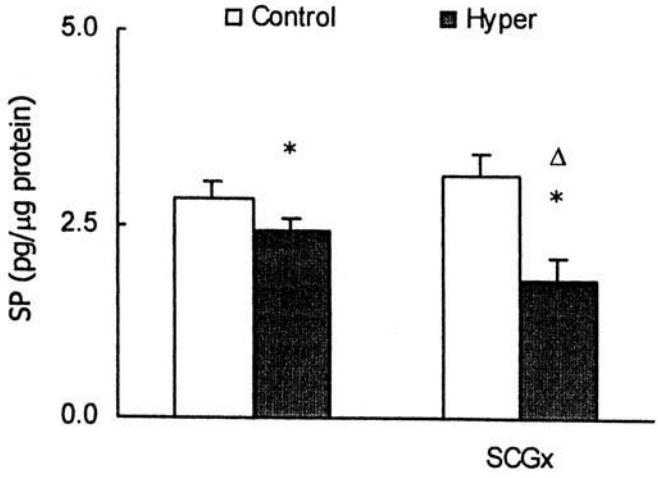

Figure 2. Effects of the Wallerian degeneration of peripheral sympathetic neurons after SCGx on SP content in the AH of control and pituitary-grafted rats. Groups of 8 to 10 rats were sacrificed between 08:30 and 09:30 hr, 12 days after SCGx or its sham operation, to avoid circadian variations of prolactin. SP was measured by a specific RIA as described in "Material and Methods." SP values were expressed as picograms per microgram protein ± SEM. $^{*}P < 0.05$ versus respective controls; $^{\Delta}P < 0.05$ versus respective groups without SCGx.

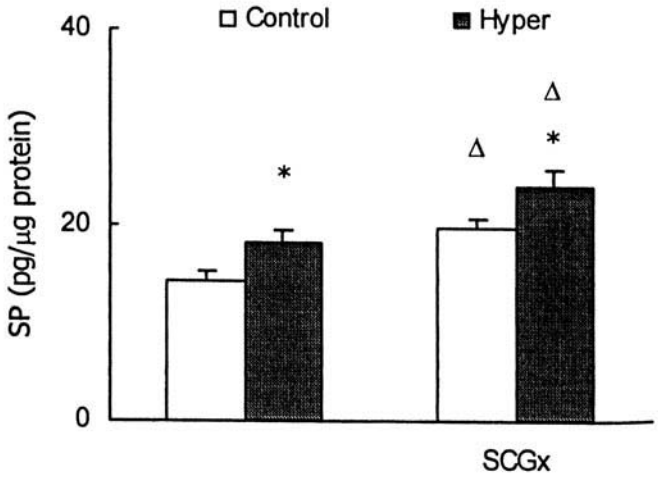

Figure 3. Effects of the Wallerian degeneration of peripheral sympathetic neurons after SCGx on SP content in the MBH of control and pituitary grafted rats. Groups of 8 to 10 rats were sacrificed between 08:30 and 09:30 hr, 12 days after SCGx or its sham operation, to avoid circadian variations of prolactin. SP was measured by a specific RIA as described in "Material and Methods." SP values were expressed as picograms per microgram protein ± SEM. $^{*}P < 0.05$ versus respective controls; $^{\Delta}P < 0.05$ versus respective groups without SCGx.

Neither hyperprolactinemia nor acute SCGx significantly affected SP content in the PG. However, Wallerian degeneration of the sympathetic terminals in hyperprolactinemic rats significantly decreased SP content in the PG ($P < 0.05$) as compared with their respective controls. An interaction between the SCGx and hyperprolactinemia was detected in this tissue (Fig. 6).

Table I shows the effects of the Wallerian degeneration of peripheral sympathetic neurons after SCGx on protein content of the areas studied. Protein content was not modified by either SCGx or hyperprolactinemia in AH, PH, or PG. However, in MBH, protein content was significantly

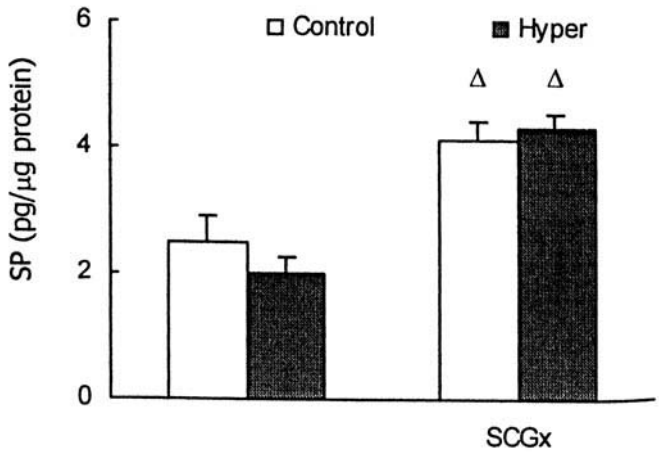

Figure 4. Effects of the Wallerian degeneration of peripheral sympathetic neurons after SCGx on SP content in the PH of control and pituitary-grafted rats. Groups of 8 to 10 rats were sacrificed between 08:30 and 09:30 hr, 12 days after SCGx or its sham operation, to avoid circadian variations of prolactin. SP was measured by a specific RIA as described in "Material and Methods." SP values were expressed as picograms per microgram protein ± SEM. $^{\Delta}P < 0.05$ versus groups without SCGx.

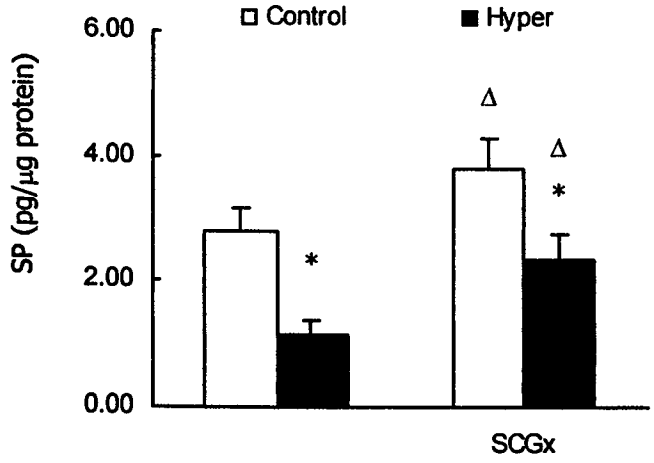

Figure 5. Effects of the Wallerian degeneration of peripheral sympathetic neurons after SCGx on SP content in the ME of control and pituitary-grafted rats. Groups of 8 to 10 rats were sacrificed between 08:30 and 09:30 hr, 12 days after SCGx or its sham operation, to avoid circadian variations of prolactin. SP was measured by a specific RIA as described in "Material and Methods." SP values were expressed as picograms per microgram protein ± SEM. $^{*}P < 0.05$ versus respective controls; $^{\Delta}P < 0.05$ versus respective groups without SCGx.

increased ($P < 0.05$) after SCGx and significantly decreased in hyperprolactinemic animals ($P < 0.05$). In ME, both SCGx and hyperprolactinemia significantly decreased this parameter ($P < 0.05$) for both comparisons.

Discussion

The data indicate that there is an interaction between prolactin and the peripheral sympathetic nerves that affects SP content in specific areas of the hypothalamus and the PG. Neonatal pituitary grafting increased plasma prolactin levels similarly to the effects of this procedure at other ages (23, 27–29). The degree of hyperprolactinemia found in neonatally grafted rats was similar to that described in previous reports from our group (12, 30), and the levels reached are high enough to induce hypogonadism in the male (28) or changes in other physiological functions (i.e., the immune function) (30). However, in female rats the increase in plasma prolactin levels may be necessary to produce similar effects (28).

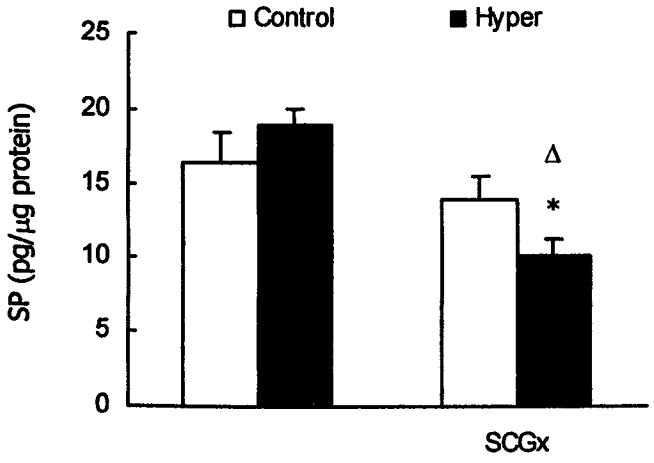

Figure 6. Effects the Wallerian degeneration of peripheral sympathetic neurons after SCGx on SP content in the PG of control and pituitary-grafted rats. Groups of 8 to 10 rats were sacrificed between 08:30 and 09:30 hr, 12 days after SCGx or its sham operation, to avoid circadian variations of prolactin. SP was measured by a specific RIA as described in "Material and Methods." SP values were expressed as picograms per microgram protein ± SEM. $^{*}P < 0.05$ versus respective controls; $^{\Delta}P < 0.05$ versus groups without SCGx.

During the Wallerian degeneration of the sympathetic terminals, an increase in plasma levels of prolactin was observed in normoprolactinemic animals, an effect opposite that described earlier (9). The discrepancies in these studies may be due to differences in the season in which the experiments were done (winter, previous studies versus spring, and this study), as seasonal differences in the response of prolactin to different manipulations were observed in previous works from the laboratory (20, 23, 31).

SCGx partially blocked the increase of prolactin levels induced by neonatal hyperprolactinemia. Although an ectopic origin of the circulating hormone was indicated in previous work (25), an *in situ* pituitary may contribute to these findings. After acute SCGx, the effects on prolactin may be mediated by the high levels of norepinephrine released by the degenerating sympathetic terminals to the hypothalamic pituitary axis (9). The increase in norepinephrine content within the hypothalamus after acute SCGx was measured in our previous studies (9).

An increase in SP in the MBH was found in neonatally hyperprolactinemic rats. Similar results were described in puerperal hyperprolactinemia (32). Prolactin also increased *in vitro* SP release and content in the MBH. Since SP increases dopamine metabolism in these areas (19), a feedback mechanism between prolactin, SP, and dopamine may occur, confirming our previous results concerning other effects of hyperprolactinemia on pituitary hormone secretion (33, 34). In contrast, SP content was decreased in the ME and was not modified in the PG in hyperprolactinemic rats. Considering that SP at the PG level increases prolactin release (17, 18), the results suggest that an equilibrium between inhibitory and stimulatory effects of SP on prolactin release may occur under these experimental conditions. Globally, the data indicate the existence of interactive mechanisms between prolactin and SP at the hypothalamic and pituitary level, thus indicating the importance of SP in the regulatory mechanism of prolactin secretion.

SCGx in hyperprolactinemic animals further increased SP content in the MBH and decreased it in the ME. This result may reflect increased inhibition of the *in situ* hypothalamic pituitary axis due to the interaction between SP and dopamine (19), which provides the main inhibitory input to the control prolactin secretion (4). Moreover, SCGx markedly decreased SP content in the PG of hyperprolactinemic rats. Since these changes were associated with the decrease in plasma prolactin levels, these results also suggest that a small percentage of the circulating prolactin that is derived from the *in situ* pituitary may be inhibited under these experimental conditions. This suggests that circulating

Table I. Effects of the Wallerian Degeneration of Peripheral Sympathetic Neurons after SCGx on Protein Content in the AH, MBH, PH, ME, and PG of Control and Pituitary-Grafted Rats

	AH	MBH	PH	ME	PG
Control	48.4 ± 2.4	57.3 ± 6.3	57.6 ± 2.5	4.6 ± 0.37	61.4 ± 8
SCGx	50.6 ± 3	78.09 ± 8.4[a]	54.3 ± 3.4	3.75 ± 0.22[a]	64 ± 5.8
Hyper	50 ± 2.3	45.8 ± 6.4[a]	52.2 ± 4.1	3.73 ± 0.28[a]	70 ± 7.5
Hyper + SCGx	53.28 ± 2.7	40.7 ± 6.6	54.3 ± 4.46	3.97 ± 0.12	61.4 ± 8

Note. Groups of 8 to 10 rats were sacrificed between 08:30 and 09:30 hr, 12 days after SCGx or its sham operation, to avoid circadian variations of prolactin. Protein content in all studied areas was determined by the Bradford method. Protein values were expressed as micrograms of protein/tissue ± SEM.
[a] $P < 0.05$ versus controls.

prolactin originates from both the *in situ* and the ectopic PG. During the Wallerian degeneration of the terminals, the increased norepinephrine release would be expected to suppress prolactin release from the *in situ* pituitary, as suggested by earlier findings (35).

However, in hyperprolactinemic rats the changes of SP contents in the hypothalamic areas studied may indicate the existence of compensatory mechanisms within the hypothalamus, which may differentially interfere with the effects of hyperprolactinemia on other neuromodulators. Results of analysis of aminoacidergic neurotransmitters within the hypothalamus of hyperprolactinemic animals (31) support this interpretation.

SCGx did not modify SP in the AH by itself, but increased the inhibitory effect induced by the neonatal hyperprolactinemia. These changes do not explain the modifications in plasma prolactin levels. However, they may account for the changes in other adenohypophyseal hormones or in neurohypophyseal hormones that were previously detected in animals subjected to SCGx (1, 2, 8–11).

In control animals, SP concentration in the PH was comparable with the levels measured in the AH, in agreement with earlier reports (36). Unexpectedly, neonatal hyperprolactinemia did not reduce the content of SP in the PH, as was observed when high circulating hormone levels were induced in older animals (B.H. Duvilanski and A.I. Esquifino, unpublished data). These results suggest that the degree of hypothalamic development conditions the response to prolactin. The changes detected after SCGx indicate that noradrenergic terminals coming from the SCG are present in the PH and they may be regulated by prolactin. This explanation is consistent with interactions between catecholamines and SP in other hypothalamic regions.

Moreover, similar interactions were detected between prolactin and dopamine (25) and between SP and prolactin (33, 34). The existence of interactive mechanisms between prolactin and the peripheral adrenergic inputs was also previously described (37).

In conclusion, these results suggest the existence of an interactive mechanism between prolactin and the sympathetic nervous system to regulate SP content in the hypothalamic pituitary axis. The changes in this neuropeptide may be modulated by the presence of high levels of norepinephrine during the Wallerian degeneration of the terminals. These results also indicate a modulatory role of SP on prolactin release.

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