Journal of Neuroscience Research 50:94–103 (1997)

Stimulatory Effect of a Specific Substance P Antagonist (RPR 100893) of the Human NK_1 Receptor on the Estradiol-Induced LH and FSH Surges in the Ovariectomized Cynomolgus Monkey

Bernard Kerdelhué,[1]* Keith Gordon,[2] Robert Williams,[2] Véronique Lenoir,[1] Valérie Fardin,[3] Paul Chevalier,[3] Claude Garret,[3] Pierre Duval,[1] Paul Kolm,[2] Gary Hodgen,[2] Howard Jones,[2] and Georgeanna Segard Jones[2]

[1]Laboratoire de Neuroendocrinologie, Faculté de Pharmacie, Paris, France
[2]Jones Institute for Reproductive Medicine, EVMS, Norfolk, Virginia
[3]Rhône Poulenc Rorer SA Département d'analgésie, Vitry-sur Seine cedex, France

Utilizing a human NK_1 receptor antagonist (RPR 100893), the present in vivo study was designed to test the hypothesis that endogenous substance P (SP) modulates the action of 17β-estradiol in inducing luteinizing hormone (LH) and follicle stimulating hormone (FSH) surges in ovariectomized cynomolgus monkey. Plasma concentrations of LH and FSH as well as NK_1 receptor antagonist and SP were measured during the development of the negative and positive feedback phases which follow a single administration of estradiol benzoate (50 μg/kg) to long-term ovariectomized monkeys. Daily administration by gastric intubation of 1 mg/kg or 10 mg/kg of the NK_1 receptor antagonist (RPR 100893) leads to detectable levels of the antagonist in the blood of treated animals for at least 6 hr after its administration. These levels are in agreement with the experimentally determined IC_{50} value of the antagonist.

The most striking finding of this study is that LH and FSH releases are enhanced during the descending arm of the estradiol benzoate-induced LH and FSH surges, which suggests that endogenous SP normally has an inhibitory role during this time. The enhancement of LH release is approximately 50%, regardless of the amount of the NK_1 antagonist used. However, the enhanced FSH release is more important. Furthermore, blockade of the NK_1 receptor with the smaller dose of the antagonist leads to a small, but significant, increase in plasma levels of SP, indicating that blockade of SP receptors leads to an increased release of SP. Collectively, these results further substantiate the link which exists between the ovarian steroid 17β-estradiol and SP systems. Also, for the first time, these results demonstrate an inhibitory involvement of the human NK_1 receptor in the 17β-estradiol-induced pseudo-ovulatory gonadotropin surges in the ovariectomized monkey. J. Neurosci. Res. 50:94–103, 1997.

Key words: NK_1 receptor; estradiol; LH; FSH; monkey

INTRODUCTION

It has been well documented that the midcycle 17β-estradiol (E_2) surge is the major event that synchronizes identified and nonidentified interactive mechanisms operating within the hypothalamo-pituitary-complex leading to the midcycle luteinizing hormone (LH) surge (Karsch et al., 1973; Nakai et al., 1978; Knobil and Hotchkiss, 1988). In the ovariectomized (OVX) monkey, a midcycle-like LH surge can be induced by the administration of E_2 benzoate (E_2B), sufficient to provoke circulating concentrations of E_2 exceeding 150 pg/ml for more than 30 hr (Knobil, 1980). It is generally accepted that pulses of GnRH constitute the signal for the tonic pulsatile secretion of LH and follicle stimulating hormone (FSH) during the cycle. However, during the midcycle gonadotropin surge, additional factors, including steroids and various neuropeptides, are implicated.

Previous studies indicate that neuropeptide Y, galanin, neurotensin, δ-sleep-inducing peptide and angiotensin II are involved in the facilitation of the midcycle LH surge in the rat, by an action at the hypothalamic and/or

*Correspondence to: B. Kerdelhué, Laboratoire de Neuroendocrinologie, Faculté de Pharmacie, 4, avenue de l'Observatoire 75006 Paris, France.

Received 31 January 1997; Revised 6 May 1997; Accepted 6 May 1997

anterior pituitary (AP) level (references in Kalra, 1993). On the contrary, substance P (SP), a neurokinin, inhibits the amplitude of the midcycle LH surge in the rat (Battmann et al., 1991). Neurokinins form a family of peptides with neurotransmitter/neuromodulator activities (references in Jessop et al., 1992). These are expressed in neurons throughout the central and peripheral nervous system, and also in endocrine anterior pituitary cells. Substance P, neurokinin A (NKA) and two extended forms of NKA, neuropeptide K (NPK) and neuropeptide γ (NPγ), are encoded by the same preprotachykinin (PPT) gene. The PPT primary transcript is subjected to differential splicing, and the different mRNA isoforms encode different combinations of tachykinins (Krause et al., 1987).

In the rat, a SP-containing hypothalamic neuronal system projects to the median eminence (Makara et al., 1986). Substance P-immunoreactive boutons contact the capillaries of the hypothalamo-pituitary portal system. Within the hypothalamus of the monkey, numerous SP-containing cells have been found in the arcuate nucleus and lateral areas as well as in the periventricular area of the dorsal tuberal region (Ronnekleiv et al., 1985). Estradiol up-regulates hypothalamic SP and NKA content (Akesson, 1994), and increases PPT gene expression (Brown et al., 1990; Priest et al., 1995). Physiological SP and NKA variations in the female hypothalamus during the estrous cycle have also been described (Parnet et al., 1990; Duval et al., 1996a). More recently, variations in levels of substance P-encoding β-, γ-PPT and substance P receptor NK-1 transcripts were documented in the rat hypothalamus throughout the estrous cycle (Gautreau et al., 1997).

The concentrations of SP in the brain, median eminence, arcuate nucleus, medial preoptic nucleus, and AP of rats (Parnet et al., 1990; Antonowicz et al., 1985; De Palatis et al., 1985) appear to be modulated by ovarian steroids. Furthermore, the number of SP-binding sites in the AP varies during the rat estrous cycle (Kerdelhué et al., 1985). Several studies have investigated the role of SP in hypothalamo-pituitary function in monkeys during the induction of a preovulatory-like LH surge following treatment with estradiol benzoate. Substance P delays the occurrence of the E_2B-induced LH surge in OVX monkeys (Jeanjean et al., 1988). In addition, a depletion of AP content of SP is noted in E_2B-treated OVX monkeys (Kerdelhué et al., 1993) by 48 hr post-E_2B, the time of the LH surge. On the other hand, E_2B exposure of OVX monkeys leads to an almost linear increase, up to 48 hr post-E_2B treatment (the time of the E_2-induced LH surge), of the in vitro hypothalamic secretion of SP (Kerdelhué et al., 1992b). An acute release of SP from hypothalami occurred following in vitro treatment with progesterone when the hypothalami had been excised 48 hr post-E_2B treatment (Kerdelhué et al., 1992b). The inhibitory effect of SP is at least exerted at the AP level in the rat (Battmann et al., 1991; Kerdelhué et al., 1978a), monkey (Kerdelhué et al., 1992a) and human (Wormald et al., 1989).

To date, three categories of neurokinin receptors which bind SP to some degree have been documented: NK_1 with primary affinity for SP, NK_2 with primary affinity for NKA and NK_3 with primary affinity for neurokinine B. NK_1 receptors have been characterized on lactotropes and gonadotropes cells at the AP level (Kerdelhué et al., 1985; Larsen et al., 1989, 1992). NK_1 mRNA encodes a seven-transmembrane domains tachykinin receptor, with preferential affinity to SP (Hershey and Krause, 1990). NK_1 is widely expressed in the central and peripheral nervous system (Hershey et al., 1991; Kiyama et al., 1993), and in the anterior pituitary (Winkler et al., 1995).

Recently, a highly potent and specific NK_1 receptor antagonist (RPR 100893) has been synthesized (Tabart and Peyronel, 1994) and characterized (Lee et al., 1994; Cutrer et al., 1995; Fardin et al., 1994a,b; Moussaoui et al., 1994). The NK_1 receptor antagonist RPR 100893 is a potent SP antagonist in models which are predictive of several pathological states, such as asthma, inflammation and pain, migraine and vascular headaches.

Utilizing this NK_1 receptor antagonist, the present in vivo study was designed to test the hypothesis that endogenous SP modulates the action of E_2 in inducing LH and FSH surges in primates. The changes in E_2, LH and FSH serum concentrations, during treatment with a specific SP antagonist of the NK_1 receptor (RPR 100893) were quantitated. Plasma levels of this NK_1 receptor antagonist as well as SP, were also measured.

MATERIALS AND METHODS

Fifteen long-term ovariectomized (OVX) adult female cynomolgus monkeys (*Macaca fascicularis*), weighting 3.5 ± 1.6 kg, were used for this study. The monkeys were housed in individual cages and fed a diet of commercial monkey chow (Agways, Elizabeth, NJ). Water was available and libitum; the lighting schedule was 12 hr of light, 12 hr of darkness, with light on from 0600–1800 hr daily; room temperature was maintained at approximately 20°C.

Fifteen primates were randomly divided into three equal groups. At 6 a.m., OVX monkeys received intragastric administrations of 1 mg/kg or 10 mg/kg of RPR 100893 or vehicule (1 ml of polyethylene glycol [PEG 400]) for 5 consecutive days. Six hr after the first administration of the vehicle, or the second doses of the NK_1 antagonist, all 15 OVX monkeys received a subcutaneous injection of estradiol benzoate (E_2B), 50 µg/kg, in

sesame oil. Blood samples (5 ml) were collected via femoral vein puncture before the administration of the NK_1 antagonist and every 6 hr for 5 days and then every 12 hr for an additional 2 days. Blood samples and treatments were performed under ketamine anesthesia (10 mg/kg, i.m.). Plasma samples were obtained and, after centrifugation, stored frozen at −20°C until assayed by radioimmunoassay (RIA) for 17β-estradiol, LH, FSH and SP.

Radioimmunoassays of 17β-Estradiol, LH, FSH and SP

17β-estradiol. The 17β-estradiol assay was performed using a commercial kit (RSL Inc., Carson, CA).

LH. The LH assay was performed using an antiserum directed against hCG (R_{13}, pool D), a ^{125}I-LH preparation from cercopithecus monkeys (WP-XV-117-3239) and a LH preparation from rhesus monkey (LH-RP_1, WP-XW-20). The intra-assay coefficients of variation was less than 9%. The sensitivity of the assay was 1.8 ng/tube.

FSH. Concentrations of monkey FSH were determined using a rabbit antibody to ovine FSH (H-31), preparations of hFSH (NIH-FSH-3) for iodination, and cynomolgus FSH (NICHD-CYN-FSH-RP1) as reference preparation. Two assays were made on all samples. The intra-assay coefficients of variation were less than 8%. The sensitivity of the assay was 1.8 ng/tube.

Substance P. Sep-Pack columns (Waters Associates, Milford, MA) filled with a stationary nonpolar phase (Sep-Pack C18) were used for the preparation of the samples prior to the SP assay, as previously described (Kerdelhué et al., 1992b). Briefly, cartridges were prepared by successive washes with 5 ml ethanol, 5 ml 8 M Urea and 10 ml of bidistilled water before the introduction of 2 ml of plasma on the column. Then the column was washed with 10 ml of H_2O and 10 ml of 4% acetic acid. Peptides retained on the column were eluted with 5 ml of a mixture of 90% ethanol and 4% acetic acid. After a speed vac evaporation (Savant Instruments Inc., Farmingdale, NY) for 12 hr the residuum was reconstituted in 0.5 ml of the RIA buffer and aliquots of 0.1 ml and 0.3 ml were used for the assay. The SP assay was performed as previously described (Cheramy et al., 1978). The intra- and interassay coefficients of variation were 10% and 12% at binding levels of 60%, respectively. The lower limit of detection was 2 pg per tube.

Plasma Substance P Receptor Antagonist (RPR 100893) Assay

The plasma concentration of NK_1 receptor binding compounds (including RPR 100893 and any active metabolite, in proportion to the affinity for a specific receptor) as determined by radioreceptor assay. The method is based on the competition between the labeled substance P (iodinated ^{125}I-Bolton Hunter Substance P, ^{125}I-BH-SP) and NK_1 receptor binding compounds present in plasma for binding sites on CHO cell clones selectively expressing human NK_1 receptors. The method involves acetonitrile deproteinization of plasma as pretreatment. A standard curve was established using seven increasing concentrations of RPR 100893 (from 5 to 320 ng/ml). The batch used for bioanalytical measurement is LJP 7194. For this reason, results were expressed as RPR 100893 equivalent. The interassay precision of the method, evaluated from two different concentrations (20 and 100 ng/ml in plasma), shows coefficients of variation lower than 13%. The limit of quantitation was 5 ng RPR 100893 equivalent per ml of plasma using 0.1 ml of sample.

Statistical Analysis

The data were analyzed by a 3 × 25 repeated measures analysis of variance (ANOVA). The 3 represents the levels of the treatment factor, control, 1.0 mg/kg and 10 mg/kg, and the 25 represents the number of repeated measurements from 0 to 174 minutes. Because of the time series structure of the data (the large number of repeated measurements), variance-covariance matrix of the 25 repeated measurements was modeled as first-order autoregressive. This takes into account the fact that measurements closer together in time are more highly correlated than measurements farther apart in time. The ANOVA tested main effects for treatment groups (differences in treatments means pooled over all times), time (trends over time for all groups combined) and the interaction of group and time (differing trends among the treatment groups).

Animal Welfare Assurance

This study was conducted following approval by the Institutional Animal Care and Use Committee of the Eastern Virginia Medical School. This committee conducts its reviews in accord with the NIH *Guide for the Care and Use of Laboratory Animals,* and *Public Health Service Policy on Humane Care and Use of Laboratory Animals.* The facilities of the Division of Animal Resources of the Eastern Virginia Medical School were and are fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

RESULTS

Serum E_2 Concentrations (Fig. 1)

For all groups, there was a significant increase in serum estradiol concentrations from 0 to 6 hr post-E_2B

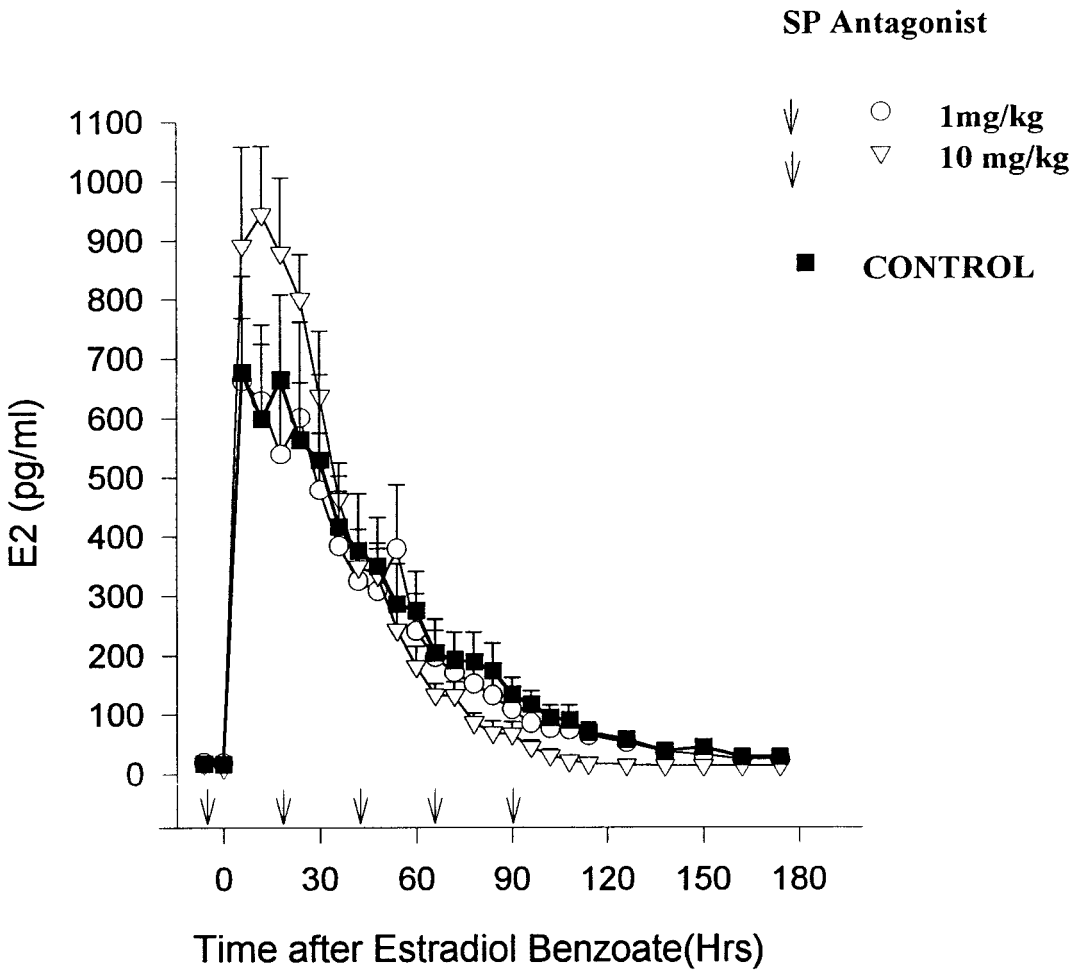

Fig. 1. Pattern of mean (± S.E.M.) plasma concentrations of E_2 in blood samples taken up every 6 hr to the time of 174 hr, from OVX cynomologus monkeys treated with one injection of E_2B (50 μg/kg) at 0 hr and with five intragastric administrations of 1 mg/kg or 10 mg/kg of RPR 100893 or vehicle, at −6 h, 18, 42, 66, and 90 hr.

treatment, and an exponential decrease after 6 hr ($P < 0.0001$ for time main effect). There was no overall difference between control and NK_1 antagonist-treated animals ($P = 0.08476$ for group main effect) or any difference in the trend between the three groups ($P = 0.7723$ for the group by time interaction).

Serum NK_1 Antagonist Concentrations (Fig. 2)

Maximum levels of NK_1 antagonist were observed 6 hr after each administration of 1 mg/kg or 10 mg/kg of the antagonist. Then levels were between 10 and 60 ng/ml after administration of 1 mg/kg and between 70 and 200 mg/ml after administration of 10 mg/kg of the antagonist.

Serum SP Concentrations (Fig. 3)

There was a significant main effect for the treatment group ($P < .0001$). Overall mean levels for control monkeys were lower than NK_1 antagonist-treated monkeys. Mean values in control animals were 7.66 ± 0.2 pg/ml as compared to 12.37 ± 0.5 pg/ml (1 mg/kg of NK_1 antagonist) and 9.01 ± 0.3 pg/ml (10 mg/kg of NK_1 antagonist). Values in the 1 mg/kg NK_1 antagonist-treated group were significantly higher than in the control group.

Serum LH Concentrations (Fig. 4)

In control animals, the pattern of mean plasma LH levels followed the expected profile of an E_2B-induced LH surge; castrate levels of LH were initially inhibited by the E_2B treatment, followed by a surge of LH with subsequent reinhibition. Maximum levels occurred by 54 hr post-E_2B treatment. A similar overall pattern of LH concentrations was present in NK_1 antagonist-treated monkeys; maximum levels were observed by 60 hr post-E_2B treatment. Markedly higher serum LH concentrations during the descending arm of the LH surge were

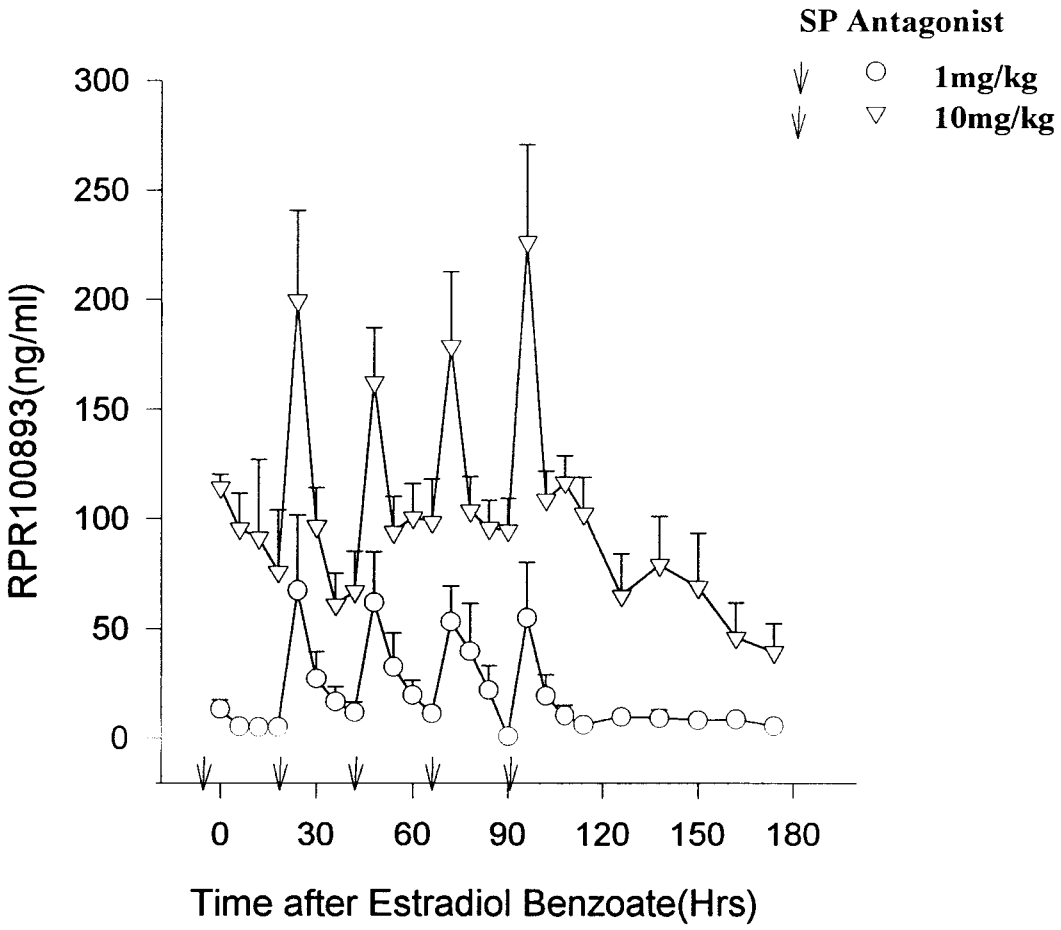

Fig. 2. Pattern of mean (± S.E.M.) plasma concentrations of NK_1 antagonist (RPR 100893) in all blood samples taken up every 6 hr to the time of 174 hr, from OVX cynomologus monkeys treated with one injection of E_2B (50 µg/kg) at 0 hr and with five intragastric administrations of 1 mg/kg or 10 mg/kg of RPR 100893 or vehicle, at −6, 18, 42, 66, and 90 hr.

present in monkeys treated with the NK_1 antagonist. These increased serum levels of LH persisted for the duration of the study. LH concentrations were 59 ± 22 ng/ml in control and 175 ± 64 and 224 ± 40 ng/ml, after treatments with 1 mg or 10 mg of NK_1 antagonist, respectively, between the 90th and 174th hr of the study. All together, there was a significant main effect for the treatment group ($P = 0.0359$). It can be seen from Figure 4 that controls have the smallest overall mean, primarily because of the values after 60 hr. The overall mean LH of controls was 156.66 ng/ml compared to 275.26 ng/ml for the 1 mg/kg and 235.54 ng/ml for the 10 mg/kg of the NK_1 antagonist.

Serum FSH Concentrations (Fig. 5)

In control monkeys, the pattern of mean plasma FSH followed the expected profile of an E_2B-induced FSH surge. Minimum levels occurred at 42 hr and maximum levels occurred by 54 hr post-E_2B. In treated animals, the time of maximum levels occurred by 60 hr in animals treated with 1 mg/kg of NK_1 antagonist and by 54 hr in animals treated with 10 mg/kg of the NK_1 antagonist. In addition, the amplitude of the FSH surge was slightly increased in animals treated with 1 mg/kg or 10 mg/kg of NK_1 antagonist. Markedly higher serum FSH concentrations during the descending arm of the FSH surge were present in monkeys treated with the NK_1 antagonist. This increase was still present by 174 hr post-E_2B treatment: Overall mean FSH concentrations were 52 ± 1 ng/ml in control vs. 68 ± 3 ng/ml in 1 mg/kg and 82 ± 10 ng/ml in 10 mg/kg of NK_1 antagonist treated animals ($P < 0.05$).

DISCUSSION

It has been well documented that administration of E_2 to OVX animals induces at the hypothalamo-pituitary

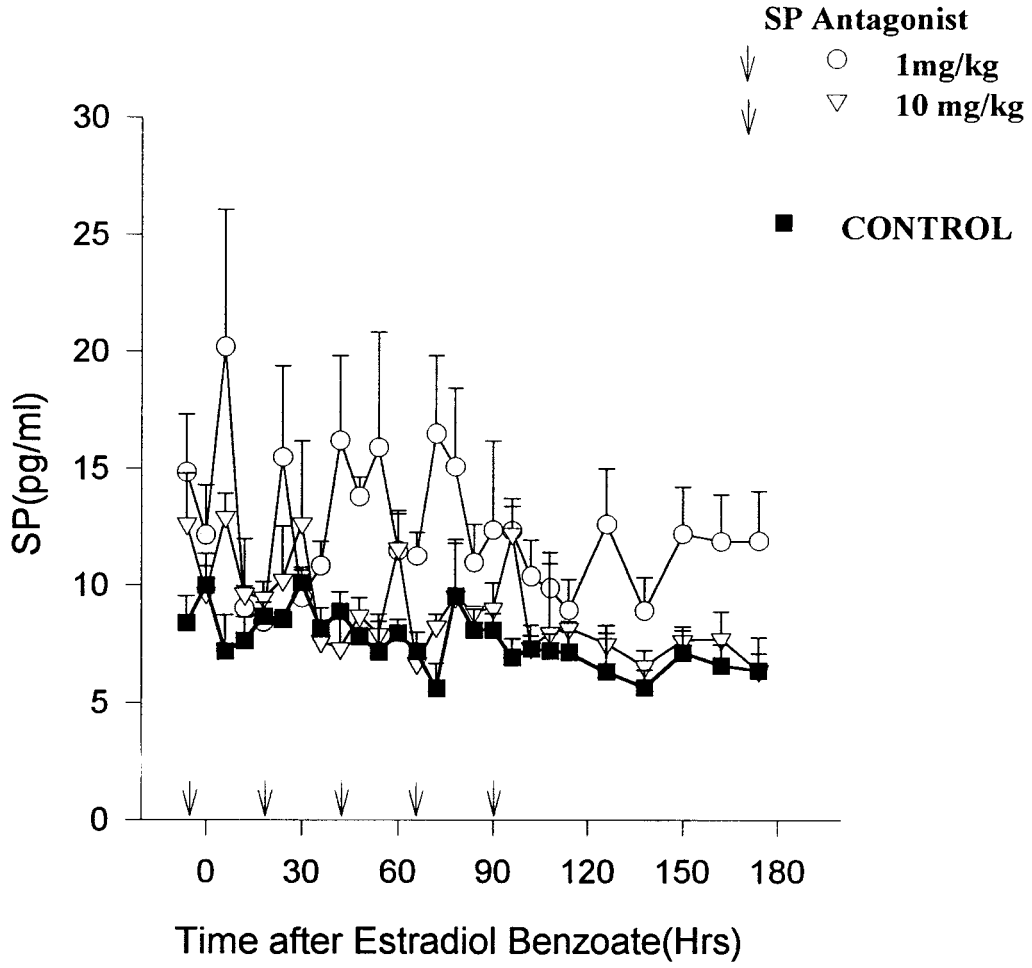

Fig. 3. Pattern of mean (± S.E.M.) plasma concentrations of SP in blood samples taken up every 6 hr to the time of 174 hr, from OVX cynomologus monkeys treated with one injection of E_2B (50 μg/kg) at 0 hr and with five intragastric administrations of 1 mg/kg or 10 mg/kg of RPR 100893 or vehicle, at −6, 18, 42, 66, and 90 hr.

level all neuroendocrine activations induced by the high level of E_2 which occur at midcycle and are necessary for LH and FSH preovulatory surges (Karsch et al., 1973; Knobil, 1980; Knobil and Hotchkiss, 1988). In this process, progesterone, either from ovarian or adrenal origin, amplifies the stimulatory action of E_2 but is not necessary for the inductive effect of E_2 (Mahesh and Brann, 1992).

The present study demonstrates for the first time an inhibitory involvement of the NK_1 receptor in the post-E_2-induced LH and FSH surges in the OVX monkey. The most striking finding is that LH and FSH concentrations are enhanced in the presence of the NK_1 receptor antagonist during the descending arm of the LH and FSH surge. This finding suggests that SP normally has an inhibitory role during this period of time. The amplitude of the LH surge was not changed but the amplitude of the FSH surge was slightly increased. Although in some experimental groups (1 and 10 mg/kg for LH and 1 mg/kg for FSH) the peak levels of LH or FSH surge occurred 6 hr later than in controls, as blood collections were made with a 6-hr interval, this difference is not statistically significant. These results are in agreement with previous results which suggested a major secretion of SP from the hypothalamus during the descending arm of an estradiol-induced LH surge (Kerdelhué et al., 1992b). Clearly, there was a clear significant dissociation of the effect of the two different doses of the NK_1 antagonist on FSH release, the higher dose (10 mg/kg) being more efficient on FSH release than on LH release.

A dissociation of LH and FSH release has been previously observed in other physiological or experimental situations such as the immunoneutralization of circulating levels of GnRH (Kerdelhué et al., 1976). In addition, the higher dose (10 mg/kg) down-regulates SP plasma levels to control levels. The reason for the lack of

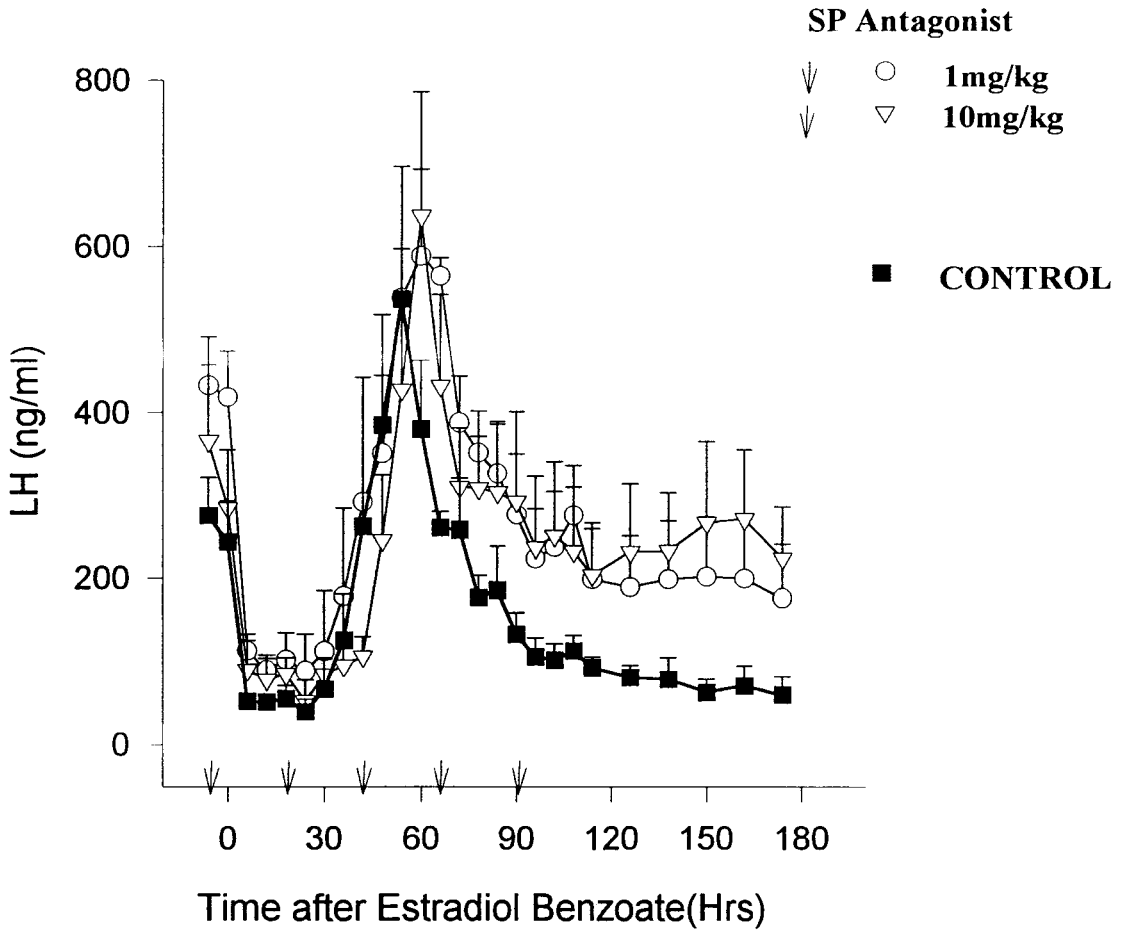

Fig. 4. Pattern of mean (± S.E.M.) plasma concentrations of LH in blood samples taken up every 6 hr to the time of 174 hr, from OVX cynomologus monkeys treated with one injection of E_2B (50 µg/kg) at 0 hr and with five intragastric administrations of 1 mg/kg or 10 mg/kg of RPR 100893 or vehicle, at −6, 18, 42, 66, and 90 hr.

effect of the NK_1 antagonist on the timing or magnitude of the LH and FSH surge after daily injections of the antagonist is not obvious, since an intracerebroventricular administration of SP delays the onset of the E_2-induced LH surge in the OVX monkey (Jeanjean et al., 1988). In the present study, the stimulatory effect of the antagonist was mainly seen after the peak levels of gonadotropins. It is possible that even though SP has much greater affinity for the NK_1 receptor, it has a weak affinity for the NK_2 and/or NK_3 receptor which can also be involved in LH release during the initial phase of LH release.

In this respect, using binding to various receptors and channels, the RPR 100893 was shown to be highly specific for NK_1 receptor up to 1 µM. In particular, it does not interact with the NK_2 or the NK_3 receptor nor with opiates receptors (µ, δ or κ) and calcium chanels (Fardin et al., 1994a). Therefore, using this NK_1 antagonist, we can assume that the effects observed in the present study are mediated through the NK_1 receptor, although it is difficult to assess the specificity of the RPR 100893 at the monkey hypothalamic level, since NK_2 receptors were not documented in the brain of various species by direct binding even if the presence of NKA has been documented.

Whether the RPR 100893 exerts its effects through the classical NK_1 receptor and/or a subsite or subtype of the NK_1 receptor sensible to the 6–11 analog of SP (septide) is not known since this compound is able to inhibit to the same extent the effect of SP and septide using in vitro experiments (guinea pig ileon and second messagers production) with a human astrocyte cell line (Fardin et al., 1994a). In fact, plasma levels of the NK_1 receptor antagonist which are in the range of 60 ng/ml (after administration of 1 mg/kg of antagonist) to 200 ng/ml (after administration of 10 mg/kg antagonist) are in agreement with receptor occupancy. A experimentally determined IC_{50} value (membrane preparation and only 30 min of incubation at 25°C) was 44 nM at the putamen

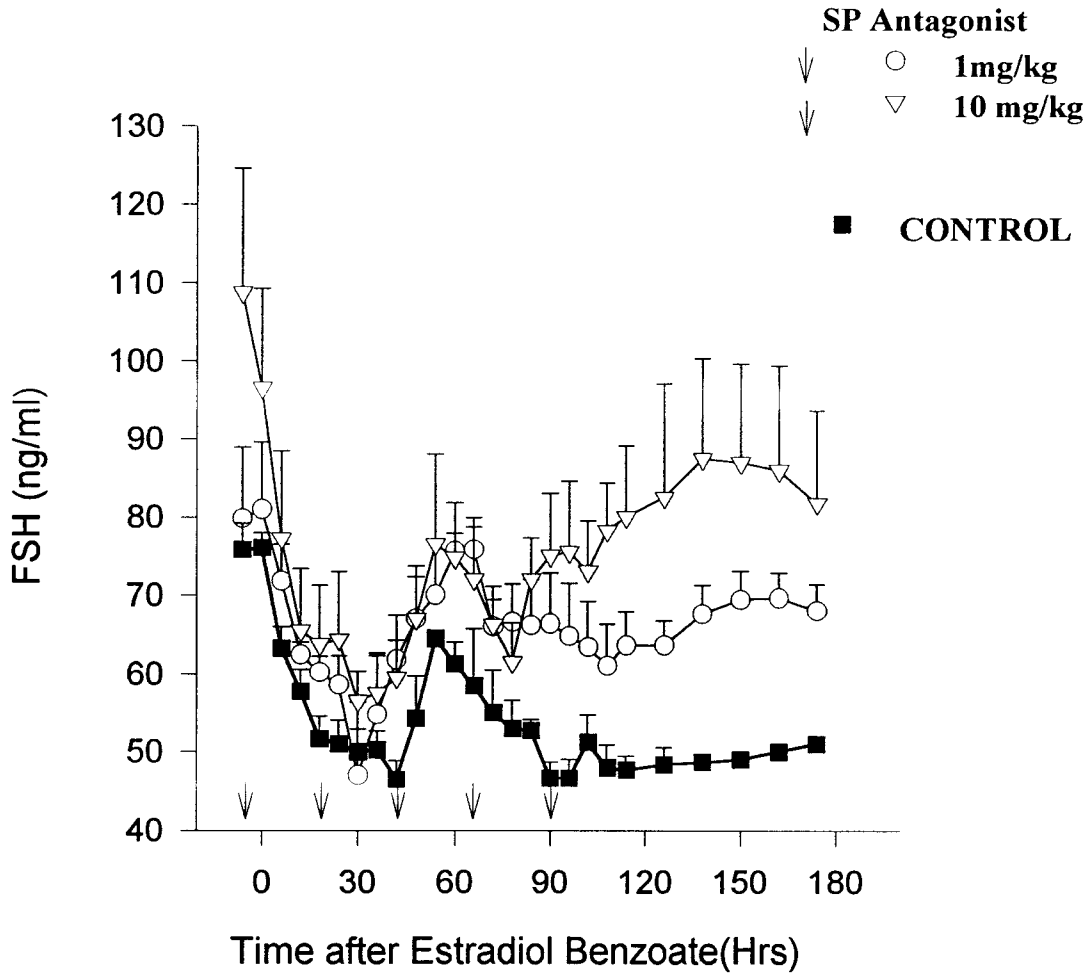

Fig. 5. Pattern of mean (± S.E.M.) plasma concentrations of FSH in blood samples taken up every 6 hr to the time of 174 hr, from OVX cynomologus monkeys treated with one injection of E_2B (50 μg/kg) at 0 hr and with five intragastric administrations of 1 mg/kg or 10 mg/kg of RPR 100893 or vehicle, at −6, 18, 42, 66, and 90 hr.

nucleus level in the monkey (Fardin et al., 1994b). In the present experiment, assuming a molecular weight of 561.73, a plasma concentration of 60 ng/ml represents a level of 107 nM and a concentration of 200 ng/ml represents a level of 356 nM. It shows that at least the plasma concentration of 60 ng/ml is in agreement with receptor occupation.

Another interesting finding of this study is the fact that the treatment of the OVX monkey with the human NK_1 receptor antagonist leads to a small but significant increase in plasma SP levels, showing a possible correlation between the occupancy of the NK_1 receptor and the secretion of SP. The fact that the RPR 100893 elevates circulating levels of substance P is difficult to explain. A phenomenon similar to the one occurring after blockade of the postsynaptic dopaminergic receptor (Lidsky and Banerjce, 1993; Seeman and Van Tol, 1994) which leads to an augmentation of dopamine release could be envisioned. However, a similar phenomenon has not been described in the case of the SPergic transmission or for any perpidergic transmission.

Elevated levels of SP were only observed for the smaller concentration of the NK_1 antagonist which is very similar to the IC_{50} for the receptor. A down-regulation process could be initiated, with a return to control values for plasma SP, for the higher concentration of the NK_1 antagonist which gives rise to NK_1 plasma values close to 10 times the IC_{50} (see above). However, the increase in circulating levels of SP observed after the administration of the smaller amount of the RPR 100893 could be a consequence of many inputs to the systemic system including a local release from peripherally situated nerve terminals spilling over to the bloodstream, or of an endocrine activity arising from the brain, the pituitary or

the gastrointestinal tract being tonically under the control of SP-mediated inhibition.

Previous studies (Wormald et al., 1989) suggest that it is very likely that the action of the human NK_1 receptor antagonist is exerted at the AP level. However, an action at the hypothalamic level cannot be completely excluded since administration of ^{14}C-RPR 100893 radioactivity really crosses the blood barrier and is extensively plasma protein-bound (results not published). Also, it was shown that oral administration of RPR 100893 inhibits up to 50% of the binding of ^{3}H-SP at the striatum level in the guinea pig (Flamand and Fardin, 1995). Furthermore, it was also shown that RPR 100893 administrated by the i.v. route decreases c-fos expression in trigeminal nucleus caudalis following noxious chemical meningeal stimulation (intracisternal capsaicin injection) in the guinea pig (Cutrer et al., 1995), suggesting an action of the NK_1 antagonist at the brain level. In addition, the stereoisomer (RPR 103253) of the RPR 100893 did not reduce the number of positive cells in the trigeminal nucleus caudalis (Cutrer et al., 1995). Also, the stereoisomer does not block the neurogenic plasma protein extravasation within guinea pig dura mater and conjonctiva (Lee et al., 1994). These data show that the nonpeptide antagonist RPR 100893 does not display nonspecific side effects as does the activation of Ca^{++} channels.

In summary, our data clearly show that NK_1 receptors are part of the hypothalamo-AP integrative network which is activated by 17β-estradiol for the LH and FSH release in the OVX monkey. It remains to be determined the extent of the stimulatory effect of the NK_1 antagonist administrated during the physiological rise of 17β-estradiol which occurs during the follicular phase, on preovulatory LH and FSH surges, the subsequent ovulation and progesterone production during the luteal phase, in the normally mentruating monkey.

ACKNOWLEDGMENTS

The authors appreciate the excellent technical assistance of Bruce Lamar, Vickie Prince, Alice Holland, Wendy Babbitt, Marron Wallace, Barbara Atkinson and Deborah Leete. We also thank Nicole Taïeb for her excellent editorial contributions and manuscript preparation.

REFERENCES

Akesson TR (1994): Gonadal steroids regulate immunoreactive tachykinin in the ventrodemial nucleus of the rat hypothalamus. J Comp Neurol 341:351–356.

Antonowicz U, Jakubowska-Naziemblo B, Cannon D, Powell D, Rohde W (1985): Immunoreactive substance P content in median eminence and pituitary gland during estrous, diestrus and after hypothalamic deafferentation. Exp Clin Endocrinol 85:155–166.

Battmann T, Mélik Parsadaniantz S, Jeanjean B, Kerdelhué B (1991): *In vivo* inhibition of the preovulatory LH surge substance P and in vitro modulation of gonadotropin-releasing hormone induced LH release by substance P, oestradiol and progesterone in the female rat. J Endocrinol 130:169–175.

Brown ER, Harlan RE, Krause JE (1990): Gonadal steroid regulation of substance P (SP) and SP-encoding mRNA in the rat anterior pituitary and hypothalamus. Endocrinology 126:330–340.

Cheramy A, Michelot R, Leviel V, Nieoullon A, Glowinski J, Kerdelhué B (1978): Effect of immunoneutralization of substance P in the cat substantia nigra on the release of dopamine from dendrites and terminals of dopaminergic neurons. Brain Res 155:404–408.

Cutrer FM, Moussaoui S, Garret C, Moskowitz MA (1995): The non-peptide neurokinin-1 antagonist, RPR 100893, decreases c-fos expression in trigeminal nucleus caudalis following noxious chemical meningeal stimulation. Neuroscience 64:741–750.

De Palatis LR, Khonam O, McCann SM (1985): Age, sex and gonadal steroid-related changes in immunoreactive substance P in the rat anterior pituitary gland. Endocrinology 117:1368–1373.

Duval P, Lenoir V, Moussaoui S, Garret C, Kerdelhué B (1996a): Substance P and Neurokinin A variations throughout the rat estrous cycle; comparison with ovariectomized and male rats: I—Plasma, hypothalamus, anterior and posterior pituitary. J. Neurosci Res 45:598–609.

Duval P, Lenoir V, Moussaoui S, Garret C, Kerdelhué B (1996b): Substance P and Neurokinin A variations throughout the rat estrous cycle; Comparison with ovariectomized and male rats: II—Trigeminal nucleus and cervical spinal cord. J. Neurosci Res 45:610–616.

Fardin V, Carruette A, Menager J, Bock M, Flamand O, Foucault F, Heuillet E, Moussaoui SM, Tabart M, Peyronel JF, Garret C (1994a): *In vitro* pharmacological profile of RPR100893, a novel non-peptide antagonist of the human NK_1 receptor. Neuropeptides 26:34.

Fardin V, Bock MD, Menager J, Carruette A, Heuillet E, Flamand O, Foucault F, Tabart M, Peyronel JF, Doble A, Garret C (1994b): RPR 100893, a Substance P antagonist from the perhydroisoindole series having preferential affinity for the human NK_1 receptor. Canadian Journal of Physiology and Pharmacology. Abstracts XIIth international Congress of pharmacology 24-29 July 1994, 72, supp. 1: P13.25.12.

Flamand O, Fardin V (1995): Estimation of brain penetration following oral administration of the NK_1 antagonist RPR 100893 using an *ex vivo* binding assay in the guinea-pig. B J Pharmacol 116:252P.

Gautreau A, Duval P, Kerdelhué B (1997): Variations in levels of substance P-encoding β-, γ preprotachykinin and substance P receptor NK-1 transcripts in the rat hypothalamus throughout the estrous cycle: A correlation between amounts of β-preprotachykinin and NK-1 mRNA. Mol Brain Res (in press).

Hershey AD, Krause JE (1990): Molecular characterization of functional cDNA encoding the rat substance P receptor. Science 247:958–962.

Hershey AD, Dykema PE, Krause JE (1991): Organization, structure, and expression of the gene encoding the rat substance P receptor. J Biol Chem 266:4366–4374.

Jeanjean B, Gordon K, Richardson DW, Jones GS, Kerdelhué B (1988): Role of substance P in negative and positive feedback effects of estradiol on LH release in the cynomolgus monkey. 21st Annual Meeting of the Society for the Study of Reproduction, Seattle WA, p 110.

Jessop DS, Chowdrey HS, Larsen PJ, Lightman SL (1992): Substance P: Multifunctional peptide in the hypothalamo-pituitary system? J Endocrinol 132:331–337.

Kalra SP (1993): Mandatory neuropeptide-steroid signaling for the preovulatory luteinizing hormone-releasing hormone discharge. Endocrine Rev 14:507–538.

Karsch FJ, Weick RF, Butler WR, Dierschke DJ, Krey LC, Weiss G, Hotchkiss J, Yamaji T, Knobil E (1973): Induced LH surges in the rhesus monkey: Strength-duration characteristics of the estrogen stimulus. Endocrinology 92:1740–1747.

Kerdelhué B, Catin S, Kordon C, Jutisz M (1976): Delayed effects of *in vivo* LH-RH immunoneutralization on gonadotropins and prolactin secretion in the female rat. Endocrinology 98:1539–1549.

Kerdelhué B, Valens M, Langlois Y (1978a): Stimulation de la sécrétion de la LH et de la FSH hypophysaires après immunoneutralisation de la Substance P endogène, chez la ratte cyclique. C R Acad Sci Paris Série D 126:977–979.

Kerdelhué B, Khar A, De Nay D, Langlois Y, Bennardo T, Liwska J, Jutisz M (1978b): Inhibition in vitro par la substance P de l'excrétion des gonadotropines induite par le LH-RH à partir de cellules adénohypophysaires de rat en culture. C R Acad Sc Paris Série D 287:879–882.

Kerdelhué B, Tartar A, Lenoir V, Abed AE, Hublau P, Millar RP (1985): Binding studies of substance P anterior pituitary binding sites: Changes in Substance P binding sites during the rat estrous cycle. Reg Pep 10:133–144.

Kerdelhué B, Tartar A, Lenoir V, El Abed A, Hubleau P, Millar RP (1985): Binding studies of substance P anterior pituitary binding sites: Changes in SP binding sites during the estrous cycle. Reg Pep 10:133–143.

Kerdelhué B, Jones GS, Gordon K, Seltman H, Lenoir V, Williams RF, Hodgen GD (1992a): Effects of Substance P and progesterone on the pituitary during the estradiol-induced LH surge in primates. Neuroendocr (Life Sci Adv), 11:81–90.

Kerdelhué B, Jones GS, Gordon K, Seltman H, Lenoir V, Williams RF, Hodgen GD (1992b): The interaction between hypothalamic GnRH, β-endorphin and substance P neuronal systems as key regulators of the LH surge: An hypothesis. Neuroendocr (Life Sci Adv), 11:91–100.

Kerdelhué B, Jones GS, Gordon K, Seltman H, Lenoir V, Millar RP, Williams RF, Hodgen GD (1993): Hypothalamo-anterior pituitary gonadotropin-releasing hormone and substance P systems during the 17 β-estradiol-induced plasma luteinizing hormone surge in the ovariectomized female monkey. Endocrinology 132:1151–1157.

Kiyama H, Maeno H, Tohyama M (1993): Substance P receptor (NK_1) in the central nervous system: Possible functions from a morphological aspect. Regul Pept 46:114–123.

Knobil E (1980): The neuroendocrine control of the menstrual cycle. Recent Prog Horm Res 36:53–88.

Knobil E, Hotchkiss J (1988): The menstrual cycle and its neuroendocrine control. In Knobil E, Neill J (eds): ''The Physiology of Reproduction.'' Vol. 2. Raven Press, New York: pp 1971–1994.

Krause JE, Chirgwin JM, Carter MS, Xu ZS, Hershey AD (1987): Three rat preprotachykinin mRNAs encode the neuropeptides Substance P and Neurokinin A. Proc Natl Acad Sci USA 84:881–885.

Larsen PJ, Mikkelsen JD, Saermark T (1989): Binding of an iodinated substance P analogue to a NK_1 receptor on isolated cell membranes from rat anterior pituitary. Endocrinology 124:2548–2557.

Larsen PJ, Saermark T, Mau SE (1992) Binding of an iodinated substance P analogue to cultured anterior pituitary prolactin- and luteinizing hormone-containing cells. J Histochem Cytochem 40:487–493.

Lee WS, Moussaoui S, Moskowitz MA (1994): Blockade by oral or parenteral RPR 100893 (a non-peptide NK_1 receptor antagonist) of neurogenic plasma protein extravasation within guinea-pig dura and conjonctiva. Br J Pharmacol 112:920–924.

Lidsky TI, Banerjce SP (1993): Acute administration of halpoperidol enhances dopaminergic transmission 265:1193–1198.

Mahesh VB, Brann DW (1992): Interaction between ovarian and adrenal steroids in the regulation of gonadotropin secretion. J Steroid Biochem Mol Biol 41:495–513.

Makara GB, Kakuscka I, Lenoir V, Kerdelhué B, Palkovits M (1986): A substance P containing hypothalamic neuronal system projects to the median eminence. Brain Res 374:399–401.

Moussaoui SM, Montier F, Carruette A, Fardin V, Floch A, Garret C (1994): *In vivo* pharmacological properties of RPR100893, a novel non-peptide antagonist of human NK_1 receptor. Neuropeptides 26:35.

Nakai Y, Plant TM, Hess DL, Keogh EJ, Knobil E (1978): On the sites of the negative and positive feedback actions of estradiol in the control of gonadotropin secretion in the rhesus monkey. Endocrinology 102:1008–1014.

Parnet P, Lenoir V, Palkovits M, Kerdelhué B (1990): Estrous cycle variations in gonadotropin-releasing hormone, substance P and β-endorphin contents in the median eminence, the arcuate nucleus and the medial preoptic nucleus in the rat. A detail analysis of proestrus changes. J Neuroendocrinol 2:291–296.

Priest CA, Vink KL, Mycevych PE (1995): Temporal regulation by estrogen of β-preprotachykinin mRNA expression in the rat ventromedial nucleus of the hypothalamus. Mol Brain Res 28:61–71.

Ronnekleiv OK, Kelly MJ, Eskay RL (1985): Distribution of immunoreactive substance P neurons in the hypothalamus and pituitary of the rhesus monkey. J Comp Neurol 224:51–59.

Seeman P, Van Tol HH (1994): Dopamine receptor phamacology (Review). Trends Pharmacol Sci 15:264–270.

Tabart M, Peyronel J-F (1994): Synthesis RPR 100893, prototype of a new series of potent and selective non peptide NK_1 antagonists: The triarylperhydroisoindolols. Bioorg Med Chem Lett 4:673–676.

Winkler A, Papsdorf G, Odarjuk J, Siems WE, Fickel J, Melzig MF (1995): Expression and characterization of the substance P (NK_1) receptor in the rat pituitary and AtT20 mouse pituitary tumor cells. Eur J Pharmacol 291:51–55.

Wormald PJ, Millar RP, Kerdelhué B (1989): Substance P receptors in human pituitary: A potential inhibitor of luteinizing hormone secretion. J Clin Endocrinol Metab 69:612–615.