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ORIGINAL ARTICLE

Oxytocin-containing neurons in the hypothalamic parvicellular paraventricular nucleus of the jerboa: No plasticity related to acute immobilization

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Abstract

OBJECTIVES AND METHODS: The presence of oxytocin (OT) and its putative participation to the phenotypic plasticity of CRH neurones in the stressed jerboa was investigated. We analysed by immunocytochemistry the OT expression within the hypothalamic parvicellular paraventricular nucleus (pPVN) of male jerboas submitted to an acute immobilization (30 min).

RESULTS: OT presence was clearly demonstrated in the pPVN of the jerboa and no significant difference in the number of OT immunolabeled cells was observed whatever the experimental conditions. Interestingly, CRH-immunoreactive neurons coexpressed OT within cell bodies and terminals in a similar way both in control and stressed animals. The level of coexpression was regionally heterogeneous and was not sensitive to the stress immobilization.

CONCLUSION: The present data reveal for the first time the occurrence of OT in hypothalamic pPVN neurons of the jerboa. Moreover, this OT expression level does not change upon an acute immobilization stress. These new data, coupled together with our previous work in the jerboa, incontestably establish a clear dichotomy between a stress-responsive CRH/CCK system and a stress non-responsive OT/VP system in the pPVN.

INTRODUCTION

The rat hypothalamic response to an environmental stress implies the corticotropin releasing hormone (CRH) neuroendocrine system of the hypothalamic parvicellular paraventricular nucleus (pPVN). The accessory neuropeptides, such as vasopressin (VP), neurotensin and cholecystokinin (CCK), probably subserve a putative complementary function to CRH in the regulation of the pituitary (Swanson *et al.* 1986; Ceccatelli *et al.* 1989). Unlike the rat, our previous data showed that jerboa CCK is not coexpressed within CRH neu-

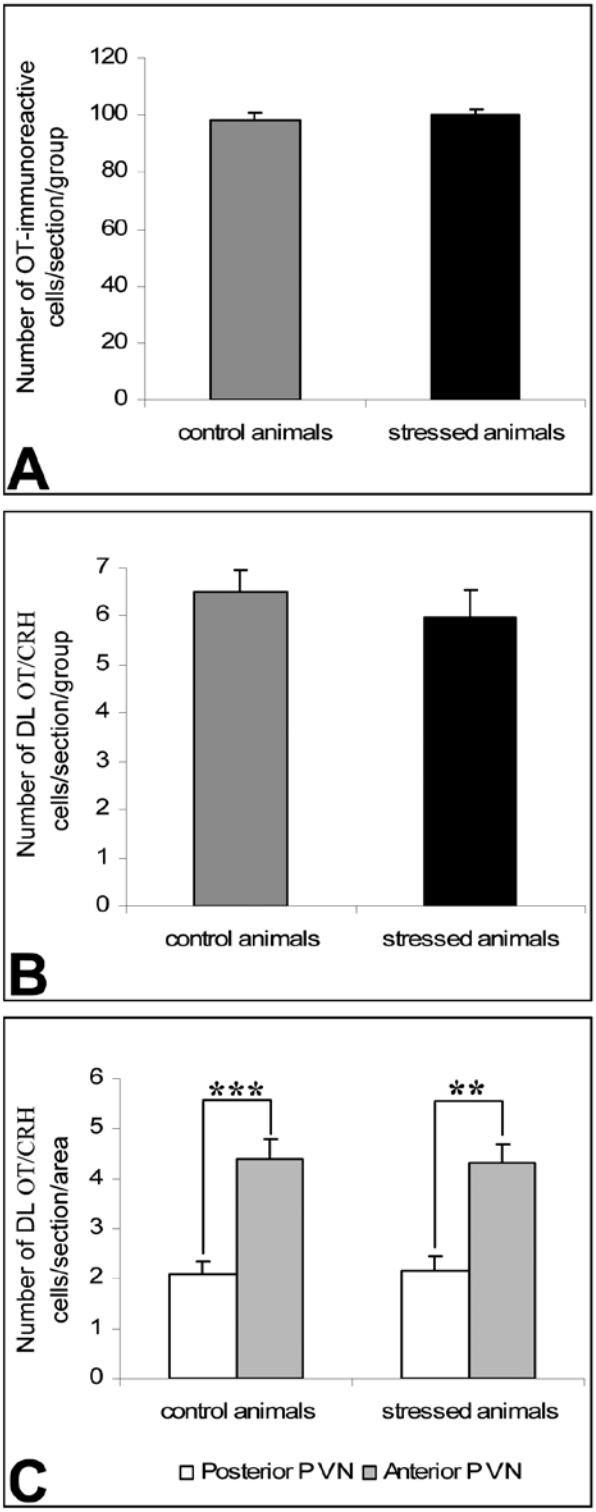

Figure 1. A) Comparison of the number of OT-ir neurons within the jerboa pPVN according to experimental groups (CC versus SC; n=5 in both groups). Values were quoted as means ± SEM. Student t-test revealed no significant change in the average number of OT-ir cells ($p<0.05$). B) Comparison between experimental groups of the number of OT/CRH double labelled (DL) cells in the pPVN. The histogram showed no significant difference in the number of DL cells between CC and SC groups ($p<0.05$). C) Comparison of the number of OT/CRH DL cells within the anterior and posterior regions of pPVN between control and stressed jerboas. Significant differences are plotted by ** for $p<0.01$ and *** for $p<0.001$. DL cells are not distributed in a homogeneous way throughout the rostrocaudal extent of the pPVN.

rons, although immobilization stress stimulates both CCK and CRH expressions in jerboas (Barakat *et al.* 2006a). Stress response regulatory mechanisms involving the pPVN CRH and CCK populations are different between jerboa and rat (Barakat *et al.* 2006a). The extreme complexity of the site has been unfolded in jerboas with a CRH/VP coexpression within cell bodies and terminals not correlated with an increase in VP-immunoreactivity following the acute immobilization stress (Barakat *et al.* 2006b). Such VP coexpression did not fluctuate following either an immobilization stress in jerboa (Barakat *et al.* 2006b) or an acute immune challenge in rat (Paulmyer-Lacroix *et al.* 1995).

About oxytocin (OT) as an additional accessory neuropeptide, previous data showed that OT mRNA synthesis can rapidly increase within pPVN following an osmotic stress known to activate the corticotrope axis (Giovannelli *et al.* 1992). The present study was aimed at investigating an eventual OT participation to the phenotypic plasticity of CRH containing neurons using the captive jerboa as an original model. The jerboa (*Jaculus orientalis*) is a hibernating nocturnal desert rodent which lives in an environment characterized by very large variations of external temperature, water scarcity and irregular food supply (Kirmiz, 1962).

A possible OT participation to the phenotypic plasticity of CRH neurons was uncovered by measuring the OT intraneuronal expression within the pPVN of jerboas submitted to an acute immobilization stress and using a double immunocytochemistry procedure to demonstrate the OT/CRH coexpression within the PVN as well as the median eminence (ME, site of CRF and OT corelease).

MATERIALS AND METHODS

Male and female jerboas (*Jaculus orientalis*) captured in the desert of the Ifkern-Boulemane region (Morocco) were housed in the animal facilities at room temperature and under natural photoperiod. The diet was supplied *ad libitum* for at least four weeks. The experiments were performed during the sexually active period (spring-summer). Two groups of adult male jerboas (130 to 170g) were sacrificed 24 hours after injection of 100 µg of colchicine into the lateral ventricle (Barakat *et al.* 2006a; Barakat *et al.* 2006b). As previously described, a control group (CC, n=5) and a group exposed to acute restraint stress for 30 min duration (SC, n=5) were carried out (Barakat *et al.* 2006a). Sacrifice occurred between 10-12H when animals are known to be less active (El Ouezzani *et al.* 1999). Animal manipulations were performed according to the recommendations of the Local Ethical Committee whose approval is in accordance with international guidelines.

Brain tissue was processed as previously described (Barakat *et al.* 2006b). The animals were anesthetized with an intraperitoneal injection of sodium pentobar-

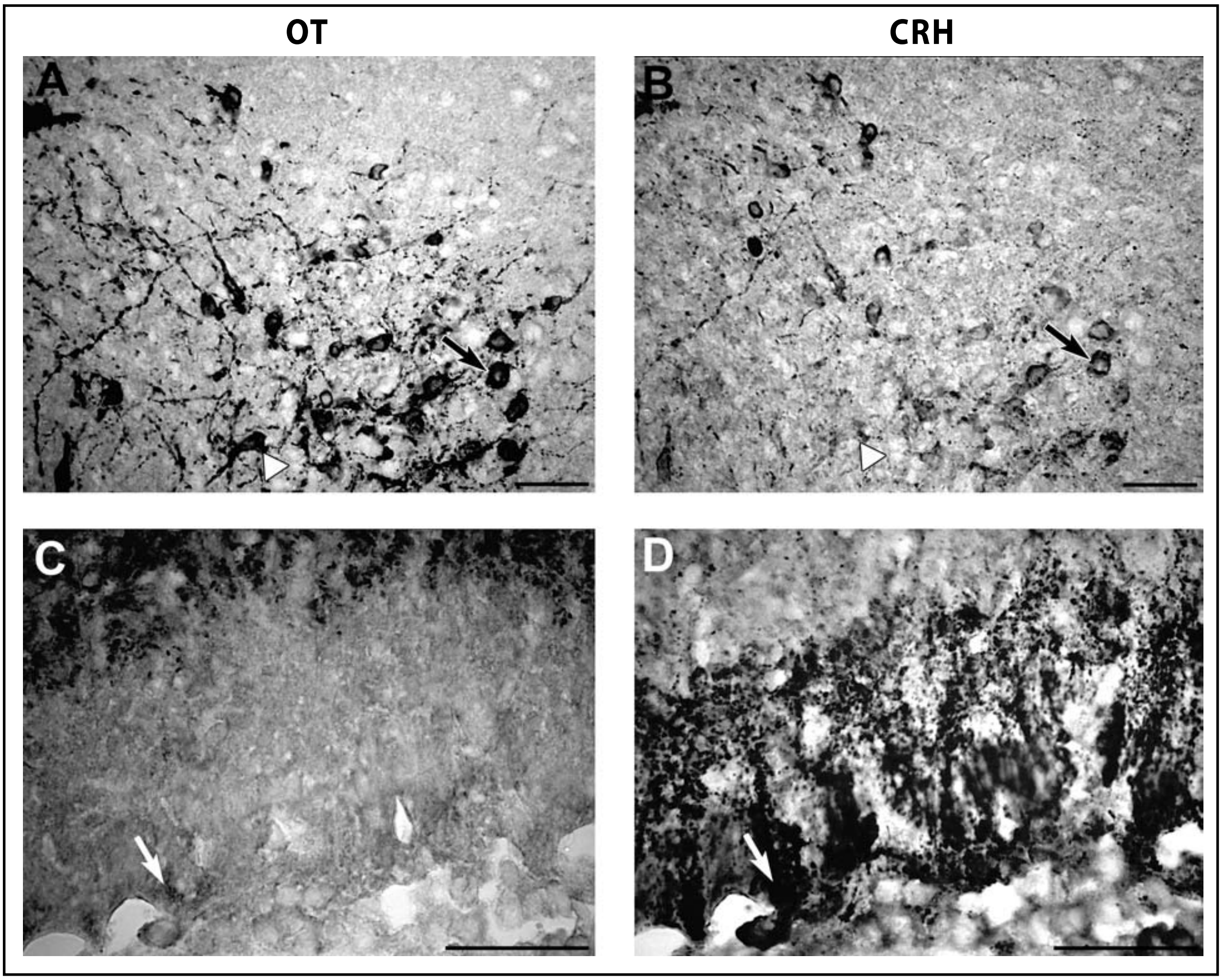

Figure 2. OT and CRH double immunohistochemistry on frontal sections of control jerboas (CC group) using the elution procedure. Efficiency of the erasing (white arrowheads) of the former OT labelling was validated (A, B). OT and CRH coexpression was detectable in neurons of the pPVN (black arrows; A, B) as well as in terminals of the external layer of ME (white arrows; C, D). Scale bars represent 50 µm.

bital (35 mg/kg) and perfused through the aorta with 50 ml saline, followed by 300 ml of a fixative solution containing 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.4). The brains were dissected out, postfixed in the same fixative for additional 24 h, and then immersed overnight in sodium Veronal buffer (pH 7.4, 0.1 M) containing 20% sucrose. The brains were embedded in Tissue-Tek and frozen in isopentane previously cooled at –60°C. Frontal sections (14 µm) were obtained with a cryostat, collected on gelatin-coated slides, and then dried at ambient temperature. Frontal sections including the pPVN were incubated overnight at room temperature in primary antisera (diluted 1:300 in CB-Triton) directed against OT [16083, tebu-bio] and CRH (Barakat *et al.* 2006a) synthetic peptides. In the end, peroxidatic activity was revealed by the 4-chloro-1-naphtol (Tramu *et al.* 1978).

Photomicrographs related to OT-immunoreactivity (ir) were taken and generated pictures digitized using a Leica DC 300 camera and ID50 software. To explore OT/CRH intraneuronal coexistence, we implemented a double immunohistochemistry using the elution procedure (Tramu *et al.* 1978). Briefly, the slides were immersed in acetone, distilled water, a vol/vol mixture composed of 0.025% KMnO4 and 0.05% H2SO4 and finally 0.1% Na2S2O5. Sections were then processed for CRH immunoreactivity using the same protocol (see above).

Labelled sections count was performed by direct microscope observation in a blind fashion. Neuroanatomical identification of hypothalamic structures was based on the Atlas of the Rat Brain (Paxinos & Watson, 1986) and on comparative data between rats and jerboas (El Ouezzani *et al.* 2000). Quantification of OT-ir cell bodies within the pPVN was performed bilaterally on four sections per animal and an average value was calculated for each animal. Comparisons of the number of OT-ir cells or double labelled cells OT/CRH according to the experimental group or to the pPVN subregion was tested by analysis of the variance homogeneity. Statistical significance was determined post-hoc by using the

Student *t*-test. The level of significance α was set to a maximum of 5%.

RESULTS

The topographical distribution of OT neurons of the hypothalamic pPVN in control jerboas is very similar to the one previously described for VP-ir cells (Barakat *et al.* 2006b). A robust density of OT-ir cell bodies occupies the parvicellular subdivisions of the PVN in addition to the well described magnocellular part. Most of the OT neurons are concentrated within the medial nuclei of the pPVN along its rostrocaudal extent. In a dorsoventral direction, oval-shaped OT neurons are located medially with the magnocellular part as a natural dorsolateral border. In spite of various experimental conditions (CC *vs* SC group), the pattern of distribution of OT-ir cells in the pPVN was unchanged.

The quantitative analysis of the effect of an immobilization stress on the expression of OT-ir in the pPVN demonstrated that the number of OT-ir neurons was similar between stressed and control animals (t=0.47, 98.18 ± 2.76 *vs* 99.86 ± 2.11, $p<0.05$; Figure 1A).

To evaluate the OT/CRH co-expression in the pPVN and the ME, the elution procedure reliability was validated by demonstrating on the same section the erasing efficiency of the former labeling before the latter labeling (Figures 2A, B; see white arrowhead). Numerous OT-ir neurons appeared immunoreactive for CRH (Figure 2A, B; see black arrow) and no significant difference in the number of double labeled cells was measured whatever the experimental group considered (t=0.72, CC 6.5 ± 0.45 *vs* SC 5.98 ± 0.57, $p<0.05$; Figure 1B). According to this result, there was an obvious matching area of distribution between OT and CRH neuropeptide. The number of OT/CRH coexpressing neurons displayed a decreasing gradient in the anteroposterior direction whatever the experimental group (CC: t=5.11, 4.4 ± 0.38 *vs* 2.1 ± 0.25, $p<0.001$; SC: t=4.93, 4.33 ± 0.34 *vs* 2.16 ± 0.28; Figure 1C) and that peculiar distribution in one subregion was not affected by immobilization stress (Ant: t=0.14, 4.4 ± 0.38 *vs* 4.33 ± 0.34; Post: t=0.16, 2.1 ± 0.25 *vs* 2.16 ± 0.28; Figure 1C).

The median eminence, site of neurohormones release, exhibited in its internal layer a very high density of OT-ir fibers coming from the magnocellular PVN (Figure 2C). The external zone of the ME expressed few OT-ir fibers with a privileged distribution around capillaries (Figure 2C). In that layer, we clearly evidenced that OT was co-expressed within CRH-containing terminals of control (Figures 2C, D) as well as stressed jerboas (*data not schown*).

DISCUSSION

The present study has shown that the pPVN of the jerboa displays an important OT-immunopositive neuronal population. Such hypothalamic site is known to play a critical role in the neuroendocrine control of the stress response (Paulmyer-Lacroix *et al.* 1995). The pattern of distribution of OT-ir cells in the pPVN well correlates the data obtained in rat (Sawchenko & Swanson, 1982). OT-containing endings occurred mainly within the internal zone of the ME although a less dense plexus of OT-ir fibers was visible in the external zone. Consequently, the pattern of distribution of OT-containing nerve endings in the jerboa ME is similar to the one of rat (Villar *et al.* 1994). Such pattern suggests a probable hypophysiotropic effect of OT in jerboa.

The acute immobilization is one form of neurogenic stressors which activates the hypothalamo-pituitary-adrenal axis. It constitutes a stressful stimulus inducing a parvicellular CRH mRNA expression increase and ACTH hypersecretion followed by hypercorticosteronemia (Wong *et al.* 2000). The present work in jerboa showed that 30 min immobilization did not affect the number of OT-immunoreactive neurons of the pPVN, as it is also the case for VP (Barakat *et al.* 2006b). However, the same stimulus provoked a robust increase in the number of CRH- and CCK-containing neurons within this nucleus, indicating a CCK neuroendocrine plasticity involved in the regulation of stress response (Barakat *et al.* 2006a). Like VP and unlike CRH and CCK, parvicellular OT expression does not depend upon an acute immobilization stress although it is the case for an osmotic stress in rat (Giovannelli *et al.* 1992). Nevertheless, previous data indicate that 2 hours acute immobilization stress was sufficient to up-regulate OT in mouse (Pirnik & Kiss, 2005). Consequently, we can not exclude that the stressor stimulus duration plays a critical role in the control of the phenotypical plasticity of pPVN neurons. This well correlates the fact that adaptative physiological mechanisms to environmental conditions might vary from one mammal species to another.

To go further, we have investigated both the presence and level of OT/CRH coexpression in the pPVN. The idea was to rule on the possible recruitment of parvicellular OT neurons in the stress-induced CRH neo-synthesis (Barakat *et al.* 2006a). Although OT-containing neurons coexpress CRH within cell bodies and terminals of the external layer of the ME, we clearly demonstrated that the number of DL OT/CRH neurons was stable between control and stressed jerboas eliminating a possible contribution of OT neurons in CRH expression. The possibility of a slight effect of stress on the parvicellular OT system was definitely discarded after the distribution analysis of DL cells which showed a clear antero-posterior gradient in both control and stressed jerboas. Immobilization stress had no impact on the number and spreading of DL OT/CRH cells suggesting a stress non-responsive eventual auto/para or endocrine role for parvicellular OT. Our results provide an additional evidence for the complexity of the phenotypical plasticity of neurons within the pPVN (Pirnik & Kiss, 2005).

In conclusion, the present data first reveals the occurrence of OT in hypothalamic parvicellular PVN neurons of the jerboa. The OT expression level does not change upon an acute immobilization stress. These data coupled with our previous works uncontestably establish a clear dichotomy in the PVN between a stress dependant CRH/CCK system and a stress non-dependant OT/VP system. This highlights differences between jerboa and rat in the neuroendocrine regulatory mechanisms of the stress response.

REFERENCES

1 Swanson LW, Sawchenko PE, Lind RW (1986). Regulation of multiple peptides in CRF parvocellular neurosecretory neurons: Implications for the stress response. *Prog Brain Res*. **68**: 169–190.

2 Ceccatelli S, Eriksson M, Hökfelt T (1989). Distribution and coexistence of corticotropin-releasing factor-, neurotensin-, enkephalin-, cholecystokinin-, galanin- and vasoactive intestinal polypeptide / peptide histidine isoleucine-like peptides in the parvocellular part of the paraventricular nucleus. *Neuroendocrinology*. **49**: 309–323.

3 Barakat Y, Pape JR, Boutahricht M, El Ouezzani S, Alaoui A, Chaigniau M, et al (2006a). Immunocytochemical detection of CCK and CRH neuropeptides in the hypothalamic paraventricular nucleus of the jerboa (Jaculus orientalis): Modulation by immobilization stress. *J Neuroendocrinology*. **18**: 767–775.

4 Barakat Y, Pape JR, Boutahricht M, El Ouezzani S, Alaoui A, Chaigniau M, et al (2006b). Vasopressin-containing neurons of the hypothalamic parvocellular paraventricular nucleus of the jerboa: Plasticity related to immobilization stress. *Neuroendocrinology*. **84**: 396–404.

5 Paulmyer-Lacroix O, Guillaume V, Anglade G, Grino M, Oliver C (1995). Régulation de la fonction corticotrope dans les situations de stress. *Ann Endocrinol.*. **56**: 245–251.

6 Giovannelli L, Shiromani PJ, Jirikowski GF, Bloom FE (1992). Expression of c-fos protein by immunohistochemically identified oxytocin neurons in the rat hypothalamus upon osmotic stimulation. *Brain Res*. **1**: 41–8.

7 Kirmiz JP (1962). *Adaptation to desert environment. A study on the Jerboa, Rat and Man*. London: *Butterworths*. 14–31.

8 El Ouezzani S, Tramu G, Magoul R (1999). Neuronal activity in the mediobasal hypothalamus of jerboa in hibernation. *Neurosci Lett*. **260**: 13–16.

9 Tramu G, Pillez A, Leonardelli J (1978). An efficient method of antibody elution for the successive or simultaneous location of two antigens by immunocytochemistry. *J Histochem Cytochem*. **26**: 322–324.

10 Paxinos G and Watson C (1986). *The rat brain in stereotaxic coordinates*. San Diego: Academic.

11 El Ouezzani S, Tramu G, Magoul R (2000). GnRH neurosecretory system and its seasonal variations in jerboa brain. *J Neuroendocrinol.* **12**: 1205–1212.

12 Sawchenko PE, Swanson LW (1982). Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. *J Comp Neurol*. **205**: 260–272.

13 Villar MJ, Meister B, Hokfelt T (1994). Reorganization of neural peptidergic systems in the median eminence after hypophysectomy. *J Neurosci*. **10**: 5996–6012.

14 Wong YN, Cassano WJ Jr, D'mello AP (2000). Acute-stress-induced facilitation of the hypothalamo-pituitary-adrenal axis. *Neuroendocrinology*. **6**: 345–365.

15 Pirnik Z, Kiss A (2005). Fos expression variances in mouse hypothalamus upon physical and osmotic stimuli: co-staining with vasopressin, oxytocin, and tyrosine hydroxylase. *Brain Res Bull*. **5**: 423–31.