JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1992, 43, 4, 367—372

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VASOPRESSIN RELEASE FROM POSTERIOR PITUITARY LOBE INCUBATED IN SITU AFTER PREGANGLIONIC STIMULATION OF THE RAT SUPERIOR CERVICAL GANGLION

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In uretane-chloralose anaesthesia the pituitary gland was exposed by transpharyngeal approach in rats. The anterior lobe was removed and the posterior lobe was incubated in situ, that is in conditions of anatomical integrity of the hypothalamus with the posterior pituitary lobe. The 15-min samples of the medium incubating the posterior pituitary lobe in situ were collected. Vasopressin (AVP) content in the incubation medium was determined by radioimmunoassay. The stimulation of preganglionic fibers of the superior cervical ganglion (SCG) with alternate short (5 s) bursts of electric pulses with short (5 s) breaks did not change AVP release. However, stimulation of preganglionic fibres with alternate long (30 s) bursts of electric pulses with long (30 s) breaks evoked an increase in AVP release after some latency . Probably, at the hypothalamic or posterior pituitary level temporal summation should occur affecting vasopressinergic neurons or their endings and evoking AVP release.

Key words: *vasopressin, superior cervical ganglion, posteriol pituitary lobe*

INTRODUCTION

In 1983 Cardinali presented a hypothesis that the superior cervical ganglion is a „small endocrine brain" (1). It innervates the posterior pituitary lobe (2, 3).

Our previous experiments showed an increase in the release of oxytocin into the fluid incubating the posterior pituitary lobe „*in situ*" after the stimulation of preganglionic fibres of the superior cervical ganglion (4). However, no influence on the vasopressin release into the cerebrospinal fluid (5).

The aim of the present study was to investigate whether the stimulation of preganglionic fibres of the superior cervical ganglion (SCG) may influence the vasopressin (AVP) release from the posterior pituitary lobe incubated *in situ*.

MATERIAL AND METHODS

The experiments were performed on 21 male rats 300—320 body weight, being the F_1 generation of cross- breeding of August strain males and Wistar strain females, from the stock of the Institute of Oncology in Warsaw. The animals were kept under constant temperature and 14:10 h light dark cycle and fed standard rat pellets and water ad libitum.

After anesthesia (50 mg/kg urethane with 6 mg/kg chloralose i. p.) the animals were placed on their back on a board and their heads were fixed by ear bars and by fixing the upper jaw with a clamp. The pituitary was exposed according to Worthington (6) and incubated *in situ* (7). The sympathetic preganglionic nerve of the SCG was exposed and a thread was placed around the nerve. Six 15-min samples of the medium incubating in situ the posterior pituitary lobe were collected. The posterior pituitary lobe was bathed in McIlwain and Rodnight's medium containing mmol of following compounds per 1 l of dist. water: NaCl 120, KCl — 4.8, $MgSO_4$ — 1.3, $CaCl_2$ — 2.8, KH_2PO_4 — 1.2, $NaHCO_3$ — 26, glucose — 10, dextrane (110 000 m. w.) — 0.1. During the collection of the fourth sample, the preganglionic fibres were stimulated by platinum wire bipolar electrodes. The electrodes were connected to a DISA Multistim and the parameters of electrical pulses were: 10 V, 20 Hz, 3 ms, in the 1st group 5 s on, 5 s off, and in the 2nd group 30 s on, 30 s off.

During the 15 min stimulation period the electrical pulses were monitored with an oscilloscope. In both groups, ipsilateral dilatation of the palpebral fissure was observed during the stimulation of preganglionic fibres.

The incubation medium was continuously collected into polypropylene tubes with 0.02 ml glacial acetic acid at about 4°C, centrifuged, transferred into glass ampoules, frozen and lyophilized. The content of AVP was determined by RIA (8) exposed in pg per min. The upper limit of RIA sensitivity (i. e. 20% depression of maximum binding) was 9.04 ± 1.09 pg/tube and 50% depression was 27.06 ± 2.40 pg/tube (both $n = 8$, mean $\pm$ SE).

For statistical analysis Student's „t" test for paired variables was used.

RESULTS

In the 1st experimental group the respective mean AVP release into the medium incubating *in situ* the posterior pituitary lobe in the three control samples amounted to: 12.6 ± 4.3 (mean $\pm$ SE) in the sample I, 9.6 ± 2.4 in the sample II and 8.4 ± 2.1 pg/min in the sample III. Stimulation of the preganglionic fibres of SCG with trains of electrical pulse of 5 s on and off did not have a significant influence on the decline of AVP release into the incubating medium as the AVP in the IVth sample amounted to 8.8 ± 2.9 pg/min and in subsequent samples: Vth 6.4 ± 1.9 and VIth 5.7 ± 1.5 pg/min.

In the 2nd experimental group the respective AVP release in the first three control samples amounted to: 10.3 ± 3.1, 11.1 ± 3.7 and 6.5 ± 2.3 pg/min.

Electrical stimulation of the preganglionic fibres of the SCG with trains of electrical pulses 30 s on and off during collection of the IVth sample produced no significant increase in the release of AVP 11.6 ± 3.9, but significant increase in the Vth sample, 14.6 ± 5.3. Release of the AVP in the VIth sample amounted to 12.7 ± 5.7 pg/min.

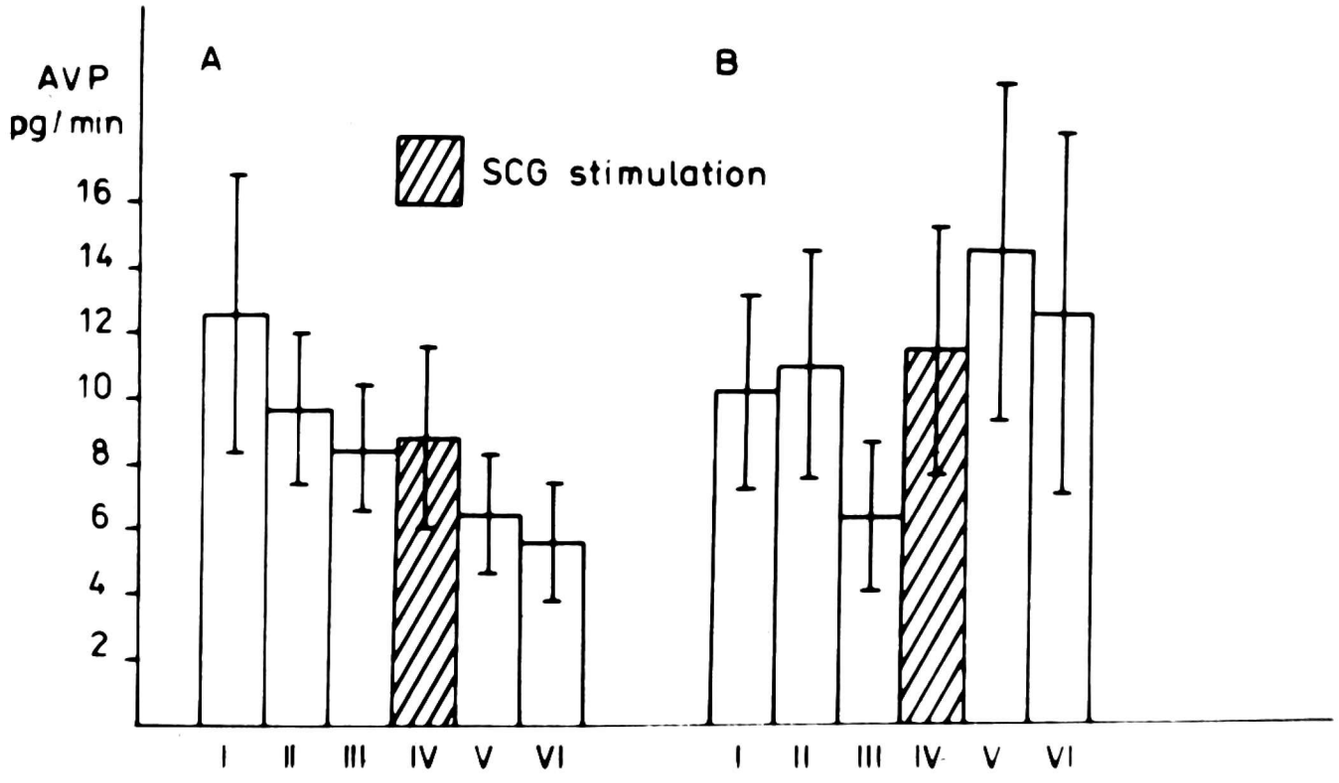

Fig. 1. AVP release into the fluid incubating the posterior pituitary lobe in situ. During collection of the IVth sample SCG was stimulated: 10V, 20Hz, 3 ms;

A. 5 s on, 5 s off. n = 10

B. 30 s on, 30 s off. n = 11

Values represent the mean $\pm$ SE.

Differences between samples:

A. II — V $p < 0.01$
II — VI $p < 0.01$
III — V $p < 0.05$

B. I — III $p < 0.02$
II — III $p < 0.05$
III — V $p < 0.05$

DISCUSSION

The method of incubation of the posterior pituitary lobe *in situ* is closer to physiological conditions of posterior pituitary hormones release as compared with incubation of the posterior pituitary lobe *in vitro*. During incubation *in situ* the connections between vasopressinergic and oxytocinergic cell bodies and their endings in posterior pituitary lobe are preserved. The circulation of the blood in the posterior pituitary lobe is also maintained to some extent.

The basal release of AVP during the experiment was not constant, but was falling with time (9), as seen as in the first experimental group. Stimuli applied during the experiment may change the baseline level of AVP release (10), as seen as in our second experimental group. The dispersion of AVP release values per time unit from the posterior pituitary lobe incubated *in situ* in different animals is specially noteworthy. It may by caused by surgery-evoked stress increasing AVP release (11, 12), and by the blood volume decrease during posterior pituitary lobe exposure (13, 14). The participation of SCG in AVP release was proved by investigation with bilateral cervical ganglionectomy which caused a change of AVP (15, 16) and Substance P (17) release from the posterior pituitary lobe.

The results obtained in the second experimental group of animals confirm the possibility of SCG participation in AVP release. However, the AVP release after stimulation of preganglionic fibres is much lower by comparison with oxytocin release in a similar experimental model (4). An about 25-fold increase of oxytocin release into the incubation medium appeared after stimulation of the preganglionic fibers with bursts of 30-sec electrical pulses alternately with 30-sec breaks. Comparison of AVP release with OXY release in the same experimental conditions leads to the conclusion that the reactivity of the vasopressinergic system is much lower as compared to the oxytocinergic system.

The frequency of electrical pulses used in our experiments is an efficient stimulus to release not only acetylcholine from preganglionic (18), and noradrenaline from postganglionic fibers (19), as well as coexisting peptides (20). Unilateral stimulation of preganglionc fibers with electrical pulses of 20 Hz, 10 V, and 3 ms for 30 sec with 30 sec breaks caused an increase in the number of synaptic vesicles in the SCG (21) and decrease in neurosecretory granules in the posterior pituitary lobe (22). The same stimulation as regards frequency, duration and amplitude of electric pulses with the same length of on and off stimulation evoked also an increase of oxytocin release into the incubation medium of the posterior pituitary lobe incubated *in situ* (4).

A lot of evidence has accumulated on the participation of adrenergic transmission in the regulation of posterior pituitary neurohormones release (23, 24). Noradrenaline released from SCG postganglionic endings in the hypothalamus and the posterior pituitary lobe (3) may enhance or inhibit the release of neurohormones, depending on the type of activated alpha- or beta-adrenergic receptors (25).

In noradrenergic neurons of the superior cervical ganglia the following peptides coexist with noradrenaline: Substance P, somatostatin, methionine enkephalin (26) and Neuropeptide Y (20). These peptides may modulate the activity of vasopressinergic system.

The difference between the first and the second experimental group are probably dependent on the duration of stimulation and breaks between them, activation or inactivation of some co-modulators present in pre- and post-ganglionic fibers, and some temporal stimulation of affecting vasopressinergic neuron on they endings and evoking the AVP release.

On the basis of results obtained, it may by presumed that the sympathetic efferents arising from the SCG probably participate in the regulation of AVP release. It is possible, that in this mechanism are involved peptides comodulators responsible for a more long-lasting effects. This problem still remains an open question and requires further investigation.

Acknowledgements: We would like to express our gratitude to Miss Beata Sulejewicz for her technical assistance during the experiments.

The study was supported by the grant MZ-X from Ministry of Health and Social Welfare.

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Received: February 10, 1992
Accepted: July 24, 1992

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