Lesion of Central Part of the Dorsomedial Nucleus Alters Vasopressin but not Corticotropin Releasing Hormone mRNA Levels in Rat Hypothalamic Paraventricular Nucleus

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Abstract. Functional significance of neural projections from the hypothalamic dorsomedial nucleus (DMN) to the paraventricular nucleus (PVN) was investigated using surgical lesion of the central part of the DMN. Under basal conditions, DMN lesion resulted in a decrease in magnocellular vasopressin (AVP) mRNA levels in the PVN, rise in pituitary proopiomelancortin (POMC) mRNA concentrations and elevated plasma corticosterone levels. Corticotropin-releasing hormone (CRH) mRNA levels remained unaffected. In sham operated animals, osmotic stress induced by hypertonic saline injection failed to modify AVP mRNA, but increased CRH and POMC mRNA levels and peripheral hormone release. The rise in CRH mRNA levels after osmotic stress was potentiated in DMN lesioned animals. Thus, the DMN participates in the control of hypothalamic peptide gene expression and pituitary adrenocorticotropic function.

Key words: Hypothalamic dorsomedial nucleus — Hypothalamic paraventricular nucleus — Vasopressin mRNA — CRH mRNA — POMC mRNA — Osmotic stress — Lesions — Rat

Introduction

The dorsomedial nucleus (DMN) acts as one of the hypothalamic integrative centers implicated in a wide spectrum of biological actions (Freeman and Banks 1980; Byrd and Bellinger 1989; Bernardis and Bellinger 1990, 1998). It has been also identified as a component of autonomic circuitries regulating cardiovascular responses (Goren et al. 1997; Soltis et al. 1998) associated with emotional arousal and behavioral changes associated with aging (Bernardis and Davis 1996).

DMN provides rich neuronal inputs to hypothalamic paraventricular nucleus (PVN) (Thompson et al. 1996), which is the principal area of the stress response

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(Aguilera 1994). Functional importance of these projections has not been clarified yet. However, their presence in close vicinity of corticotropin-releasing hormone (CRH) and vasopressin (AVP) producing neurons (Day et al. 1999) might suggest their involvement in the modulation of the CRH hypophysiotrophic (Aguilera 1998) and AVP magnocellular neurosecretory systems. To verify this suggestion the activity of CRH and AVP neurons was studied in animals bearing DMN lesion. The most densely packed DMN subdivision, the central one, was selected for the study. Basal activity of CRH and AVP neurons and their responsiveness to intraperitoneal (i.p.) injection of hypertonic saline (HS) were assessed by changes in CRH and AVP mRNA levels evaluated by *in situ* hybridization histochemistry. In addition, pituitary POMC mRNA expression and plasma ACTH and corticosterone levels were analysed.

Materials and Methods

Animals and in vivo procedures

Adult male Sprague-Dawley rats (300–350 g) (Charles River Wiga, Snilzfeld, Germany) were used, housed three or four *per* cage under standard conditions of lighting (6.00–18.00 h) and temperature (23 °C) with free access to food and water. The animals were divided into sham operated and DMN lesioned groups. Each group contained one control and two hypertonic saline (HS) injected subgroups sacrificed 30 min or 4 h after the intraperitoneal (i.p.) injection of 5 ml of 1.5 mol/l NaCl. Experimental protocol has been approved by the Ethical Committee of the Institute of Experimental Endocrinology.

DMN lesion

Rats were anesthetized with sodium pentobarbital (50 $mg \cdot kg^{-1}$) and fixed in a Kopf stereotaxic apparatus with a 5° of nose-down position. DMN lesion was performed by help of a specially designed wire knife placed 2.7 mm caudal to Bregma. The knife was lowered 10.25 mm deep and rotated 2–3 times to produce a round lesion (0.8 1.0 mm in diameter) in the central portion of the DMN (Palkovits and Brownstein 1988). In sham operated animals the knife was only lowered up to the hypothalamic level without making any rotatory movement. Next 12 days the animals were kept in their home cages to achieve postoperation recovery. The completeness of the lesions was evaluated by light microscopic examination of 20 μm thick thionin stained coronal sections over the whole hypothalamus. Only rats with appropriate lesions and undamaged PVN area were accepted.

In situ hybridization

After decapitation the brains were removed and kept frozen at −70 °C until processed. Then 12 μm thick sections were cut on the cryostat and thaw-mounted onto poly-L-lysine-coated slides. The hybridization was performed using [^{35}S]deoxy-ATP labeled (1200 Ci $mmol^{-1}$; NEN, DuPont, Boston, MA, USA) 48-mer oligonucleotides by terminal deoxynucleotidyl transferase (Boehringer Mannheim GmbH

Wien, Austria) with a specific activity 12×10^6 cpm·pmol^{-1}. The probes were complementary to the bases corresponding to amino acids 22–37 of rat/human pro-CRH, 16 carboxyterminal aminoacids of rat AVP-neurophysin, and 102–117 of rat POMC (a gift from Dr. G. Aguilera, USA), synthesized by Synthecell (Rockville, MD, USA). The procedure was performed essentially as previously described (Škultétyová et al. 1998). The hybridized brain sections were exposed to Hyperfilm-β max (Amersham, Piscataway, NJ, USA) and the autoradiographic hybridization signals were quantified using a computerized image analysis system (Imaging Research, Inc., St. Catherines, Ontario, Canada). The comparison between the groups was performed after subtracting the background signal from the values of at least 6 sections *per* animal at a minimum of 5 rats *per* group.

Hormone measurements

Plasma ACTH was analyzed by a radioimmunoassay as described previously (Ježová et al. 1987). The specific antibody was kindly provided by G. B. Makara (Budapest, Hungary). Corticosterone was measured in dichloromethane extracts of plasma (10 μl) by a radioimmunoassay according to the previously described procedure (Ježová et al. 1994). Plasma osmolality was measured by cryoscopy (Osmomat 030, Germany).

Statistical analysis

Data have been statistically evaluated by two way analysis of variance (ANOVA) followed by post-hoc Tukey test (calculations were made using Jandel SigmaStat statistical software) or unpaired Student's t-test.

Results

Surgical ablation of the central portion of the DMN resulted in a significant reduction of the body weight in comparison with sham-operated controls (328 ± 5.4 g and 299 ± 3.5 g in control and lesioned animals, respectively) (Student's t-test) as measured 12 days after the surgery.

Lesion of the DMN had no impact on basal levels of CRH mRNA in the parvicellular subdivision of the PVN (Fig. 1). Two way ANOVA revealed a significant rise of CRH mRNA levels in response to osmotic stress ($F = 33.5$, $p < 0.001$) 4 h after a single injection of hypertonic saline. As revealed further by Tukey test, this rise was significantly potentiated ($p < 0.05$) in animals with lesion of the central portion of the DMN (Fig. 1).

Lesion of the DMN induced a significant decrease in basal AVP gene expression in magnocellular neurons of the PVN (Fig. 1). The difference in AVP mRNA levels between sham-operated controls and lesioned animals was statistically significant ($F = 14.0$, $p < 0.001$). HS injection significantly increased plasma osmolality in both sham and DMN lesioned animals 30 min and 4 h after the injection (Tab. 1). However, AVP mRNA levels in the PVN of lesioned animals were not influenced by HS administration (Fig. 1).

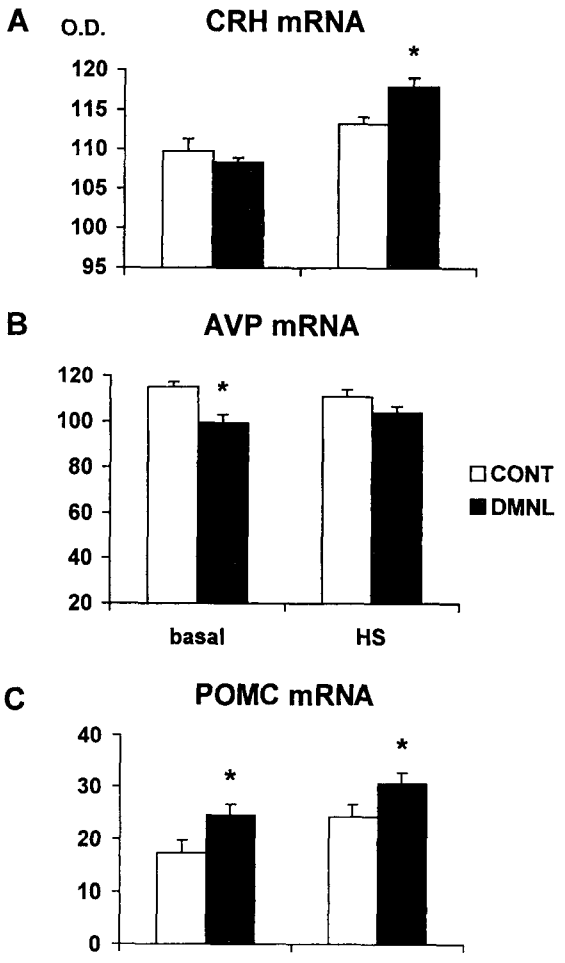

Figure 1. Gene expression of peptides in rats with central lesion of the DMN Levels of mRNAs were determined by *in situ* hybridization in parvocellular part of the PVN (CRH), **A.** magnocellular part of the PVN (AVP), **B.** and the anterior pituitary (POMC), **C.** 4 h after HS injection (O D , optic density, CONT, sham-operated controls, DMNL, lesioned DMN, HS, hypertonic saline injected rats) Results are displayed as mean ± S E M and each column represents an average of 5–8 values Two way ANOVA comparison (see results) followed by Tukey test (CRH mRNA $*p <$ 0 05, HS lesion *vs* HS sham control, POMC mRNA $*p < 0$ 05, basal sham control *vs* basal lesioned and HS sham control *vs* HS lesioned, AVP mRNA $*p <$ 0 05, basal lesion *vs* basal sham control)

The changes in the anterior pituitary POMC mRNA levels showed a statistically significant difference for surgical intervention under basal conditions (sham *vs* lesion, $F = 4.8$, $p < 0.05$) and stress exposure (basal *vs* stress, $F = 4.6$, $p < 0.05$). In DMN-lesioned animals, both basal and HS-stimulated pituitary levels of POMC mRNA 30 min (not shown) as well as 4 h after osmotic stress were higher in comparison with those seen in controls (Fig. 1).

Osmotic stress resulted in a significant elevation in plasma ACTH ($F = 20.0$, $p < 0.001$) and corticosterone ($F = 47.5$, $p < 0.001$) concentrations in sham control and DMN-lesioned animals (Tab. 1). The rise in both hormone levels was statistically significant at 30 min after HS injection. Thereafter, concentrations of ACTH in both groups decreased, while those of corticosterone remained signifi-

Table 1. Changes in plasma ACTH, corticosterone and osmolality after i p injection of 5 ml of 1 5 mol/l NaCl in sham-operated control rats and rats with lesion of the central part of the DMN

		0 min	30 min	4 h
ACTH (pg/ml)	sham	215 ± 78	1823 ± 415**	360 ± 109
	lesion	396 ± 109	1983 ± 351**	621 ± 141
corticosterone (μg/100 μl)	sham	1 1 ± 0 4	30 7 ± 2 6**	28 7 ± 1 5**
	lesion	4 3 ± 1 0#	38 5 ± 4 2**	24 0 ± 4 6**
osmolality (mmol/kg)	sham	318 6 ± 2 4	352 3 ± 5 5*	342 3 ± 2 8*
	lesion	320 6 ± 2 9	359 5 ± 5 7*	338 6 ± 3 9*

** $p < 0\ 001$ (against 0 min), * $p < 0\ 01$ (against 0 min), # $p < 0\ 05$, lesion *vs* sham (unpaired Student's t-test)

cantly elevated even 4 h after HS injection ($p < 0\ 001$) No differences between the values in sham-operated and DMN lesioned animals were revealed by two way ANOVA statistical comparison However, when evaluated separately (Student's t-test) basal, unstressed plasma ACTH levels unchanged while corticosterone levels were significantly elevated ($p < 0\ 05$) in rats with DMN lesion in comparison with sham-operated controls (Tab 1)

Discussion

The present study demonstrates involvement of the DMN in the modulation of PVN functions as revealed by changes in CRH and AVP mRNA levels under basal and stress conditions

It is well recognized that the basal as well as stress-induced activity of CRH neurons is under the control of brainstem ascending projections and that the main extrahypothalamic inputs to the PVN have noradrenergic origin (Szafarczyk et al 1988, Kiss and Aguilera 1992, Kiss et al 1996, Day et al 1999, Palkovits et al 1999) Although the present experiments cannot identify chemical character of DMN projections directed to the PVN, they provide further support that not only extra- but also intra-hypothalamic circuits play an important role in the regulation of CRH neurons It has been shown that the DMN exerts topographically diverse inputs to the PVN (Cullinan et al 1996) The present data demonstrating potentiation of CRH mRNA response to HS in animals with partial lesioning of the DMN indicate that the central area of the DMN has a functional impact on CRH biosynthesis

Increased activity of CRH neurons in the PVN is known to evoke pituitary POMC mRNA expression and plasma ACTH and corticosterone release However, an increase in these parameters was found in DMN lesioned animals even under

basal conditions, without concomitant changes in CRH mRNA levels Thus, it may be suggested that mechanisms other than those affecting CRH gene expression in the PVN are involved in the modulation of pituitary and adrenocortical hormone release by the DMN The neural circuits responsible for these interactions are unclear, but GABA-ergic and serotoninergic neurons are the most likely candidates Local administration of bicuculline, the $GABA_A$ receptor antagonist, into the DMN enhanced ACTH and corticosterone release (Keim and Shekhar 1996) Another possibility is a PVN independent activation of POMC gene expression by DMN serotoninergic neurons The latter suggestion is substantiated by the presence of serotoninergic neurons in the DMN (Arezki et al 1985), serotoninergic innervation of the anterior pituitary (Shannon and Moore 1987, Carvajal et al 1991) and modulation of pituitary-adrenocortical function by serotoninergic system manipulations (Calogero et al 1995)

Magnocellular vasopressinergic perikarya provide anatomical basis of the hypothalamic-neurohypophyseal system and vasopressin release is known to be stimulated by osmotic challenges including administration of HS (Kiss and Aguilera 1993) However, the activation of magnocellular VP gene expression apparently requires a prolonged exposure to osmotic stress, while in accordance with our previous findings (Ježová et al 1995) we failed to observe any changes in VP mRNA levels in response to HS in both control and DMN lesioned animals An unexpected finding of the present study was the reduction of magnocellular AVP gene expression under basal conditions by central lesion of the DMN It should be noted that experimental situations inducing a decrease in magnocellular VP mRNA levels are rather rare Therefore, it is likely that the DMN represents a hypothalamic regulatory center participating in maintaining basal activity of AVP gene expression in the magnocellular part of the PVN

In summary, the present results show that the DMN has an impact on peptide gene expression in both parvocellular CRH and magnocellular AVP neurons The involvement of the DMN in the control of the pituitary adrenocorticotropic function seems to include also components independent of the activation of CRH gene expression in the PVN The mechanisms involved in the decrease in AVP mRNA levels in DMN lesioned animals remain to be elucidated

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