

ROLE OF SUBSTANCE P IN CENTRAL CONTROL OF OVULATION IN FEMALE RATS

E. POTARGOWICZ, W.Z. TRACZYK

Department of Physiology, Institute of Physiology and Biochemistry, Medical University of Lodz, 90-131 Lodz, Poland

Objective. To investigate the influence of substance P on gonadotropin release from the pituitary as evaluated according to the final effect, i.e. the ovulation.

Methods. Stainless steel tube was implanted into the 3rd cerebral ventricle and substance P (SP), C-terminal hexapeptide of Substance P (SP₆₋₁₁), Gn-RH and adrenaline were infused on the 3th day of the cycle (proestrus). The oviducts were then isolated on the day of estrus and the ova were counted.

Results. SP or SP₆₋₁₁ inhibited the ovulation which was not prevented by intracerebroventricular (i.c.v.) capsaicin pretreatment. The i.c.v. administration of anti-SP antibodies or Met-Enkephalin did not had any effect on the ovulation. The i.c.v. administration of noradrenaline on the 7-8th day of pseudopregnancy induced the ovulation which was prevented by the injection of SP immediately before noradrenaline.

Conclusions. It is suggested that noradrenergic neurons which control the ovulation by influencing the release of Gn-RH into pituitary portal vessels are affected by SP-ergic neurons.

Key words: Substance P – Capsaicin – Noradrenaline – Met-Enkephalin – Ovulation – Pseudopregnancy – 3rd Cerebral Ventricle

Substance P (SP) was found in great concentration in the hypothalamus (BROWNSTEIN et al. 1976), but little is known about its function. FISHER et al. (1974) did not observe any influence of SP on gonadotropin hormone (GH) release from the pituitary *in vitro*, but they showed its slight activity on the release of LH and FSH. However, opposite results were obtained by VIJAYAN and McCANN (1979) who found no effect of SP *in vitro*, while its intravenous injection decreased plasma LH level in ovariectomized female rats. However, KERDELHUE et al. (1978a,b) showed that the administration of SP into the cerebral ventricles blocked the preovulatory surge of LH and FSH in rats. The effects of alteration of pituitary gonadotropin functions (i.e. prolongation of vaginal estrus) were observed also after the implantation of Hexa SP substance 6-11 into the hypothalamus (KACPRZAK and TRACZYK 1980).

The fluctuations of SP and Gn-RH concentration in the median eminence on consecutive days of estrus cycle were also reported, the highest content being noticed in diestrus and the lowest in estrus (ANTONOWICZ et al. 1982; JAKUBOWSKA-NAZIEMBLO et al. 1985). However, opposite results were obtained by PARNET et al. (1990) who found that the highest SP content during the proestrus. The parallel fluctuations of SP and Gn-RH content suggest a possible correlation between these peptides. In addition, the interactions between SP and Gn-RH neurons were suggested on the basis of immunohistochemical data. SP immunoreactive cell bodies in the arcuate nucleus whose axons projected to the external layer of the median eminence were been demonstrated (HOFFMAN 1985). SP administrated into the 3rd cerebral ventricle decreased Gn-RH content in the medial basal hypothalamus (MBH) in ovariectomized estrogen

implanted but not in ovariectomized female rats (WALCZEWSKA et al. 1996).

Materials and Methods

Animals. Female rats weighing 200-280 g and bred in this Department were used. The animals were F₁ generation of a cross strain of female Wistar and male Buffalo rats from the stock of the Institute of Oncology in Gliwice (Poland). They were kept under 14:10 hours light:dark cycle and fed standard laboratory food and water *ad libitum*. The studies were carried out in the animals in which 4 day regular estrous cycles were observed after the implantation of a cannula into the 3rd cerebral ventricle.

Determination of ovulation. The estrous cycle was determined by routine microscopic examination of vaginal smears. The ovulation was evaluated by counting the ova in oviducts. After two consecutive and regular estrous cycles the appropriate compound was infused into 3rd cerebral ventricle on the 3th day of the cycle that is during the proestrus at 13.00h. The oviducts were then isolated at 11.00 h on the day of estrus under urethane anesthesia (20 % solution of urethane in a dose of 0.5 g/100 g b.w. was injected ip). First, the oviducts were ligated on the side of the uterus, dissected and placed on a watch glass. The ova inside the ampulla were then counted with the aid of stereomicroscope. In order to eliminate the error the wall of the ampulla was disrupted and, after washing out, the ova were counted again.

Implantation of a cannula into the 3rd cerebral ventricle. The cannula was a stainless steel tube of 0.6 mm external diameter with a platinum mandrin. After the implantation under hexobarbital anesthesia (80 mg/kg ip) it was fixed to the skull with dental cement. The cannula was implanted according to the atlas by DE GROOT (1963) in such a way that its end was in the 3rd cerebral ventricle 7 mm anterior to the frontal zero plane, in the midline sagittal plane and 5.5 mm deep from the skull surface.

Infusion into 3rd cerebral ventricle. The following peptides and drugs were infused with the aid of Hamilton syringe joined to the cannula with polyethylene tubing in a volume of 10 µl/min: Substance P (SP) (Schwarz-Mann, Lot 2303); C-terminal hexapeptide fragment of Substance P (SP₆₋₁₁) (synthesized at the Department of General Chemistry,

Institute of Physiology and Biochemistry, University of Medicine in Lodz); Gn-RH (Peninsula labs., Lot 90-0223) dissolved in dimethylsulfoxide (Serva) and 0.9 % NaCl; noradrenaline (Fluka, Buchs, Switzerland)

Pseudopregnancy induction. Females in the proestrus phase were placed together with vasectomized males in a separate cage and left there overnight in order to induce pseudopregnancy which was ascertained on the basis of the absence of cornified cells in the vaginal smears taken at 9.00 h on the fifth day after placing with the male. The criteria for pseudopregnancy in female rats were: arrest of the estrus cycle and constant presence of leucocytes in the vaginal smears for 7 or 8 subsequent days. The investigated compounds were infused into the 3rd cerebral ventricle on day 7 or 8 of pseudopregnancy.

Control of the distribution of infused fluid in cerebral ventricles. After removal of the oviducts the anesthetized female rats were infused with 10 µl of 1 % trypan blue into the 3rd cerebral ventricle through the cannula. Ten minutes later they were decapitated and the skull was placed in 10 % formalin solution. After one week the brains were removed from the skulls and the area of the brain where the cannula had been implanted was cut a freezing microtome into 50 µm sections in frontal plane. If the end of the cannula was in proper positions the staining of the ependyma was observed. The rats in which no staining of the 3rd ventricle ependyma was observed and no trace of the cannula in the 3rd ventricle was found were excluded.

The experiments were performed in fifteen groups of rats:

[1]. i.c.v. infused with 10 µl 0.9 % NaCl containing 0.2 % dextrane (40 000 m.w.); [2]. ip injected with pentobarbital (3.5 mg/100 g) (Vetbutal, Biowet, Poland); [3]. ip injected with 3.5 mg/100 g of pentobarbital followed after 10 min by i.c.v. infusion of 10 µl of 10 nmol Gn-RH; [4]. i.c.v. infused with 10 µl of 20 nmol SP followed by i.c.v. 10 µl of 10 nmol Gn-RH; [5]. i.c.v. infused with 10 µl of 2 nmol SP; [6]. i.c.v. infused with 10 µl of 2 nmol SP₆₋₁₁; [7]. i.c.v. infused with 10 µl diluted rabbit SP antiserum on the proestrus day at 13.00 h; [8]. i.c.v. infused with 10 µl of rabbit SP antiserum on dioestrus day II and proestrus day at 13.00

T a b l e 1
The ovulation after infusion into 3rd cerebral ventricle on proestrus day in female rats with regular estrous cycles.

Group	n	Infused compound	Time of infusion (h)	Number of animals ovulating tested	Average number of ova	Inhibition of ovulation (per cent)
1	9	Vehicle	13.00	8/9	10.3	11.1
2	12	Pentobarbital i.p.	13.00	0/12	0	100.
3	17	Pentobarbital i.p. + Gn-RH	12.45 13.00	13/17	10.5	23.5
4	9	SP + Gn-RH	12.50 13.00	7/9	11.7	22.2
5	13	SP	13.00	4/13	9.6	69.2
6	11	SP 6-11	13.00	3/11	11.0	72.7
7	8	Anti-SP antibodies	13.00	7/8	11.2	12.5
8	9	Anti-SP antibodies	13.00	7/9	10.2	22.2
9	8	Anti-SP antibodies	13.00	8/8	11.6	0
10	9	SP	13.00	5/9	12.6	44.4
11	27	Met-enkephalin	13.00	22/27	11.0	18.5

Significant difference (0.05 level) between group No. 1 versus groups No. 2, No. 5 and No. 6.

h; [9]. i.c.v. infused with 10 µl diluted rabbit SP antiserum on dioestrus I, dioestrus II and proestrus day at 13.00 h; [10]. i.c.v. infused with 10 µl of 20 nmol SP in the second oestrus cycle (that is on the 8th day after pretreatment) with 300 µg of capsaicin dissolved in dimethylsulfoxide and 0.9 % NaCl, infused into each lateral cerebral ventricle just before the implantation of the cannula into the third cerebral ventricle; [11]. i.c.v. infused with 10 µl of 2 nmol met-enkephalin; [12]. i.c.v. infused with 10 µl of vehicle solution consisting of 0.9 % NaCl with 0.2 % dextrane (40 000 m.w). on the 7th or 8th day of pseudopregnancy; [13]. i.c.v. infused with 10 µl of 40 µg noradrenaline on the 7th or 8th day pseudopregnancy; [14]. i.c.v. infused with 10 µl of 100 µg noradrenaline on the 7th or 8th day of pseudopregnancy; [15]. i.c.v. infused with 10 µl of 20 nmol SP and after 10 min i.c.v. infused with 10 µl of 100 µg noradrenaline.

Group No.1 was a control for groups No.2 to 11 and group No.12 was a control for groups No.13 to 15.

Statistical evaluation. The differences between groups in the number of ovulating animals and in the number of ova were evaluated by the nonparametrical Wald-Wolfowitz runs test according to BLACKLOCK (1961).

Results

As shown in Tab. 1, eight rats ovulated in the control group of 9 animals (group 1) which had the vehicle infused into the 3rd cerebral ventricle. An intraperitoneal injection of pentobarbital prevented the ovulation completely ($P < 0.05$) in all 12 animals treated (group 2).

The infusion of Gn-RH into the 3rd ventricle of such animals, in which the expected ovulation was blocked by the pretreatment with pentobarbital (group 3), induced the ovulation in 13 of 17 females.

Between the groups 3 and 2 there was a significant difference, but no such difference was found between the groups 1 and 3. The infusion of Gn-RH into the 3rd cerebral ventricle (group 4) after the infusion of SP induced the ovulation in 7 out of 9 female rats. These values differed significantly as compared to the group 5 where only SP was infused, but there was no significant difference in comparison to the control group (group 1).

The effects of SP infusion on the ovulation were investigated in group 5 in which a significant difference from the control (group 1) was found. The infusion of SP₆₋₁₁ (group 6) also caused an inhibition of ovulation. The comparison between groups 6 and

T a b l e 2
Ovulation after infusion into the 3rd cerebral ventricle on the 7th or 8th dau of pseudopregnancy in female rats

Group	n	Infused compound	Time of infusion	Number of animals ovulating/ tested	Average number of ova	Inhibition of ovulation (percent)
12	10	Vehicle	13.00	0/10	0	100.0
13	11	Noradrenaline 40 µg	13.00	6/11	11.5	45.4
14	11	Noradrenaline 100 µg	13.00	8/11	12.3	27.2
15	11	SP 20 nmol + Noradrenaline 100 µg	13.00 13.10	0/11	0	100.0

Significant difference (0.05 level) between group No.12 and No.13 or No.14.

5 did not show any significant difference, but there was a significant difference when group 6 was compared with group 4. The infusion of SP (group 5) and its fragment SP₆₋₁₁ (group 6) caused an inhibition of ovulation. These values differed significantly as compared to the control (group 1 and 4). The comparison between groups 6 and 5 did not show any significant difference. The infusion of anti-SP antibodies was performed in groups 7 to 9. The comparison of groups 7, 8 and 9 with the control (group 1) revealed no significant differences. The difference between the animals pretreated with capsaicin (group 10) and group 5 was not significant. Infusion of met-enkephalin into 3rd cerebral ventricle (group 11) did not interfere with the ovulation.

As shown in Tab. 2, the infusion of vehicle into the 3rd cerebral ventricle on the 7th or 8th day of pseudopregnancy (group 12) did not induce ovulation in any of 10 tested animals. This group served as control for the groups 13, 14 and 15. The infusion of 40 µg or 100 µg noradrenaline into 3rd ventricle induced ovulation in a significant number of animals. However, the infusion of SP preceding noradrenaline infusion (group 15) significantly abolished the ovulation inducing effect of the latter.

Discussion

The administration into the cerebral ventricle of SP or SP₆₋₁₁ (group 5 and 6) resulted in a significant but not complete inhibition of spontaneous ovulation. The systemic administration of pentobarbital and that the ovulation suppressing activity was related to the C-terminal fragment of the SP molecule. Gn-RH administered into the 3rd cerebral ventricle

abolished the suppression of ovulation induced with SP or pentobarbital. These results suggest a hypothalamic level of SP activity. The obtained results were consistent with those of KERDELHUE et al. (1978 b) who demonstrated that SP administered into the lateral cerebral ventricle in rat on the day of proestrus completely blocked the pre-ovulatory secretion of LH and FSH. However, opposite results were obtained by ARISAWA et al. (1990) who found the stimulatory effect of SP on gonadotropin release after its intracerebroventricular injection in ovariectomized (OVX) and OVX estrogen-primed rats and the inhibitory effect of SP after its microinjection into the medial preoptic area of conscious intact and orchidectomized adult rats.

Effect of i.c.v. administration of anti-SP antibodies, capsaicin and met-enkephalin on ovulation.

Intraventricular infusion of anti-SP antibodies on the subsequent days of the estrus cycle had no effect on spontaneous ovulation. In our experiments the mean number of ova in the oviducts of the animals belonging to the groups 7,8 and 9 did not differ from that in the controls. There may be various reasons why the administration of antibodies into the 3rd cerebral ventricle did not cause any changes in ovulation. First of all we should consider that SP is not involved in the hypothalamic mechanism of spontaneous ovulation control during regularly occurring estrus cycles. Actually, the removal of SP from the cerebrospinal fluid by immunoneutralisation had no effect on spontaneous ovulation. KERDELHUE et al. (1978 a) obtained the abolition of SP induced blocking of pre-ovulatory gonadotropin release by means of intraperitoneal administration of anti-SP antibodies.

The effect may be also dependent on the route of administration of the antibodies. Thus, the i.c.v. infusion of the antibodies may be less effective than systemic administration. The results obtained by CHIHARA et al. (1978) confirmed such a possibility.

Capsaicin pretreatment preceding by eight days SP administration (group 10) did not prevent the ovulation suppression by administration of the latter, but ovulation occurred in a lower percent of animals than in the not pretreated group (group 5). Presumably some transient release of SP into

the cerebrospinal fluid just after capsaicin administration decreased the sensitivity of some neurons to SP. The studies on capsaicin as a neurotoxin depleting selectively SP (HELKE et al. 1981; BUCK et al. 1982; RUSSEL et al. 1984; BURKS et al. 1985) showed that this compounds decreased the content of SP in the dorsal part of pineal spinal cord and medulla oblongata, while other areas of central nervous system (mesencephalon, hypothalamus and substantia nigra), did not show any significant changes. However, according to HAJOS et al. (1986) eight days after s.c. administration of a large dose of capsaicin the noradrenaline concentration in the preoptic regions and hypothalamus was elevated. This may explain why in our experiments the administration of SP was less effective in capsaicin pretreated animals than in the group not pretreated.

The release of Gn-RH from Gn-RH neuron terminals in the median eminence is under the neural control of classic transmitters and neuropeptides (OEVEs et al. 1986; WEINER et al. 1988)

Some interaction of SP and Met-enkephalin in ovulation control could be expected because the axons containing both peptides terminate on the dendrites of catecholaminergic neurons (PICCKEL et al. 1979). Other workers have also shown that met-enkephalin indirectly inhibited LH release from the pituitary gland (VAN VUGT et al. 1981; WANG et al. 1983). We did not observe the influence of 2 nmol met-enkephalin i.c.v. on the ovulation. The finding that the decrease in plasma level of LH after treatment with met-enkephalin was transient (WANG et al. 1983) implies that any effect of this compound in our study would have been short lasting.

Intracerebroventricular administration of noradrenaline stimulated the release of gonadotropins to the extent sufficient to induce ovulation in the ani-

mals which have been anovulatory for various reasons (TIMA and FLERKO 1974). Accordingly, the ovulation was induced by noradrenaline in pseudopregnant animals and the effects of SP on the ovulation induced in such a way were investigated. Our results confirmed the efficacy of noradrenaline in inducing ovulation and the number of ovulating rats was dependent on the administered dose of NA (groups 13 and 14).

Infusion of SP immediately preceding the infusion of noradrenaline completely abolished its ovulation inducing effect thus indicating that SP administered i.c.v. interfered in some way with the activity of noradrenergic neurons promoting the release of Gn-RH (group 15).

Such a possibility seems to be more likely, since it has been demonstrated that SP can influence monoaminergic neurons in the brain. MAGNUSSON et al. (1976) observed an increase in the production of DOPA in various parts of the brain after SP injection. They also demonstrated an accelerated turnover of noradrenaline, dopamine and serotonin in brain tissue after i.c.v. injection of this peptide.

Such an influence on the turnover of monoamines is a proof of physiological interactions between them and SP. Histochemical investigations (PALKOVITS et al. 1974; KELLY et al. 1982) demonstrated that the axons of Gn-RH-ergic, SP-ergic and NA-ergic neurons extend to the median eminence. Thus, there is a possible interaction between these fibres (Fig. 1).

SP infused into the 3rd ventricle did not prevent the pregnancy in female rats, neither did pentobarbital (ASLANOWICZ-ANTKOWIAK 1987). Considering these results it may be supposed that SP is involved only in the mechanism of inhibition of spontaneous ovulation, but not in the copulation induced reflex ovulation. Later studies from our laboratory indicate that injection of SP into the internal carotid artery caused a significant increase in Gn-RH concentration in pituitary portal blood plasma. SP acts on pituitary gonadotropin release at the hypothalamus level through the interneural connections and/or perhaps in a paracrine manner (WALCZEWSKA et al. 1998).

Acknowledgements

The study was conducted under contract C.P.B.P. No. 06.03.3.1. with the Polish Academy of Sciences

and supported by the Ford Foundation, New York, Grant 770-0540. The authors thank Dr.A. Szymanski for statistical evaluation of the data, Dr.W.Koziolekiewicz (Department of General Chemistry, Medical University of Lodz) for the synthesis of SP₆₋₁₁, Dr.A.Lipkowski (Faculty of Chemistry, Warsaw, University) for the synthesis of Met-enkephalin and Dr.A Babinska (Department of Biophysics Medical University of Lodz) for producing rabbit anti-SP antibodies.

References

- ANTONOWICZ U, JAKUBOWSKA-NAZIEMBLO B, CANNON O, POWELL D: Immunoreactive Substance P content in median eminence and pituitary gland during oestrus, dioestrus and after anterior hypothalamic deafferentation. *Endokrinologie* **79**, 25-34, 1982
- ARISAWA M, DE PALATIS L, HO R, SNYDER GD, YU WH, PAG, MCCANN SM: Stimulatory role of substance P on gonadotropin release in ovariectomized rats. *Neuroendocrinology* **51**, 523-529, 1990
- BLALOCK HM, JR: *Social Statistics*. McGraw-Hill, New York, Toronto 1969
- BROWNSTEIN HJ, ARIMURA A, SCHALLY AV, PALKOWITS M, KIZER JS: The effect of surgical isolation of the hypothalamus on its luteinizing hormone-releasing hormone content. *Endocrinology* **98**, 662-665, 1976
- BUCK SH, MILLER MS, BURKS TF: Depletion of primary afferent Substance P by capsaicin and dihydrocapsaicin without altered thermal sensitivity in rats. *Brain Res* **253**, 216-220, 1982
- BURKS TF, BUCK SH, MILLER MS: Mechanisms of depletion of Substance P by capsaicin. *Fed Proc* **44**, 2531-2534, 1985
- CHIHARA K, ARIMURA A, COY DH, SCHALLY AY: Studies on the interaction of endorphins, Substance P, and endogenous somatostatin in growth hormone and prolactin release in rats. *Endocrinology* **102**, 281-290, 1978
- DE GROOT L: *The rat forebrain in stereotaxic coordinates*. Second Printing. N.V. Noord-Hollandsche Uitgevers Maatschappij. Amsterdam 1963
- FISHER GH, HUMPHRIES J, FOLKERS K, PERNOV B, BOWERS CY: Synthesis and some biological activities of Substance P. *J Med Chem* **17**, 843-846, 1974
- HAJOS M, SVENSSON K, NISSBRANDT H, OBAL F, JR, CARLESON A: Effects of capsaicin on central monoaminergic mechanisms in the rat. *J Neural Transm* **66**, 221-242, 1986
- HOFFMAN GE: Organization of LHRH cells: Differential opposition of neurotensin, substance P and catecholamine axons. *Peptides* **6**, 439-461, 1985
- JAKUBOWSKA-NAZIEMBLO B, WLODZIMERSKA B, TRACZYK WZ: Effects of intraventricular administration of Substance P and its hexapeptide fragments on the estrus cycle in female rats. *Acta Physiol Pol* **30**, 83-85, 1979
- KACPRZAK E, TRACZYK WZ: Effect of implantation of Substance P fragment 6-11 into diencephalon on the estrus cycle in female rats. In: *Neuropeptides and Neural Transmission* (Eds. C. Ajmone Marsan, W.Z. Traczyk), pp.141-151, Raven Press, New York 1980
- KATO V, CHIHARA K, OHGO S, IWASAKI V, ABE H, IMURA H: (1976). Growth hormone and prolactin release by Substance P in rats. *Life Sci* **191**, 441-446, 1976
- KELLY NJ, RONNEKLEIV OK, ESKAY RL: Immunocytochemical localization of luteinizing hormone-releasing hormone in neurons in the medial basal hypothalamus of the female rat. *Exp Brain Res* **49**, 97-106, 1982
- KERDELHUE B, YALENS N, LANGLOIS V: Stimulation de la secretion de la LH et de la FSH hypophysaires apres immunoneutralisation de la Substance P endogene, chez la ratte cyclique. *CR Acad Sci Paris, Serie D*, **286**, 977-979, 1979a
- KERDELHUE B, YALENS N, LANGLOIS V: Substance P inhibits the secretion of gonadotropins in female rats. 60th Annual Meeting of Endocrine Society, p.186, Miami 1978b
- KOEVES K, MOLNAR J: Effect of various hypothalamic deafferentations injuring different parts of the Gn-RH pathways on ovulation Gn-RH content of the median eminence and plasma LH and FSH levels. *Neuroendocrinology* **44**, 172-183, 1986
- MAGNUSSON T, CARLSSON A, FISHER GH, CHANG C, FOLKERS K: Effect of synthetic Substance P on monoaminergic mechanisms in brain. *J Neur Transm* **38**, 89-93, 1976
- PALKOVITS N, BROWNSTEIN N, SAAVEDRA JM, AXELROD J: Norepinephrine and dopamine content of hypothalamic nuclei of the rat. *Brain Res* **77**, 137-149, 1974
- PARNET P, LENOIR V, PALKOVITS M, KERDELHUE B: Estrous cycle variations in gonadotropin-releasing hormone, substance P and beta-endorphin contents in the median eminence, the arcuate nucleus and the medial preoptic nucleus in the rat: A detailed analysis of proestrus changes. *J Neuroendocrinol* **2**, 291-296, 1990
- PICKEL V, JOH RS, RATS DJ, LAWMAN SE, MILLER RJ: Electron microscopic localization of Substance P and

- enkephalin in axon terminals related to dendrites of catecholaminergic neurons, *Brain Res* **160**, 397-400, 1979
- RUSSEL LC, BURCHIEL KJ: Neurophysiological effects of capsaicin. *Brain Res Rev* **61**, 165-176, 1994
- TIMA L, FLERKO B: Ovulation induced by norepinephrine in rats made anovulatory by various experimental procedures. *Neuroendocrinology* VOLUME IS MISSED, 346-354, 1974
- VIJAYAN S, McCANN SM: In vivo and in vitro effects of Substance P and neurotensin on gonadotropin and prolactin release. *Endocrinology* VOLUME IS MISSED, 64-69, 1979
- VAN YUGT DA, AYLSWORTH C, SYLVESTER PW, LEUNG PC, MEITES J: Evidence for hypothalamic noradrenergic involvement in naloxane induced stimulation of luteinizing hormone release. *Neuroendocrinology* **33**, 261-264, 1981
- WALCZEWSKA A, DZIEDZIC B, JAKUBOWSKA-NAZIEMBLO B, TRACZYK WZ: Gonadotropin-releasing hormone (Gn-RH) content in the medial basal hypothalamus after Substance P injection into the 3rd cerebral ventricle in female rats. *Endocrine Regul* **30**, 99-107, 1996
- WALCZEWSKA A, SZKUDLAREK U, JAKUBOWSKA-NAZIEMBLO B, DZIEDZIC B, TRACZYK WZ: Increase of gonadotropin-releasing hormone concentration in pituitary portal blood after substance P administration in male rats *JOURNAL IS MISSED* **67**, 219-227, 1998
- WANG Y, KUBO K, KIYOTA V, TONI N, SASAKI T: Endorphin actions to inhibit LH release and facilitate lordosis reflex in relation to estrogen feedback in the CNS. *Proc. of XXIX. Congress Internat. Union of Physiol. Sci.*, Vol. XV, p. 367, Sydney 1995
- YANG HY, HONG JS, COSTA S: Regional distribution of Leu and Met enkephalin in rat brain. *Neuropharmacology* **16**, 303-307, 1977
- WEINER RJ, FINDELL PR, KORDON C: (1988). Role of classic and peptide neuromediators in neurocrine regulation of LH and prolactin. In: *The Physiology of Reproduction* (Eds. E. Knobil, J. Neil et al.), pp. 1236-1278, Raven Press, New York 1988
- Corresponding author:** Dr. Elsbietta Potargowicz
Department of Physiology
Institute of Physiology and Biochemistry,
Medical University of Lodz,
Lindleya 3
90-131 Lodz, POLAND
Phone: (4842) 678 2661
Fax (4842) 678 2661
- Accepted:** June 15, 1999

BOOK REVIEW

INTERNATIONAL TEXTBOOK OF DIABETES MELLITUS

Editors: **K.G.M.M. Alberti** (Newcastle u.T.), **P. Zimmet** (Caulfield) and **R.A. DeFronzo** (San Antonio),
Honorary Editor: **H. Keen** (London), Second Edition, Volume I and II, 1827 pages, John Wiley & Sons,
Chichester 1997

This is an excellent result of the giant project to bring together all present knowledge on diabetes presented by 188 outstanding experts and cut into more than 100 subject headings. The topics discussed in this king size manual include a broad spectrum of aspects from the molecular genetics, morphology, physiology, molecular basis of insulin action and immunopathogenesis, to the diagnosis, epidemiology, dietary management, drug treatment, implantable pumps, islet transplantation, glucose sensors, self monitoring (blood glucose, glycated hemoglobin, lipids), computer-assisted education of diabetic patient, special problems in management (brittle diabetes, childhood, pregnancy, aging), acute disturbances (hypoglycemia, ketoacidosis, infections, surgery, vascular events), chronic microvascular (nephropathy, retinopathy, peripheral neuropathy etc.) and macrovas-

cular complications (coronary heart disease, clotting disorders, hypertension), diabetic foot and public health problems (organisation of care in various continents, economics of diabetes, social rights, primary prevention).

At the same time, such broad spectrum of topics defines the broad spectrum of the readers and professions (physicians of all medical fields, social workers, economists, biochemists, pharmacologists etc.) which may benefit from this diabetes bible. This comprehensive work, in addition to excellent and valuable scientific and medical information, presents up to date references and high quality of technical arrangements including up to additional 300 pages of indexes facilitating to find any subtopic on advancing broad field of diabetes.

Pavel Langer