*Neuroscience* 139 (2006) 779–789

# CONTINUOUS I.C.V. INFUSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR MODIFIES HYPOTHALAMIC–PITUITARY–ADRENAL AXIS ACTIVITY, LOCOMOTOR ACTIVITY AND BODY TEMPERATURE RHYTHMS IN ADULT MALE RATS

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**Abstract—Brain-derived neurotrophic factor is a neurotrophin belonging to the nerve growth factor family, which is involved in the differentiation and survival of many types of neurons. It also participates in neuroprotection and neuronal plasticity in adult rats. Our previous studies showed that a single brain-derived neurotrophic factor injection modifies hypothalamic–pituitary–adrenal axis activity in adult male rats. To investigate the effect of chronic brain-derived neurotrophic factor administration on some physiological parameters, adult rats were implanted with osmotic micropumps to deliver brain-derived neurotrophic factor continuously for 14 days in the lateral ventricle (12 μg/day/rat). mRNA levels were evaluated by *in situ* hybridization analysis, peptide contents and plasma hormone concentrations by radioimmunoassay. Animals were also equipped with telemetric transmitters to study locomotor activity and temperature rhythms modifications, since hypothalamic–pituitary–adrenal axis is known to modulate these two parameters. Decreased body weight was used as a control of brain-derived neurotrophic factor access to hypothalamic areas as already documented.**

**In the hypothalamus the continuous brain-derived neurotrophic factor treatment increases: (i) the mRNA steady state levels of corticotropin releasing hormone and arginin-vasopressin in the paraventricular nucleus, the supraoptic nucleus, and the suprachiasmatic nucleus; (ii) the surface of corticotropin releasing hormone and arginin-vasopressin mRNA signals in these nuclei as detected by *in situ* hybridization, and (iii) the corticotropin releasing hormone and arginin-vasopressin contents. The plasma concentrations of adrenocorticotropic hormone and corticosterone were decreased and increased, respectively. Finally, this treatment increased daily locomotor activity and temperature, and provoked some circadian perturbations. These results obtained after chronic brain-derived neurotrophic factor administration extend data on the brain-derived neurotrophic factor involvement in the hypothalamic–pituitary–adrenal axis regulation and illustrate its effects on the locomotor and temperature rhythms. They also allow demonstrating that the regulation of the hypothalamic–pituitary–adrenal axis by brain-derived neurotrophic factor differs according to the brain-derived neurotrophic factor administration mode, i.e. acute injection or chronic administration. © 2006 Published by Elsevier Ltd on behalf of IBRO.**

**Key words: CRH, AVP, PVN, ACTH, corticosterone, Alzet osmotic micro-pump.**

Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth factor (NGF) family (Lewin and Barde, 1996) and its main biological effects are mediated by specific tyrosine kinase (TrkB) receptors (Bothwell, 1995). Besides their classic effects on neuronal differentiation and survival (Lindsay et al., 1994), neurotrophins, and particularly BDNF, are important molecular mediators that translate neuronal activity into biochemical and structural plasticity (Thoenen, 1995; McAllister et al., 1999). Many types of insults induce modifications in neurotrophin mRNA expression in the CNS (Lindvall et al., 1994; Tapia-Arancibia et al., 2004). Recently, we showed that immobilization stress induces a rapid increase in BDNF mRNA expression in the hippocampus, hypothalamus and pituitary (Givalois et al., 2001; Rage et al., 2002; Marmigere et al., 2003), three regions highly involved in the stress response and homeostasis maintenance. The major neuroendocrine response to stress is exerted via activation of the hypothalamic–pituitary–adrenal (HPA) axis, leading to rapid secretion of adrenocorticotropic hormone (ACTH) that triggers glucocorticoid release from adrenal glands, which are essential for stress adaptation. The paraventricular nucleus (PVN) is the crucial hypothalamic nucleus for stress responses and possesses special plasticity capacities to respond to physiological stimuli (Theodosis and Poulain, 1987; Givalois et al., 2000) and to adapt cells to meet the stress challenge (Bartanusz et al., 1993). Basically, it presents two main anatomical divisions, the parvocellular and the magnocellular regions (Whitnall, 1993) although the parvocellular region can be subdivided into different functional groups of cells. The corticotropin-releasing hormone (CRH) is the major physiological stimulus for ACTH secretion and is essentially synthesized in the parvocellular portion of the PVN. Besides, arginin-vasopressin (AVP) is also synthesized in this portion and acts synergistically with CRH potentiating its ability to induce ACTH (Whitnall, 1993). AVP is also synthesized in other regions of the hypothalamus, i.e. in the PVN magnocellular region, in the supraoptic nucleus (SON) and in the suprachiasmatic nucleus (SCN) (Kiss, 1988). AVP derived from these neurons

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*Abbreviations:* ACTH, adrenocorticotrophin; AVP, arginin-vasopressin; BDNF, brain-derived neurotrophic factor; CORT, corticosterone; CRH, corticotropin-releasing hormone; HPA axis, hypothalamic–pituitary–adrenal axis; ME, median eminence; OD, optical density; PVN, paraventricular nucleus; RIA, radioimmunoassay; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus.

0306-4522/06$30.00+0.00 © 2006 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2005.12.028

is also involved in ACTH control (Swanson, 1987), but is specifically affected by osmotic stress (Zingg et al., 1986) and involved in circadian rhythms (Buijs et al., 1998). These different hypothalamic nuclei contain high levels of BDNF and TrkB mRNA (Tapia-Arancibia et al., 2004). More recently, we have shown that immobilization stress induces a rapid increase in BDNF mRNA expression in the PVN, which precedes the activation of CRH and AVP neurons, suggesting that BDNF could be involved in the regulation of the HPA axis (Givalois et al., 2004b). Therefore, we showed that a single i.c.v. injection of BDNF modified the HPA axis activity (Givalois et al., 2004b), suggesting that BDNF could be involved in adaptative processes, which might be vital for the functioning of neurons that must support the allostatic overloading underlying the stress response.

Here, we investigate the effect of chronic BDNF administration and analyzed some of the physiological parameters eventually modified by this treatment, notably the different components of the HPA axis. For this purpose adult rats were implanted with osmotic micro-pumps that delivered continuously BDNF in the lateral ventricle during 14 days. Since the HPA axis and particularly CRH neurons are involved in the regulation of locomotor activity and body temperature (Rowsey and Klugger, 1994; Linthorst et al., 1997) and a previous report suggested the involvement of BDNF in biological rhythms (Martin-Iverson et al., 1994), we have also examined the effect of continuous BDNF treatment on the locomotor activity and body temperature rhythms.

## EXPERIMENTAL PROCEDURES

### Animals

Adult male Sprague–Dawley rats (Depré, St Doulchard, France) were housed for 1 week before experiments under constant temperature (21±1 °C) and lighting (light on from 7:00 a.m. to 7:00 p.m.) regimens. Food pellets and water were freely available throughout the experiment. Procedures involving animals and their care were conducted in conformity with French laws on laboratory animals that are in compliance with international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, 12 December 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985). The Animal Welfare Committee at the University of Montpellier 2 approved all protocols and all efforts were made to minimize the number of animals used and potential pain and distress. All experiments were performed in conscious rats (five to six in each group) between 9:00 a.m. and 2:00 p.m., i.e. during the diurnal trough of the circadian HPA axis rhythm.

### Experimental design

*Surgery.* The animals were divided into three groups: control, sham and treated. The control group included intact, untreated rats and the sham group that consisted of rats having an osmotic pump (Alzet model 2002; 200 μl; 0.5 μl/h; Charles River, France, L'Arbresle, France) filled with the vehicle (NaCl 0.9%) implanted into the lateral ventricle. The treated group was implanted with the osmotic pump filled with BDNF (1 μg/μl; kindly provided by Regeneron Pharmaceuticals Inc., Tarrytown, NY, USA). These animals received 12 μg/day of BDNF for 14 days (Siuciak et al., 1996; Pencea et al., 2001). Before surgery, each rat was intramuscularly anesthetized with a ketamine (80 mg/kg) and xylazine (10 mg/kg) mixture. For the osmotic pump implantation, animals were placed on a stereotaxic apparatus (David Kopf) to implant a cannula into the right lateral ventricle (1 mm posterior; 1.5 mm lateral; −3.5 mm height; flat skull position with bregma as reference), according to the atlas of Paxinos and Watson (1997). The cannula was sealed with dental cement and connected to an Alzet pump with medical grade vinyl tubing. The pump was placed in a s.c. pocket in the dorsal region and filled the day before surgery. The pumps and tubing were incubated at 37 °C overnight in a sterile saline solution to prime them. Animals were weighed every day since the BDNF dose was selected on the basis of previous experiments known to induce weight loss (Siuciak et al., 1996; Pencea et al., 2001).

*Locomotor activity and temperature rhythms.* Ten days before the beginning of the experiments, a single telemetric transmitter (PhysioTel, TA 10TA-F40; Data Science International, Arden Hills, MN, USA), equipped with a thermistor encapsulated in a biocompatible silicon elastomer, was implanted intraperitoneally under deep anesthesia (ketamine/xylazine). After the recovery period, the animals were individually recorded for 7 days to establish, for each rat, its own control period. The corresponding receiver (RA1010; DSI, St. Paul, MN, USA) was fixed under the animal's cage and connected via a BMC100 consolidation matrix (DSI) to a Dataquest III computerized data analyzer. Then, these animals were anesthetized and stereotaxically implanted in the lateral ventricle with an osmotic pump containing the vehicle (sham group) or the BDNF solution (BDNF group), as described. The animals were then recorded during the 14-day treatment period and telemetric data were analyzed. This system allows measurement of continuous locomotor activity and body temperature variations, as previously reported (Assenmacher et al., 1995). On the basis of integrated periodograms, cosinor functions were individually calculated for each animal by the analyzer program and corresponded to the best sinusoidal fit of the experimental data series. Several rhythm parameters were calculated: (1) the number of cycles per 24 h; (2) the minimal and maximal values [calculated from sinusoid functions]; (3) the acrophase [the local time of the peak of sinusoid functions]; (4) the mesor [the average hourly calculated for each animal from sinusoid functions]; and (5) the amplitude [the difference between the values at the acrophase and the mesor]. All of these parameters allow defining the rhythmic type and constitute the essential basic and classic rhythm parameters.

*Preparation of cRNA probes.* The pGEM4 plasmid containing a 1.2 kb EcoR1 fragment of rat CRH cDNA and pSP2 VAS subclone used for specific hybridization of rat AVP were kindly provided by Dr. G. Pelletier. Plasmids were linearized with *Hind*III (Promega, Charbonniere, France) and transcribed with SP6 RNA polymerase to generate antisense cRNA. Transcription was performed using a TransProbe SP kit (Amersham, Orsay, France) and [$\alpha^{35}$S] UTP (200 μCi; NEN, Paris, France) for *in situ* hybridization, as previously reported (Givalois et al., 2000, 2001; Rage et al., 2002). Radioactive sense cRNA copies (negative control) were also prepared to verify the specificity of each probe. Hybridizations with these probes did not reveal any positive signal. Transcription was performed using a TransProbe SP kit (Amersham) and [$\alpha^{35}$S] UTP (200 μCi; NEN).

*Semi-quantitative* in situ *hybridization.* On the day of the experiment, the animals were deeply anesthetized with an i.m. injection of 0.2 ml of a mixture of ketamine hydrochloride (80 mg/kg) and xylazine (10 mg/kg) and then rapidly perfused transcardially with 4% paraformaldehyde in 0.2 M phosphate buffer. Brains were removed and postfixed in the same fixative for 4 h at 4 °C, and then placed in 15% sucrose in the 0.2 M phosphate buffer overnight at 4 °C. Thereafter, the tissues were quickly frozen in