JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1993, 44, 2, 155—159

B. STEMPNIAK, J. W. GUZEK

INSULIN-LIKE IMMUNOREACTIVITY (IRI) IN THE RAT HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM: effect of dehydration and haemorrhage *)

Department of Pathophysiology, School of Medicine, Lodz, Poland

Hypothalamic IRI was not affected in haemorrhaged rats, but diminished considerably in the dehydrated ones. In the neurohypohysis, IRI was distinctly higher both in dehydrated and haemorrhaged rats, i.e., under disorders which stimulated vasopressin and/or oxytocin release. It is suggested that insulin-like substance(s) may be someway involved in regulation of vasopressin or oxytocin secretion.

Key words: *Insulin-like immunoreactivity, hypothalamus, neurohypophysis*

INTRODUCTION

Insulin as well as insulin-like growth factors (IGF-I and IGF-II, both resembling insulin by amino-acid structure, biological effects and receptor affinity) are present in the brain of mammals. The relatively highest insulin-like immunoreactivity (IRI) has been found in the hypothalamus (1). Several studies have shown that insulin and/or IGFs may exert regulatory (neuromodulatory?) functions within the central nervous system. Brain insulin as well as brain insulin receptors were noted to be controlled independently from peripheral insulin (2). In the rat, insulin-binding sites are localized mainly in the arcuate, dorsomedial and paraventricular nuclei (3); such a distribution suggests that insulin and/or IGFs might interact with the peptide- and/or amino-secreting neurones in these areas.

This report deals with the effect of dehydration or haemorrhage on the insulin-like immunoreactivity (IRI) in the hypothalamus and neurointermediate lobe in the rat.

---

*) Conducted under contract No. 06.03.3.5. with the Polish Academy of Sciences.

MATERIAL AND METHODS

Animals

Adult male Wistar rats weighing 280-320 g were used. They were fed standard laboratory diet and kept at about +22°C; a 14-h light, 10-h dark cycle was provided (artificial illumination 6.00 a.m. — 8.00 p.m). Before experiment, they were given tap water ad libitum.

Experimental design

The rats were divided into three groups: A — intact controls (i.e., euhydrated animals); B — animals dehydrated, i.e. having access to standard rat pellets only and not receiving drinking water for four days; C — animals haemorrhaged. The rats of the group C were anaesthetized by i.p. injection of 10 per cent urethane (1.4 ml/100 g b.w.), immobilized on an operating board and the femoral vein was cannulated. They were haemorrhaged (1.0 ml per 100 g b.w.) and killed 20 min. after bleeding.

Experimental procedure.

All animals were fasted for 15 h and then decapitated at 9.00—10.00 a.m. The brain with intact pituitary was quickly removed, the infundibular stalk cut up and the neurointermediate lobe separated. From the brain, hardened in the freezer, the hypothalamus was dissected as follows: rostral limit — frontal plane situated at the anterior margin of the optic chiasma; caudal limit — frontal plane just behind the mammillary bodies; lateral limits — sagittal planès passing, on both sides, just through the hypothalamic fissures. The depth was about 1.5—2.0 mm from the base of the brain. The wet weight of such block of tissue, containing hypothalamus and a part of thalamus, was 74.3±3.4 mg (mean±S.E.M.).

For the determination of IRI, the hypothalami and neurointermediate lobes were extracted as recommended by Baskin et al (1); as shown by separate estimations, the losses of insulin during extraction procedure were 16.2±3.3% (mean±SEM). For RIA assay, the extracts were reconstituted in phosphate buffer and measured for IRI with a RIA-kit (POLON, Otwock-Swierk, batch No. 04007). A sample of the extract was taken for determination of protein by the method of Folin-Ciocalteu as modified by Lowry (4); bovine serum albumin (BSA, batch No. 30688, POCH, Cracow) was used as standard. Control estimations of insulin-like immunoreactivity in the blood plasma have been also carried out in an additional group of intact animals. The latter were killed by decapitation to collect trunk blood into heparinized tubes. IRI in the plasma was shown to be 36.0±5.2 μU/ml (mean ± S.E.M.; n = 5); this finding is consisted with other respective data (1).

Hypothalamic and neurohypophysial IRI was finally expressed: a) in microunits of insulin per whole hypothalamus or neurointermediate lobe, and b) in microunits of insulin per 1 mg protein. All results are reported as mean ± SEM and evaluated using the Wilcoxon's test (5).

RESULTS

The results are summarized in *Table 1.* In animals dehydrated for four days, IRI appeared to diminish in the whole hypothalamus (by about 35% in comparison with intact controls) but to increase in the whole neurointermediate lobe (by about 42%). The haemorrhage resulted in an increase of IRI in the

neurointermediate lobe only (by about 50%), its hypothalamic content being not affected significantly under such conditions. When expressed per whole hypotalamus or neurointermediate lobe, IRI values were highly correlated with respective results calculated per 1 mg protein (*Table 1*).

Table 1. The hypothalamic (Hth) and neurohypophysial (NH) insulin-like immunoreactivity in dehydrated or haemorrhaged male rats (mean ± S.E.M.)

Group of animals (No. of animals in parentheses)	Hypothalamus			Neurointermediate lobe		
	μU/Hth (a)	μU/mg protein (b)	correlation coefficient (a) vs (b)	μU/NH (c)	μU/mg protein (d)	correlation coefficient (c) vs (d)
A) intact controls (8)	14.4±0.9	1.5±0.1	$r = 0.95$ $p < 0.001$	18.7±1.3	39.7±2.9	$r = 0.85$ $p < 0.01$
B) animals dehydrated (8)	9.3±0.9	0.7±0.1	$r = 0.71$ $p < 0.05$	26.6±2.5	65.4±8.6	$r = 0.96$ $p < 0.001$
C) animals haemorrhaged (7)	14.8±1.4	1.2±0.2	$r = 0.96$ $p < 0.001$	28.6±1.6	67.3±7.8	$r = 0.98$ $p < 0.001$
Significance as estimated by Wilcoxon's test: (A) versus (B) (A) versus (C)	 $p < 0.01$ NS	 $p < 0.01$ NS		 $p < 0.05$ $p < 0.01$	 $p < 0.05$ $p < 0.01$	

DISCUSSION

The content of IRI substance(s) in the rat neurointermediate lobe seems to be quite high: the wet weight of the rat neurointermediate lobe being about 1 mg, as much as 18.7 mU (equivalent to 748 ng or 120 pmol of insulin) per 1 g fresh tissue is to be assumed, on the average, for IRI content in this structure. Such a concentration is considerably higher when compared with the respective value found in the brain: for the hypothalamic block, 194 μU/g has been found in this experiment. The latter result seems to be similar to some findings reported from other laboratories (6).

The present findings don't allow to distinguish the possible involvement and utilization of hypothalamo-neurohypophysial IRI in the modulation of vasopressinergic and/or oxytocinergic neurones from possible changes in local IRI content as brought about by other mechanisms set in motion under conditions of present experiment. Yet, contrary to the hypothalamus — which is known to embrace a number of various regulatory events, some of them

(e.g., regulation of feeding behaviour) being thought to affect the local IRI substance(s) — in the neurohypohysis storage and release of vasopressin, oxytocin and neurophysins seem to complete the list of local functional tasks. For the estimation of possible relationship between IRI and vasopressinergic as well as oxytocinergic neurones, the neurohypohysial IRI seems therefore to be more informative than the hypothalamic one.

If neurohypophysial IRI is co-secreted with vasopressin and/or oxytocin or if it is locally utilized during hormonal release, its regional content shall decrease under conditions which stimulate the hormonal secretion. On the contrary, if the functional task for neurohypophysial IRI is to favour the inhibition of hormonal discharge, IRI ressources would not be used under dehydration or haemorrhage, i.e., its local content shall then either not change or even increase. Indeed, the latter instance has been found in the experiment here reported. In this respect, it may be interjected that the increase in plasma vasopressin on dehydration (7) or after haemorrhage (8) has been shown to attenuate markedly in animals injected i.c.v. with insulin. Insulin diminished vasopressin and oxytocin release from the neurointermediate lobe in vitro (9). Thus, present findings seem to support the view that insulin and/or insulin-like substance(s), available in place, are involved in some regulatory events related to vasopressin and/or oxytocin release, also at the neurohypophysial level. In the neurohypophysis, IRI seems to favour the inhibition of hormonal release. Neuromodulation appears to be the most likely mechanism for the phenomena in question. As far as we know, however, there is no evidence to date whether IRI-substances(s) are localized, in the hypothalamus, within the same (i.e., vasopressinergic or oxytocinergic perikaryons) or different (e.g., some hypothalamic regulatory interneurones) cells as vasopressin or oxytocin. To our knowledge, there are also no data as to the structural and/or functional relation of IRI to the hypothalamo-neurohypophysial fibers and endings at the neurohypophysial level.

Acknowledgments. The authors wish to thank Professor Marek Pawlikowski, Head of the Institute of Endocrinology, School of Medicine, Lodz, for kind hospitality in his laboratory. We are also indebted to Dr. Hanna Pisarek for her assistance in the RIA estimations.

REFERENCES

1. Baskin DG, Porte D Jr., Guest K. Dorsa MD. Regional concentrations of insulin in the rat brain. *Endocrinology* 1983; 112: 898-903.
2. Underhill LH, Rosenzweig JL., Roth J, Brownstein MJ. Scott Young W, Havrankova J. Insulin and insulin receptors in the nervous system of mammals. *Front Horm Res* 1982; 10: 96-110.

3. Corp ES, Woods SC, Porte D Jr., Dorsa DM, Figlewicz DP, Baskin DG. Localization of ^{125}I-insulin binding sites in the rat hypothalamus by quantitative autoradiography. *Neurosci Lett* 1986; 70: 17-22.
4. Lowry OH, Rosebrough NJ, Farr AL. Randall RJ. Protein measurement with the Folin phenol reagent. *J biol Chem* 1951; 193: 265-275.
5. Diem K, Lentner, C. Wissenschaftliche Tabellen. 7 Auflage, Ciba — Geigy Ag, Basle 1971; 193.
6. Havrankova J, Schmechel D, Roth J, Brownstein M. Identification of insulin in the rat brain. *Proc Natl Acad Sci* 1978; 75: 5735-5741.
7. Forsling ML, Stempniak B, Guzek JW. Insulin and neurohypophysial function in the euhydrated and dehydrated rat. *J Endocrinol* 1991; Suppl 129: A302.
8. Forsling ML, Stempniak B, Guzek JW. The effect of intracerebroventricular administration of insulin on the neurohypophysial response to haemorrhage in the anaesthetized rat. *J Physiol* 1992; 446: 272P.
9. Guzek JW, Lewandowska A, Stempniak B. Insulin and release of neurohypophysial hormones: in vivo and in vitro studies. *Exp Clin Endocrinol* 1988; 92: 43-50.

Received: September 24, 1992
Accepted: February 2, 1993

Author's address: B. Stempniak, Department of Pathophysiology, School of Medicine, 90-136 Łódź, ul. Narutowicza 60, Poland.