JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1992, 43, 3, 279—298

W. Z. TRACZYK [1], K.-Y. F. PAU [2], A. H. KAYNARD [2], H. G. SPIES [2]

MODULATORY ROLE OF SUBSTANCE P ON GONADOTROPIN AND PROLACTIN SECRETION IN THE RABBIT *

[1] Department of Physiology, Institute of Physiology and Biochemistry, Medical University of Lodz, Poland, and
[2] Reproductive Biology and Behavior, Oregon Regional Primate Research Center, Beaverton, Oregon, USA.

Substance P (SP) is present in large quantities in the brainstem and hypophysiotropic areas of the brain, but its roles in gonadotropin and prolactin secretion are controversial. The aim of this study was to measure luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin (PRL) release from the pituitary after either intracerebroventricular (ICV) injection or infusion of SP or its C- and N-terminal fragments in intact (INT) and ovariectomized (OVX) conscious rabbits. A single injection of SP into the 3rd cerebral ventricle (3CVT) in INT and OVX rabbits augmented plasma LH concentrations, especially when SP was applied during the initial phase of an LH peak. Injection of SP during the declining phase of LH release was not effective. Injection of SP into the 3CVT was followed by increased plasma PRL concentrations in OVX but not in INT rabbits. Both SP 1-11 and SP 1-7 failed to alter LH, FSH, and PRL secretion when the peptides were slowly infused into the 3CVT, although ICV infusion of SP 6-11 did cause a delayed increase in LH release. The results support a stimulatory role of SP on LH and prolactin release. The results further indicate that although the stimulatory effect of SP on LH is ovarian steroid-independent, in the absence of ovarian steroids, SP is stimulatory only during the rising phase of an LH pulse. A dual role of SP-ergic transmission in modulating LH secretion is discussed.

K e y W o r d s: *substance P, hexa-substance P 6-11, substance P 1-7, gonadotropins, prolactin, 3rd cerebral ventricle*

---

* The work described in this paper was conducted in part at the Oregon Regional Primate Research Center, supported by the National Institutes of Health Grants RR-00163, HD-16631, and HD-18185.

INTRODUCTION

A high Substance P (SP) content in the hypothalamus (1) and rich dispersion of immunoactive SP cell bodies and nerve fibers throughout the mediobasal hypothalamus (MBH) and pituitary gland provide morphological indications that SP may affect pituitary hormone synthesis and/or secretions (2). For example, Rønnekleiv et al. (3) reported that immunoreactive SP neurons are widely distributed lateral to and within the arcuate nucleus and median eminence (ME) in gonadectomized rhesus monkeys. They noted dense concentrations of immunoreactive SP fibers in the external layer of the ME, in the infundibular stalk close to the portal vessels, and in the neurohypophysis. While differences between species in SP immunoreactive cell distribution in the MBH should be considered, this evidence in simians favors SP actions on the pituitary; however, its effects on adenohypophyseal hormone release are still contradictory (2).

Four years after SP was isolated, purified, and chemically characterized from extracts of bovine hypothalamus by Chang and Leeman (4), the effects of SP on luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were studied in vitro (5). Synthetic SP in doses of 10 and 100 μg per 1 ml of incubating medium caused release of LH and FSH from pituitaries of 20-day old female rats. The authors explained the gonadotropin-releasing activities of SP by suggesting conformational compatibility between the structures of SP and gonadotropin-releasing hormone (GnRH) receptors. However, recently Arisawa et al. (6) have reported no in vitro effects of SP on gonadotropin release from anterior pituitary gland cells that were harvested from estradiol-primed ovariectomized (OVX) rats. After systemic administration in adult female rats, SP has varied effects on pituitary gonadotropins (6) and prolactin (PRL) release (7-9). According to Arisawa et al. (6) intravenous injection of SP induces LH release, but only in estradiol-primed OVX rats. The same dose of SP is not effective on FSH release in either estradiol-primed or nonprimed OVX rats.

Effects of intracerebroventricular (ICV) SP administration on release of gonadotropins and PRL from the pituitary gland was also reported in rats (9-11) and in monkeys (12). Injection into the 3rd cerebral ventricle (3CVT) of 1 or 5 μg of SP significantly increased plasma LH levels in estradiol-primed OVX rats, but caused little change in plasma FSH (6). Elevations in serum PRL were usually noted. The microinjection of SP into the medial preoptic area in intact or orchidectomized male rats induced a decrease in plasma LH and FSH levels and an elevation in plasma PRL concentrations (13).

The contradictory data on the role of SP in gonadotropin secretion in spontaneously-ovulating animals, i.e., rats and monkeys, favored the use of a reflex ovulating species, i.e., rabbits, in the current experiments.

MATERIALS AND METHODS

Animals and Surgery

Adult female New Zealand White rabbits (3.5—4.5 kg b. w.) were housed in single cages in a temperature-controlled (21 ± 1°C) and light-regulated (lights on 07.00 and off 20.00) room for at least 30 days prior to surgical intervention. Experiments were performed on two groups of animals; the first group was intact (INT) and the second group was bilaterally ovariectomized (OVX). The surgical procedures consisted of two consecutive weekly interventions in OVX animals or one intervention in INT animals. Deep surgical anesthesia was induced by deep muscular injection of a mixture containing ketamine (40 mg/kg), acepromazine (0.5 mg/kg), and xylazine (5 mg/kg) maintained by inhalation of 2.5% halothane and 30% nitrous oxide. The animals of the OVX group underwent bilateral ovariectomy in the initial surgical intervention. One week later these animals and those of the INT group underwent cranial surgery. Each animal was mounted in the stereotaxic frame (14) and stereotaxically implanted with a 3rd cerebral ventricular (3CVT) guide cannula that consisted of stainless steel tubing (22-gauge) in a preconstructed nylon holder which was mounted to the skull by stainless steel screws and dental cement. The guide cannula was fitted with a stylette of 0.35-mm diameter stainless steel wire. Immediately after ventricular cannula fixation, a catheter (Intravenous Medical Vinyl Tubing, Bolab Inc., Havasu City, AZ, catalog no. BB317-85, size V-6), was inserted into the right jugular vein and positioned under the skin of the neck so that the closed end of the catheter could be wound around the 3rd ventricle (3VT) cannula nylon-holder. The patency of the catheter was maintained by heparin (1,000 IU; Upjohn Co., Kalamazoo, MI). After the surgery each rabbit received an intramuscular injection of 300,000 units of antibiotics (Flo-Cillin, Bristol Labs., Syracuse, N.Y.) and was returned to its cage for recovery.

Third Cerebral Ventricular Injections or Infusions

One week after the last surgery each rabbit was transferred from the animal quarters to the laboratory and placed in a plexiglass box, pre-designed to facilitate blood collection and 3CVT injections or infusions. The box allowed the animal free access to food and water but restricted body rotation.

Both INT and OVX animals were subjected to experiments in which either 13 or 25 blood samples were collected during 3 h or 6 h, respectively. The sequential 15-min blood samples (1.0 ml) were obtained via the previously implanted jugular catheter after it had been connected to a heparin-saline filled (10 IU per ml) polyvinyl extension set. Blood samples were placed into ice-cold heparinized (50 IU per tube) glass tubes and centrifuged at 4°C for 30 min

(1,000 g). The plasma was harvested and stored at −20°C until radioimmunoassay for LH, FSH, and PRL.

Four experiments were performed according to the following protocols.

Experiment 1: Single bulus injection of SP 1-11 into the 3VT.

Five INT and five OVX females received a single slow (30 sec) 3CVT injection of 25 μg (18.5 nmol) of SP in a volume of 100 μl of Krebs-Ringer phosphate buffer (KRP) just after the 13th consecutive, 15-min interval 1-ml blood sample. The stylette was removed from the 3CVT guide cannula and an inner cannula (29-gauge stainless-steel tubing) was inserted. The inner cannula protruded 1.0 mm beyond the tip of the outer cannula into the 3VT. Prior to injection the inner cannula and 30 mm of connecting polyethylene tubing (PE-20, Clay Adams, Parsippany, NJ) was pre-filled with KRP buffer and attached to the needle of a Hamilton microsyringe containing the peptide solution.

Experiment 2: Continuous 3VT infusion of SP 1-11.

In six INT and five OVX female rabbits, the stylette was replaced by an inner cannula that was connected by PE-20 tubing to a pump. Sterile KRP buffer (100 μl) and 100 μl KRP that contained 18.5 nmol (25 μg) of SP (Peninsula Labs., Inc., Belmont, CA, Code 7451, Lot 008534) was infused into the 3VT during the first and second 3 h, respectively. The experiment was repeated twice in some rabbits.

Experiment 3: Continuous 3VT infusion of SP 6-11.

In four INT and five OVX female rabbits 100 μl of KRP with 18.5 nmol of SP 6-11 (Peninsula Labs., Inc., Code 7457, Lot 016490) was infused during 3 h beginning immediately after collection of the first blood sample.

Experiment 4: Continuous 3VT infusion of SP 1-7.

In three INT and four OVX female rabbits, 18.5 nmol of SP 1-7 (Peninsula Labs., Inc., Code 7489, Lot 010485) was infused in 100 μl KRP during 3 h beginning immediately after one blood sample.

After completion of the experimental program each rabbit was deeply anesthetized and 100 μl of 1% trypan blue was injected into the 3VT through the implanted cannula. The brain was removed from the calvarium and carefully dissected to verify the position of the cannula tip. In all animals the cannula tip was in the lumen of the 3VT since the ependyma of the 3VT, aqueduct and 4th ventricle were stained with trypan blue.

Hormone Determinations

Plasma LH was measured by a homologous rabbit LH radioimmunoassay involving antiserum AFP-8-1-28 at a final dilution of 1:480,000 and AFP-559-B as reference standard, both provided by Dr. Albert F. Parlow (Harbor-UCLA Medical Center, Torrance, CA). Rabbit LH EX-253-B provided by Dr. Harold Papkoff (University of California, San Francisco, CA) was used as ligand for iodination. The sensitivity of this assay ranged from 0.15 to 0.75 ng/ml, and binding curves between different serum pools from OVX rabbits and the standard were parallel. The coefficients of variation within and between assays were 10% and 12%, respectively.

Plasma FSH and PRL were measured by homologous rabbit FSH and PRL radioimmunoassays. Purified rabbit FSH (AFP-538-C) and PRL (AFP-1974-C) were provided by Dr. Albert F. Parlow and the National Institutes of Health (NIH) Pituitary Hormone Agency Distribution Program. The AFP-538-C and AFP-1974-C were used both for iodination and as the reference standard. When antisera AFP-4-7-21-76 for FSH and AFP-18102677 for PRL were used at final dilutions of 1:48,000 and 1:400,000, the sensitivities of the assays ranged from 0.5 to 1.0 and from 0.2 to 0.9 ng/ml, respectively. The coefficients of variation between and within assays were 9% and 6% for FSH and 11% and 8% for PRL, respectively.

Statistical Analysis

In each experiment, changes in plasma hormonal concentrations were analyzed statistically by analysis of variance and Duncan's multiple-range test. Levels of P equal to or less than 0.05 were considered as significant.

RESULTS

Experiment 1: A single injection of SP 1-11 into the 3VT.

Injection of 18.5 nmol of SP 1-11 into the 3CVT in intact female rabbits induced a brief, but significant, increase in plasma LH concentration 15 min after SP injection (fig. 1A). The nature and magnitude of the plasma LH response after ICV injection of SP varied between animals with the clearest LH increase occurring in two animals (Nos. 273 and 286) with stable LH patterns before the injection (fig. 1B).

The ICV injection of SP 1-11 had no influence on FSH and PRL blood plasma concentrations in INT animals.

Injection of 18.5 nmol of SP 1-11 ICV in five OVX rabbits induced an increase in blood plasma LH concentrations at 15 min (sample 14) and 30 min

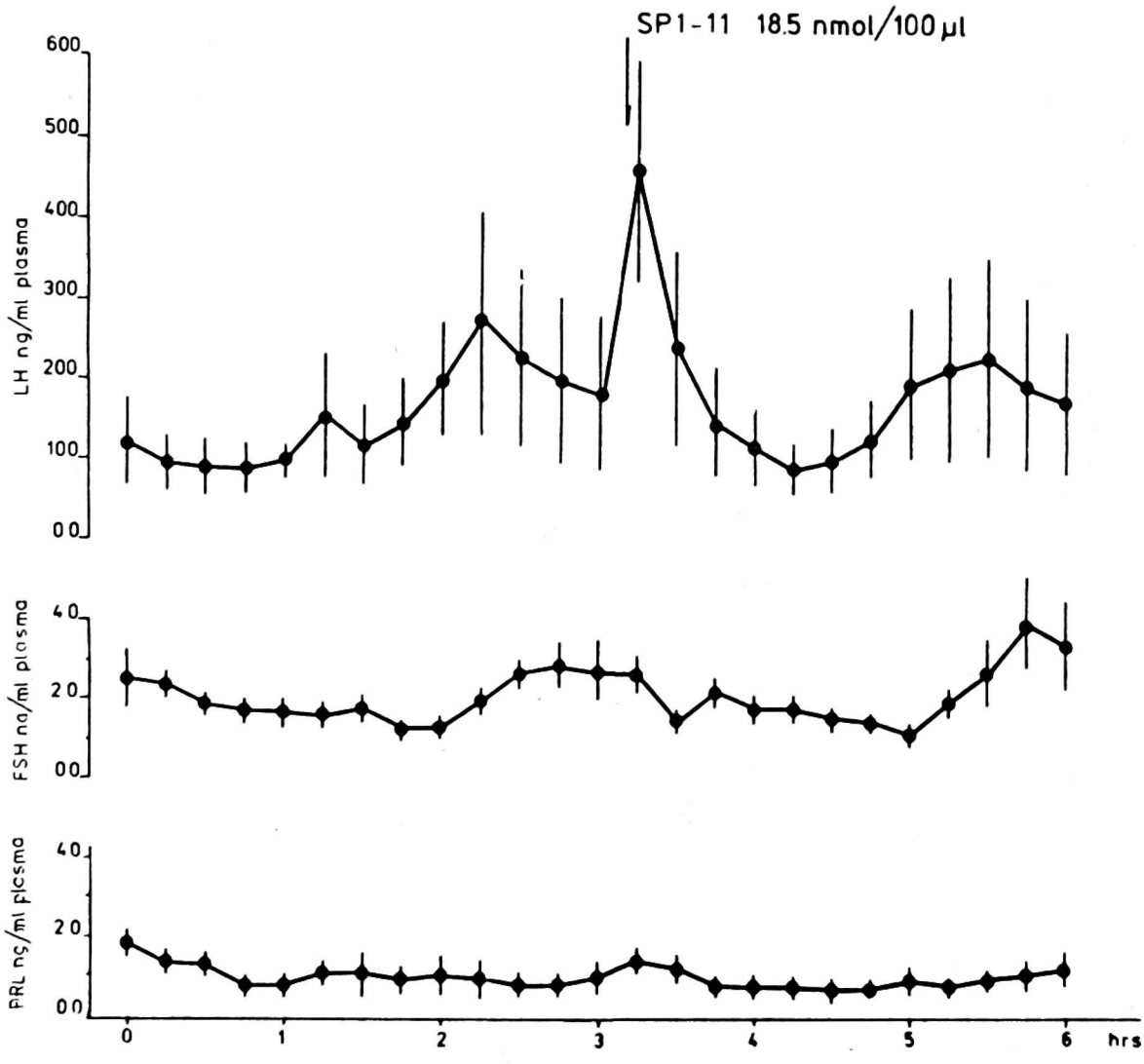
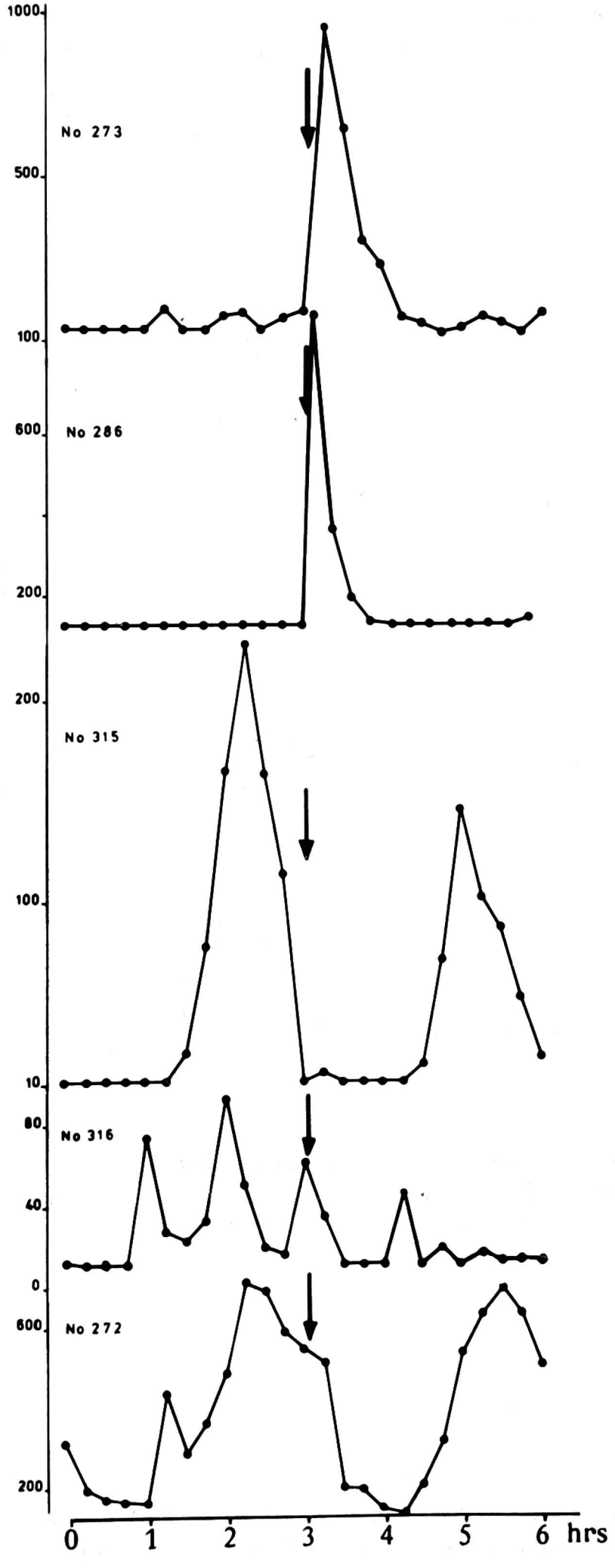


Fig. 1A. Blood concentrations of pituitary hormones (LH, FSH, and PRL) in 5 intact female rabbits before and after a single 3CVT injection of 18.5 nmol of SP 1-11 immediately after collection of the 13th blood sample. Blood samples were collected every 15 min during 6 h. Mean values are depicted by the solid line connecting individual points and SEM are shown in vertical bars.

Fig. 1B. Blood plasma concentrations of LH, ng/ml, in 5 individual intact female rabbits. The ICV injection of 18.5 nmol of SP 1-11, marked by arrow, was immediately after the 13th blood sample.


1000
500
100
No 273
600
200
No 286
200
100
10
No 315
80
40
0
No 316
600
200
No 272
0
1
2
3
4
5
6
hrs

(sample 15) after injection (fig. 2A). Pulses of LH in blood plasma of OVX rabbits occurred spontaneously and were readily detected in our radioimmunoassay (fig. 2B). These LH pulses were augmented in height and width after ICV injection of SP in three animals (nos. 228, 229, and 266; fig. 2B).

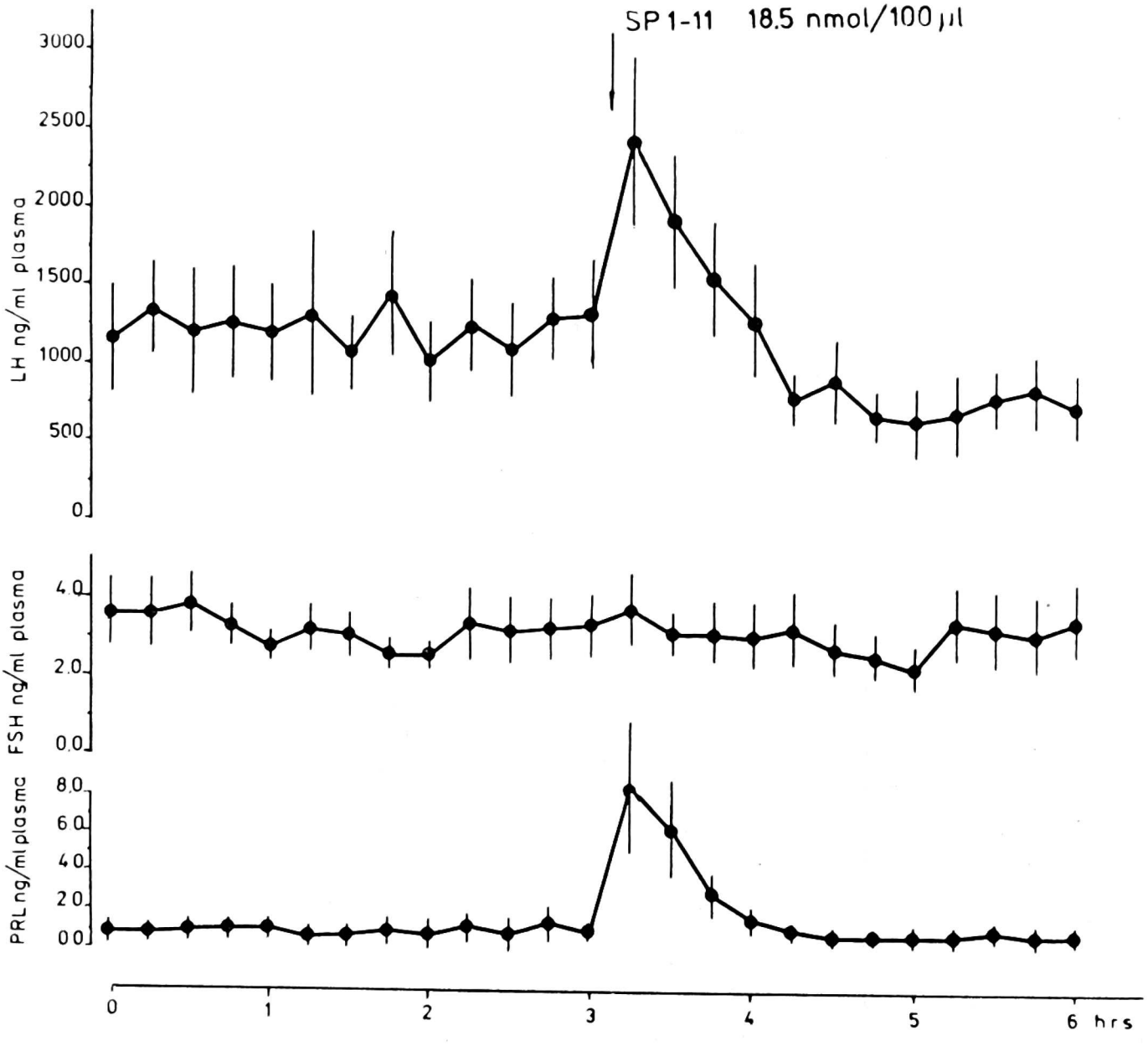
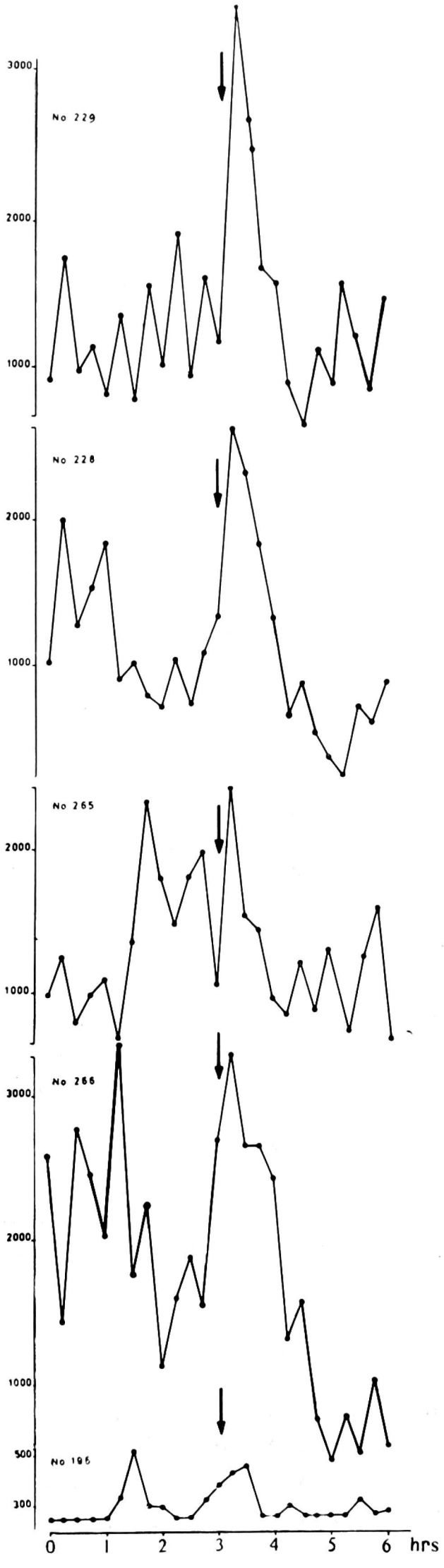


Fig. 2A. Plasma concentrations of pituitary hormones (LH, FSH, and PRL) in 5 OVX rabbits before and after a single 3CVT injection of 18.5 nmol of SP 1-11 immediately after blood sample number 13. Mean values (dots) and SEM (vertical bars) are shown.

Fig. 2B. Plasma concentrations of LH, ng/ml, in 5 individual OVX rabbits. The ICV injection of 18.5 nmol of SP 1-11, marked by arrow, was done just after collection of the 13th blood sample.


3000
2000
1000
No 229
No 228
No 265
No 266
No 196
500
300
0
1
2
3
4
5
6
hrs

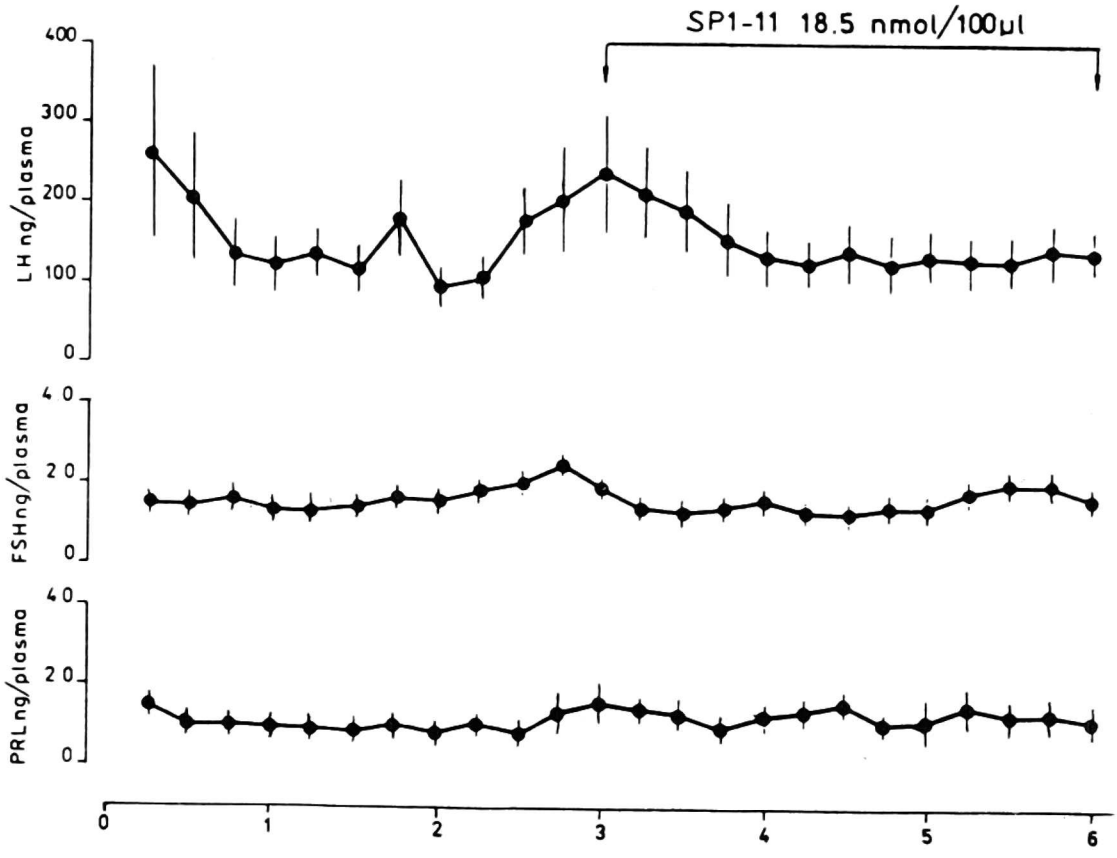


Fig. 3. Blood plasma concentrations of pituitary hormones (LH, FSH, and PRL) from 10 experiments that were performed on 6 intact female rabbits. During the first 3 h 100 µl of KRP buffer was infused into the 3CVT and blood samples were collected at 15-min intervals. The 3VT infusion of 18.5 nmol of SP 1-11 in 100 µl of KRP buffer was started just after collection of the 12th blood sample. Mean values (dots) and SEM (vertical bars) are shown.

The ICV injection of SP 1-11 in OVX rabbits had no influence on the blood plasma FSH concentration, but induced an increase in plasma PRL concentrations 15 and 30 min after SP injection ($p < 0.05$, fig. 2A).

Experiment 2: ICV infusion of SP 1-11.

Continuous ICV infusion of 18.5 nmol of SP 1-11 had no significant effects on LH, FSH, and PRL secretions in either intact (fig. 3) or OVX animals (fig. 4).

Experiment 3: ICV infusion of SP 6-11.

Infusion of 18.5 nmol of the C-terminal fragment of Substance P (SP 6-11), induced a significant increase of LH release after 2.5 h of infusion in four intact animals; this effect was evident by the time that five-sixths of the total dose of SP 6-11 had reached the cerebral ventricles (figs. 5A and 5B). The mean

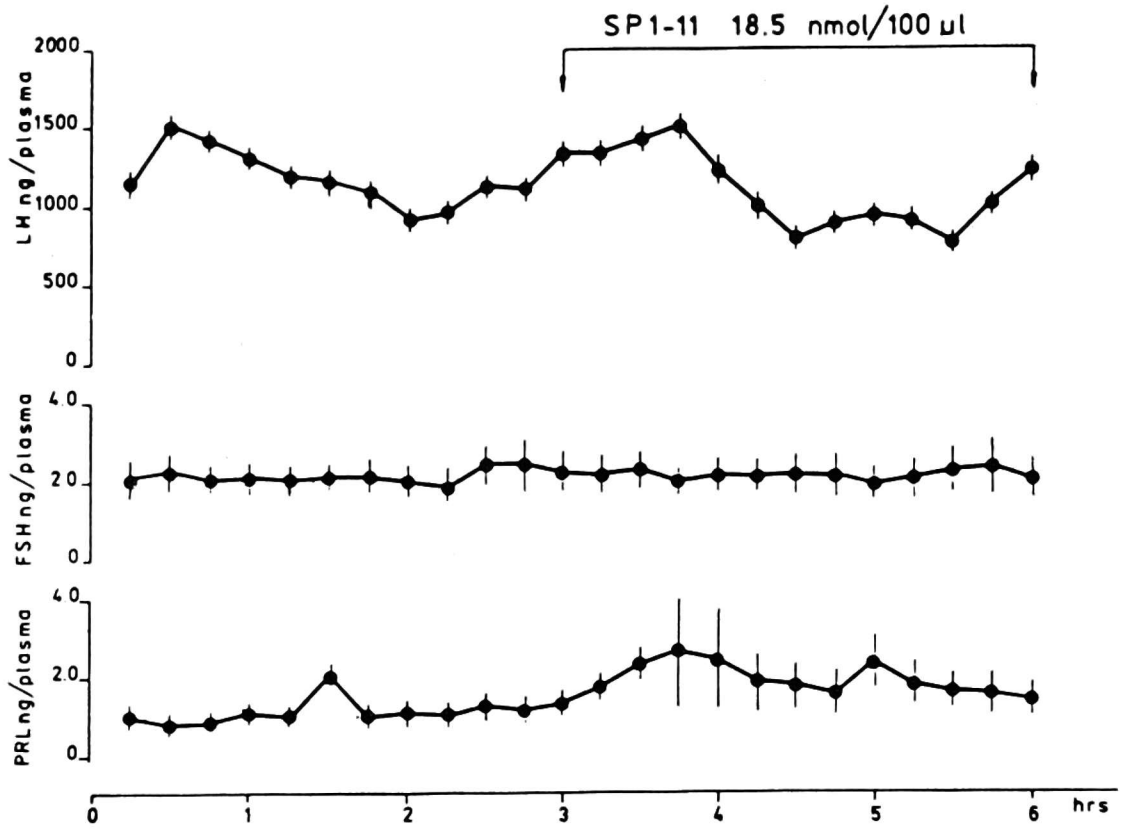


Fig. 4. Blood plasma concentrations of pituitary hormones (LH, FSH, and PRL) from 8 experiments that were performed with 5 OVX rabbits. The experiment was repeated twice in three animals. During the first 3 h 100 µl of KRP buffer was infused into the 3CVT and blood samples were spaced at 15-min intervals. Just after blood sample no. 12, the 3CVTinfusion was altered to include 18.5 nmol of SP 1-11 in 100 µl of KRP buffer. Mean values and SEM are shown.

concentrations of LH in blood plasma in samples 12 and 13 were higher ($p<0.05$) than those in the preceding 11 samples. The infusion of this fragment had no significant effect on either FSH or PRL secretion in intact rabbits.

In OVX females, ICV infusion of the hexapeptide SP 6-11 did not alter the release of any of the three pituitary hormones (fig. 6).

Experiment 4: ICV infusion of SP 1-7.

The ICV infusion of 18.5 nmol of the N-terminal fragment of Substance P (SP 1-7), was not effective in altering plasma levels of LH, FSH, and PRL in either intact ($n=3$) or OVX ($n=4$) animals (figs. 7 and 8).

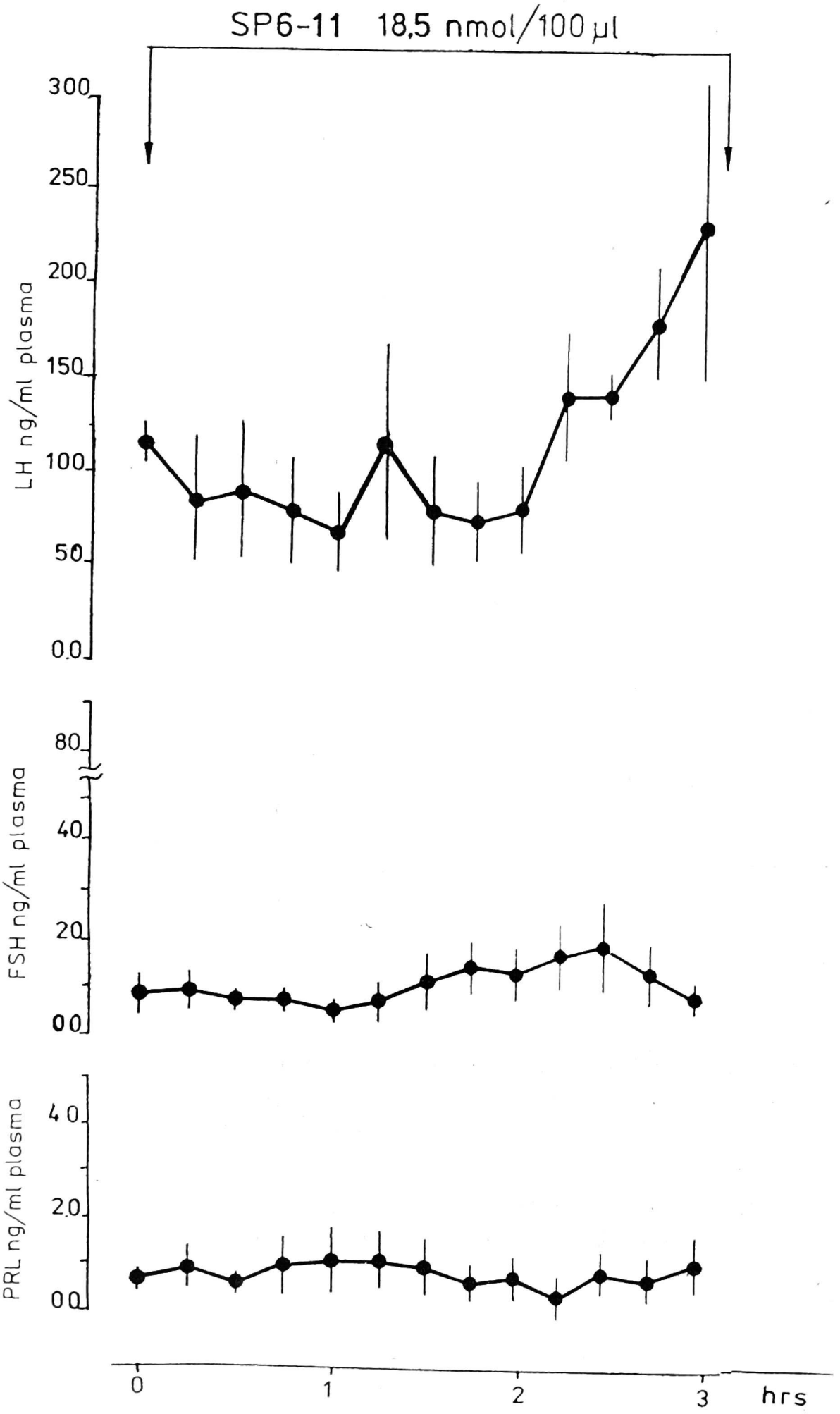


Fig. 5A. Plasma concentrations of LH, FSH, and PRL in 4 intact female rabbits during a 3-h infusion of 18.5 nmol of hexapeptide SP 6-11 into the 3CVT. In blood samples 12 and 13, the mean concentration of plasma LH was significantly higher ($p < 0.05$) than all preceding samples. Mean values (dots) and SEM (vertical bars) are shown.

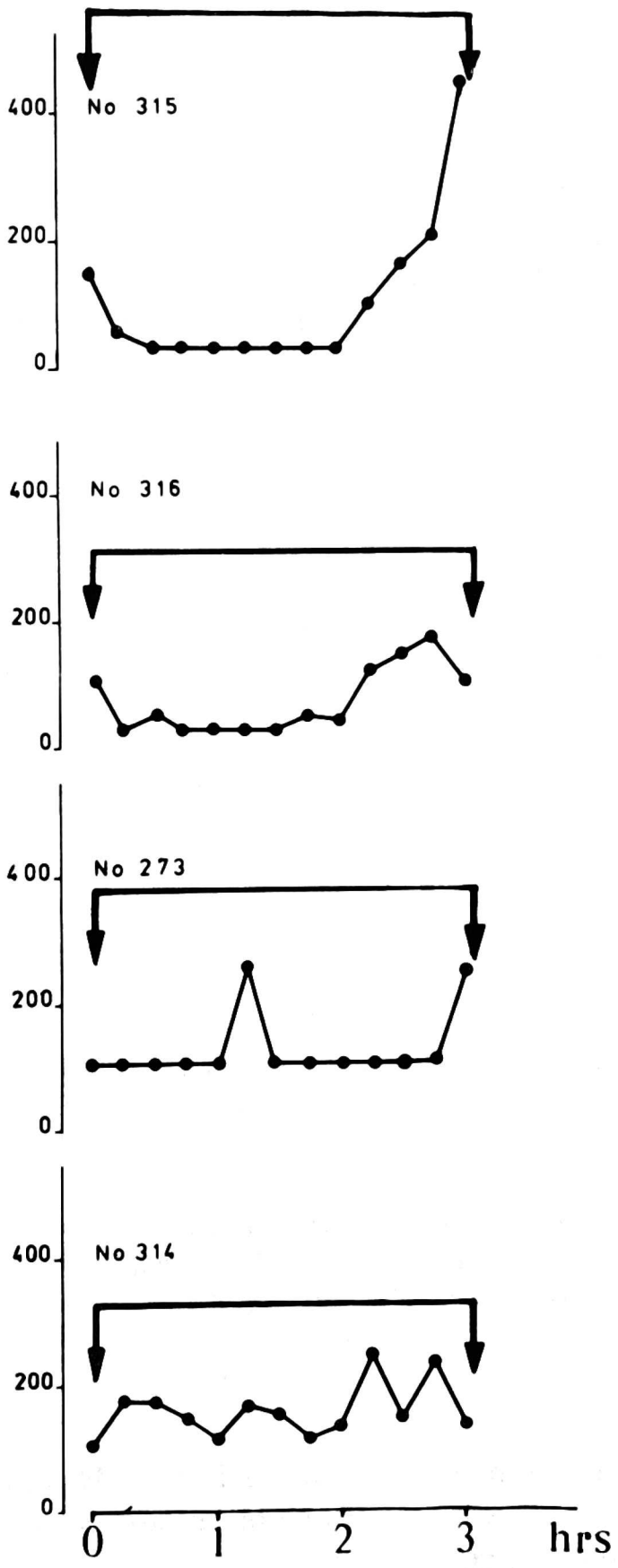


Fig. 5B. Blood plasma concentrations of LH in ng per 1 ml in 4 individual intact females during a 3-h ICV infusion of 18.5 nmol of SP 6-11.

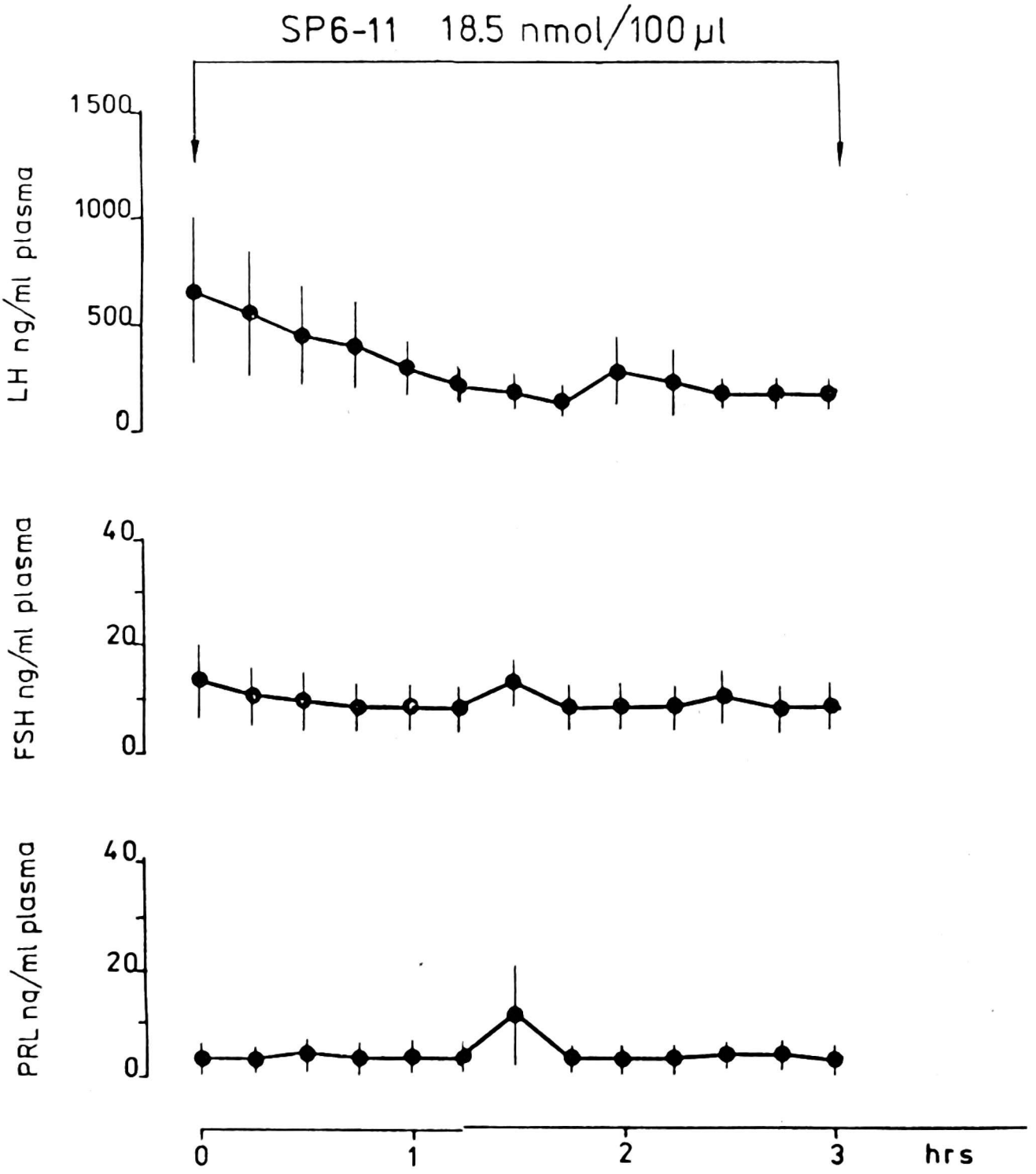


Fig. 6. Blood plasma concentrations of LH, FSH, and PRL in 5 OVX rabbits during a 3-h infusion of 18.5 nmol of hexapeptide SP6-11 into the 3CVT. Points that are connected by a line present the mean values and SEM are depicted by vertical bars.

DISCUSSION

The patterns of spontaneous release of LH, FSH, and PRL in intact and OVX female rabbits in our experiments resembles those that have been published elsewhere (15-18).

The injection of SP 1-11 into the 3CVT induced an elevation of blood plasma PRL for 30 min in OVX animals, but it failed completely in intact animals. Infusion of SP 1-11, SP 6-11, or SP 1-7 in the same volume and

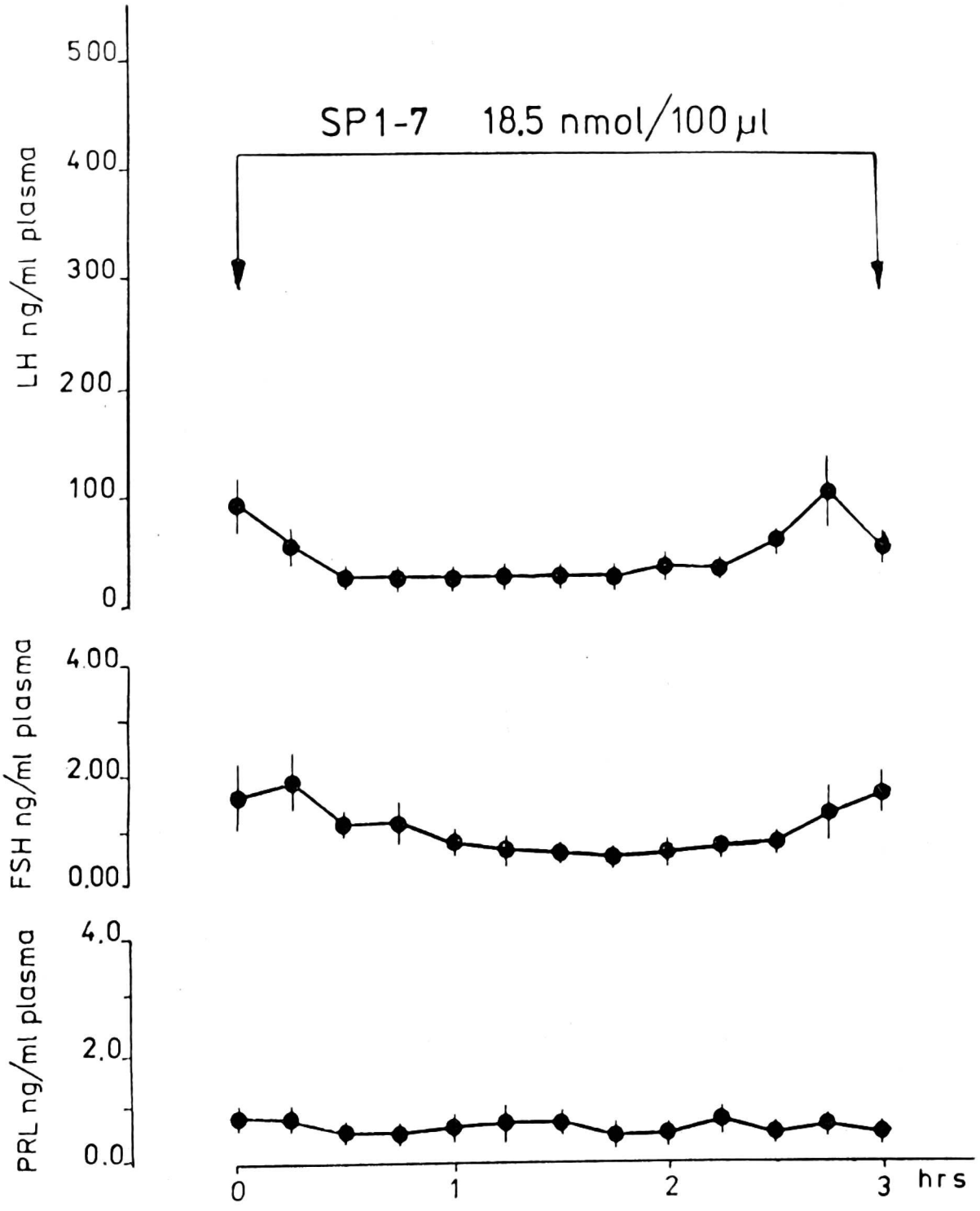


Fig. 7. Plasma LH, FSH, and PRL concentrations in 3 intact female rabbits during a 3-h infusion of 18.5 nmol of heptapeptide SP 1-7 into the 3CVT. The line and connecting points reflect mean values and the vertical bars show SEM.

amounts into intact and OVX animals was also ineffective. SP 1-11 injected into the 3VT probably reached the lactotrophs in the anterior pituitary gland in a concentration high enough to induce PRL secretion as a direct stimulus of lactotrophs has been found in vitro (9). Furthermore other workers have noted an increase in plasma PRL after intravenous injection of SP (8), even in rats with extensive hypothalamic destruction (7). The presence of SP-like immunoreactive nerve fibres in the adenohypophysis (19) and endogenous

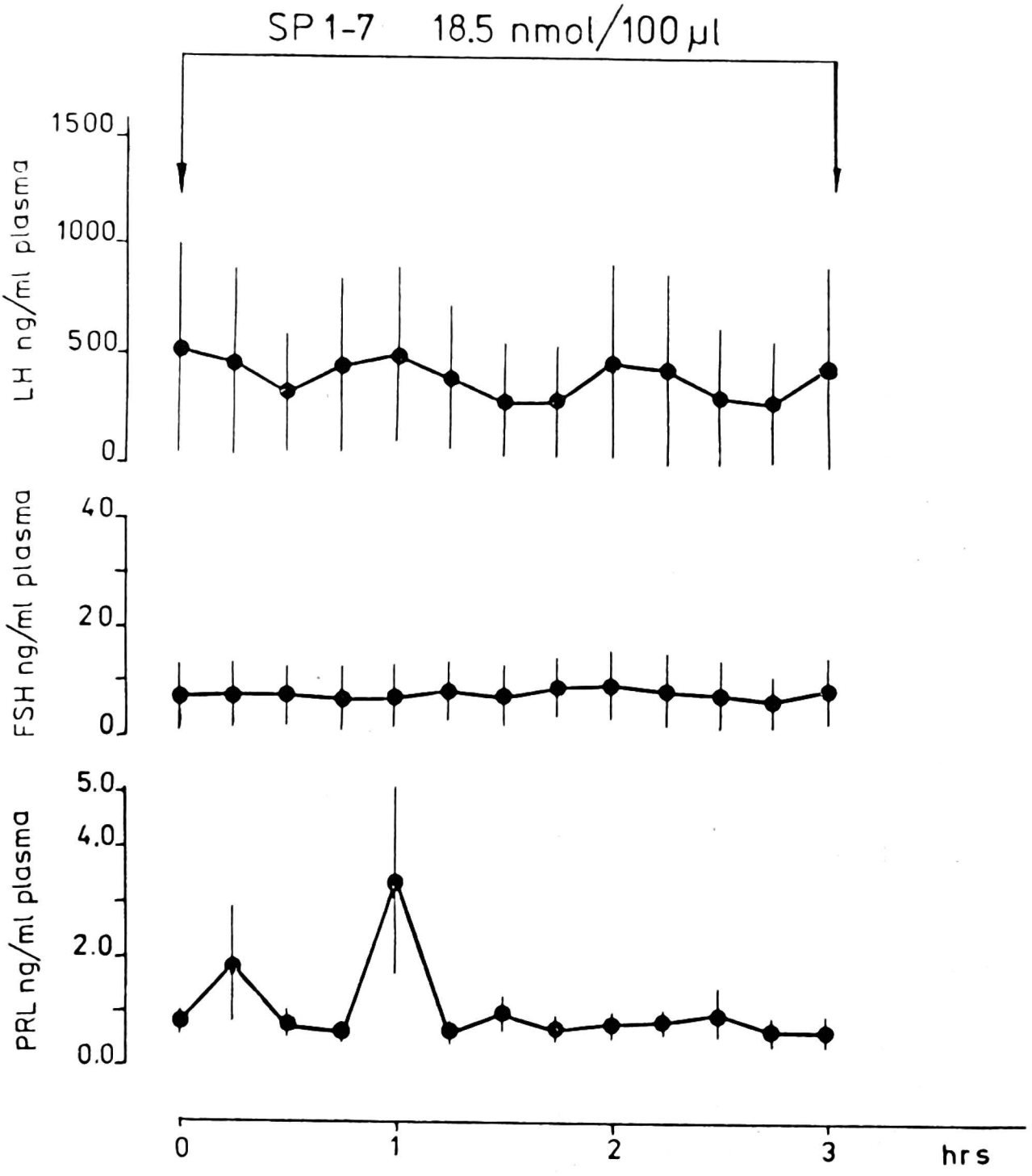


Fig. 8. Plasma concentrations of LH, FSH, and PRL in 4 OVX rabbits during 3 h of infusion of 18.5 nmol of heptapeptide SP 1-7 into the 3CVT. Mean values (dots) and SEM (vertical bars) are illustrated.

SP-like immunoreactivity in the lactotrophs and gonadotrophs (20) also support such an explanation. Why the presence of the ovarian hormones inhibits the direct lactotroph stimulation by SP in female rabbits is unclear. However, one possible explanation may be that central SP triggers an ovarian-dependent hypothalamic mechanism that overrides the direct effect of SP on PRL secretion.

Intracerebroventricular administration of SP 1-11 by injection or infusion and SP 6-11 or SP 1-7 by infusion had no significant effect in our experiments

on plasma levels of FSH in intact and OVX female rabbits. This agrees with the data of other authors in estrogen-primed OVX rats (6) and in intact female rhesus monkeys (12).

Some evidence suggest that SP does not act directly on gonadotrophs. For example, in *in vitro* incubation of pituitaries with several doses of SP, i.e., from 0.5 μg per 1 ml of medium (9, 11) to 1.34 μg per 1 ml of medium (6), have no effect on LH and FSH release. In another study however, a higher concentration of SP, that is 10 μg per 1 ml of medium, did induce gonadotropin release in vitro (5). *In vivo,* the intraventricular injection of SP induces plasma LH increase in rats (6, 9), but this route of administration is ineffective in the female rhesus monkey (12). Moreover, microinjection of SP into the medial preoptic area in male rats inhibits LH and FSH secretion (13). Apparently, the effects of SP on LH secretion depend on many factors including sex and species. The presented data suggest that SP 1-11 augmented spontaneous LH pulses when the peptide was injected before or during the rising phase of an LH pulse. SP injections during the declining phase of spontaneous LH pulses or after them were not effective. Thus our data support a stimulatory SP effect on LH release in a reflex-ovulator, and further indicate that the response of LH to SP also depends on the pulsatile pattern of spontaneous LH secretion.

The mechanism of the augmentation of LH pulses could reside within the pituitary, the hypothalamus, or higher central sites. The possibility of simultaneous actions of SP at several levels cannot be ruled out. Our results favor an extrahypothalamic site of SP action on LH release, considering the volume, concentration and duration of the 3CVT application that we utilized. Application of 18.5 nmol of SP in a volume of 100 μl was effective when injected within 30 sec (experiment 1), but ineffective when the same amount and the same volume was infused over 3 h (experiment 2). The injected SP may have exerted its activities on the structures neighboring not only the 3CVT but also the cerebral aqueduct and 4CVT. Infusion of a given amount of SP over 3 h may permit a high concentration at the site of infusion; that is in the 3CVT. In other parts of the ventricular system the concentration of SP maybe much lower, as the infused fluid is diluted by the cerebrospinal fluid formed in the ventricles and is also degraded by enzymes. Some significant increase of LH release in the late intervals of infusion and blood collection in experiment 3 in intact female rabbits may indicate that the C-terminal hexapeptide of SP is more active than the whole SP molecule (23) or penetrates deeper into the extrahypothalamic structures. The lack of activity of SP 6-11 in OVX female rabbits may be explained as follows. Its effect is exerted through the brainsterm noradrenergic system and the release of norepinephrine in the hypothalamus occurs primarily in the presence of ovarian hormones (22).

Our experimental data in conjunction with the data of other workers allow us to hypothesize a dual role of SP-ergic transmission in LH release from the

pituitary. SP-ergic transmission, by acting through noradrenergic and GnRH-ergic neurons, may augment LH release during the initial phase of its surge. An opposite activity of SP-ergic transmission is exerted in the declining phase of LH release. At this stage of the event, SP release from neural endings in the septo-preoptic area (24) may inhibit directly GnRH release from GnRH-ergic neurons.

Acknowledgments: We thank Terry Oyama, Margaret Stobie, Perry Gliessman, and Helmy Hawash for technical assistance; William Baughman and Gregory Katayama for their surgical assistance; Anna Kliszko for help in drawing the figures. We are grateful to Drs. William Ellinwood, Harold Papkoff, Albert F. Parlow, and the National Hormone and Pituitary Program of the NIH for providing reagents for rabbit pituitary hormone radioimmunoassays. This study was supported by the NIH Exchange Visitor Program No. G050111 and the Institute of Pharmacology Polish Academy of Sciences in Cracow grant CPBP 06.03.

REFERENCES

1. Brownstein M, Mroz E, Kizer J, Palkovits M, Leeman S. Regional distribution of substance P in the brain of the rat. *Brain Res* 1976; 116: 299—305.
2. Aronin N, Coslovsky R, Leeman SE. Substance P and neurotensin: their roles in the regulation of anterior pituitary function. *Ann Rev Physiol* 1986; 48: 537—549.
3. Rønnekleiv OK, Kelly MJ, Eskay RL. Distribution of immunoreactive substance P neurons in the hypothalamus and pituitary of the rhesus monkey. *J Comp Neurol* 1984; 224: 51—59.
4. Chang MM, Leeman SE. Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. *J Biol Chem* 1970; 245: 4784—4790.
5. Fisher GH, Humphries J, Folkers K, Pernow B, Bowers CY. Synthesis and some biological activities of substance P. *J Med Chem* 1974; 17: 843—846.
6. Arisawa M, De Palatis L, Ho R, et al. Stimulatory role of substance Pongonadotropin release in ovariectomized rats. *Neuroendocrinology* 1990; 51: 523—529.
7. Kato Y, Chihara K, Ohgo S, Iwasaki Y, Abe H, Imura H. Growth hormone and prolactin release by substance P in rats. *Life Sciences* 1976; 19: 441—446.
8. Rivier C, Brown M, Vale W. Effect of neurotensin, substance P and morphine sulfate on the secretion of prolactin and growth hormone in the rat. *Endocrinology* 1977; 100: 751—754.
9. Vijayan E, McCann SM. In vivo and in vitro effects of substance P and neurotensin on gonadotropin and prolactin release. *Endocrinology* 1979; 105: 64—68.
10. Chihara K, Arimura A, Coy DH, Schally AV. Studies on the interaction of endorphins, substance P, and endogenous somatostatin in growth hormone and prolactin release in rats. *Endocrinology* 1978; 102: 281—290.
11. Vijayan E, McCann SM. Effects of substance P and neurotensin on growth hormone and thyrotropin release in vivo and in vitro. *Life Sciences* 1980; 26: 321—327.
12. Eckstein N, Wehrenberg WB, Louis K, et al. Effects of substance P on anterior pituitary secretion in the female rhesus monkey. *Neuroendocrinology* 1980; 31: 338—342.
13. Picanco-Diniz DLW, Valenca MM, Franci CR, Antunes-Rodrigues J. Role of substance P in the medial preoptic area in the regulation of gonadotropin and prolactin secretion in normal or orchidectomized rats. *Neuroendocrinology* 1990; 51: 675—682.

14. Sawyer CH, Everett JW, Green JD. The rabbit diencephalon in stereotaxic coordinates. *J Comp Neurol* 1954; 101: 801—824.
15. Pau KYF, Spies HG. Effects of cupric acetate on hypothalamic gonadotropin-releasing hormone release in intact and ovariectomized rabbits. *Neuroendocrinology* 1986; 43: 197—204.
16. Orstead KM, Spies HG. Inhibition of hypothalamic gonadotropin-releasing hormone release by endogenous opioid peptides in the female rabbit. *Neuroendocrinology* 1987; 46: 14—23.
17. Khorram O, Pau KYF, Spies HG. Bimodal effects of neuropeptide Y on hypothalamic release of gonadotropin-releasing hormone in conscious rabbits. *Neuroendocrinology* 1987; 45: 290—297.
18. Pau KYF, Gliessman PM, Oyama T, Spies HG. Disruption of GnRH pulses by anti-GnRH serum and phentolamine obliterates pulsatile LH but not FSH secretion in ovariectomized rabbits. *Neuroendocrinology* 1991; 53: 382—391.
19. Ju G, Liu SJ, Zhang X. Peptidergic innervation of the pars distalis of the adenohypophysis. *Neuroendocrinology* 1991; 53 (suppl 1): 41—44.
20. Morel G, Chayvialle JA, Kerdelhue B, Dubois PM. Ultrastructural evidence for endogenous substance-P-like immunoreactivity in the rat pituitary gland. *Neuroendocrinology* 1982; 35: 86—92.
21. Magnusson T, Carlsson A, Fisher GH, Chang D, Folkers K. Effect of synthetic substance P on monoaminergic mechanisms in brain. *J Neural Transmission* 1976; 38: 89—93.
22. Pau KYF, Spies HG. Estrogen-dependent-effects of norepinephrine on hypothalamic gonadotropin-releasing hormone release in the rabbit. *Brain Research* 1986; 399: 15—23.
23. Traczyk WZ, Kubicki J. The pressor response to substance P and hexapeptide [pGlu^6] SP_{6-11} injections into the cerebral ventricles in rats. *Neuropharmacology* 1980; 19: 607—611.
24. Tsuruo Y, Kawano H, Hisano S, Kagotani Y, Daikoku S, Zhang T, Yanaihara N. Substance P- containing neurons innervating LHRH-containing neurons in the septo-preoptic area of rats. *Neuroendocrinology* 1991; 53: 236—245.

Received: January 22, 1992
Accepted: March 17, 1992

Author's address: W. Z. Traczyk, Department of Physiology, Institute of Physiology and Biochemistry, Medical University of Łódź, ul. Lindleya 3, 90-131 Łódź, Poland