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Research Report

Restraint stress activates nesfatin-1-immunoreactive brain nuclei in rats

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ABSTRACT

Nesfatin-1 is a newly discovered peptide that was reported to reduce food intake when injected centrally. We recently described its wide distribution in rat brain autonomic nuclei which implies potential recruitment of nesfatin-1 by stress. We investigated whether restraint, a mixed psychological and physical stressor, activates nesfatin-1-immunoreactive (ir) neurons in the rat brain. Male Sprague-Dawley rats were either subjected to 30 min restraint or left undisturbed and 90 min later brains were processed for double immunohistochemical labeling of Fos and nesfatin-1. Restraint induced significant Fos expression in neurons of the supraoptic nucleus (SON), paraventricular nucleus (PVN), locus coeruleus (LC), rostral raphe pallidus (rRPa), nucleus of the solitary tract (NTS), and ventrolateral medulla (VLM). Double Fos/nesfatin-1 labeling revealed that Fos-ir neurons comprised 95% of nesfatin-1-ir cells in the SON, 90% in the VLM, 80% in the LC, 48% in the caudal NTS, 57% in the rRPa, 48% in the anterior parvicellular PVN, 27% in the medial magnocellular PVN, 18% in the lateral magnocellular PVN and 10% in the medial parvicellular PVN. These data demonstrate that nesfatin-1 neurons are part of the hypothalamic and hindbrain neuronal cell groups activated by restraint suggesting a possible role of nesfatin-1 in the response to stress.

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1. Introduction

The recently discovered 82 amino acid peptide nesfatin-1 and its precursor nucleobindin2 (NUCB2) were first described to induce dose-dependent anorexigenic effects in rats and mice upon 3rd ventricular or intraperitoneal injection during the dark phase (Oh-I et al., 2006; Shimizu et al., 2009). In addition, we showed that nesfatin-1 acts centrally to inhibit food intake through the activation of brain corticotropin-releasing factor $(CRF)_2$-receptor (Stengel et al., 2009c). There is also a report that central injection of nesfatin-1 induces anxiety- and fear-related behaviors in the rat (Merali et al., 2008). Recent neuroanatomical studies depicted the occurrence of nesfatin-1 immunoreactivity in cell bodies of autonomic regulatory nuclei in the forebrain, hindbrain and spinal cord along with other forebrain nuclei involved in stress response and cognitive function (Foo et al., 2008; Goebel et al., 2009). Collectively, these data suggest a potential role of the peptide in stress-recruited circuitries which so far has not been explored. Emotional or psychological stressors (processive) are integrated by the brain and activate cortical limbic and pontine structures that then impact on the hypothalamus (Dayas et al., 2001b; Senba and Ueyama, 1997).

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Restraint is considered primarily an emotional/processive stress paradigm that does not induce pain or direct physical insult (Dayas et al., 1999; Herman and Cullinan, 1997). The combination of a given stressor and successive Fos immunohistochemistry in the brain is a widespread approach to visualize neuronal activation and to characterize brain areas involved in the processing of stressors (Dayas et al., 2001a; Dragunow and Faull, 1989; Sagar et al., 1988).

In the present study, we investigated whether acute exposure to restraint, known to alter gut function through central modulation of autonomic outflow (Taché and Bonaz, 2007), activates nesfatin-1 immunoreactive (ir) neurons with an emphasis on hypothalamic and hindbrain nuclei involved in autonomic regulation of visceral function (Saper, 2002; Taché et al., 1995). This was achieved using double immunohistochemical detection of the immediate early gene Fos and nesfatin-1 immunoreactivity with an antiserum raised against the nesfatin-1 fragment corresponding to rat NUCB2 amino acid residues 1–82.

2. Results

2.1. *Restraint stress activates nesfatin-1-immunoreactive neurons in hypothalamic nuclei in conscious ad libitum fed rats*

As expected, Fos immunostaining was low in the rat forebrain of undisturbed freely fed control rats (Figs. 1–3). Restraint for 30 min significantly increased the number of Fos-ir neurons/section compared to non-stressed controls in the medial parvicellular paraventricular nucleus of the hypothalamus (mpPVN, 91.7±18.8 *vs.* 1.1±0.6, $p<0.001$; Figs. 1C and 2E–F) and lateral magnocellular PVN (lmPVN, 45.2±16.0 *vs.* 0.7±0.3, $p<0.05$; Figs. 1D and 2E–F) and to a smaller extent in the medial magnocellular PVN (mmPVN, 28.9±7.7 *vs.* 0.3±0.2, $p<0.01$; Figs. 1B and 2C and D), anterior parvicellular PVN (apPVN, 13.9±1.4 *vs.* 1.5±0.7, $p<0.001$; Figs. 1A and 2A and B), and supraoptic nucleus (SON, 21.0±6.7 *vs.* 2.4±1.4, $p<0.05$; Fig. 3) as monitored 90 min after the end of the stress. Restraint did not significantly increase Fos immunoreactivity in neurons of the ventral bed nucleus of the stria terminalis (BNST) and of subcortical and cortical limbic structures such as the septum and cingulate, piriform, and entorhinal cortex and in the amygdaloid nuclei (data not shown because no double labeling was observed in these areas).

In control rats, nesfatin-1-ir neurons were prominently localized (number/section) in the lmPVN (166.3±9.1; Figs. 1D and 2E), SON (141.0±7.8; Fig. 3), mmPVN (106.3±5.5; Figs. 1B and 2C), and, to a lesser extent, in the mpPVN (36.9±7.6; Figs. 1C and 2E). Rats exposed for 30 min to restraint had similar numbers of nesfatin-1-positive neurons as the control group monitored 90 min after the end of stress (Figs. 1–3). Restraint significantly increased the number of double labeled cells in the SON (19.8±6.6 *vs.* 2.2±1.3, $p<0.05$; Fig. 3C), apPVN (6.6±0.8 *vs.* 0.0±0.0, $p<0.001$; Fig. 1A), mmPVN (7.7±2.4 *vs.* 0.0±0.0, $p<0.01$; Fig. 1B), lmPVN (8.0±2.8 *vs.* 0.5±0.3, $p<0.05$; Fig. 1D),

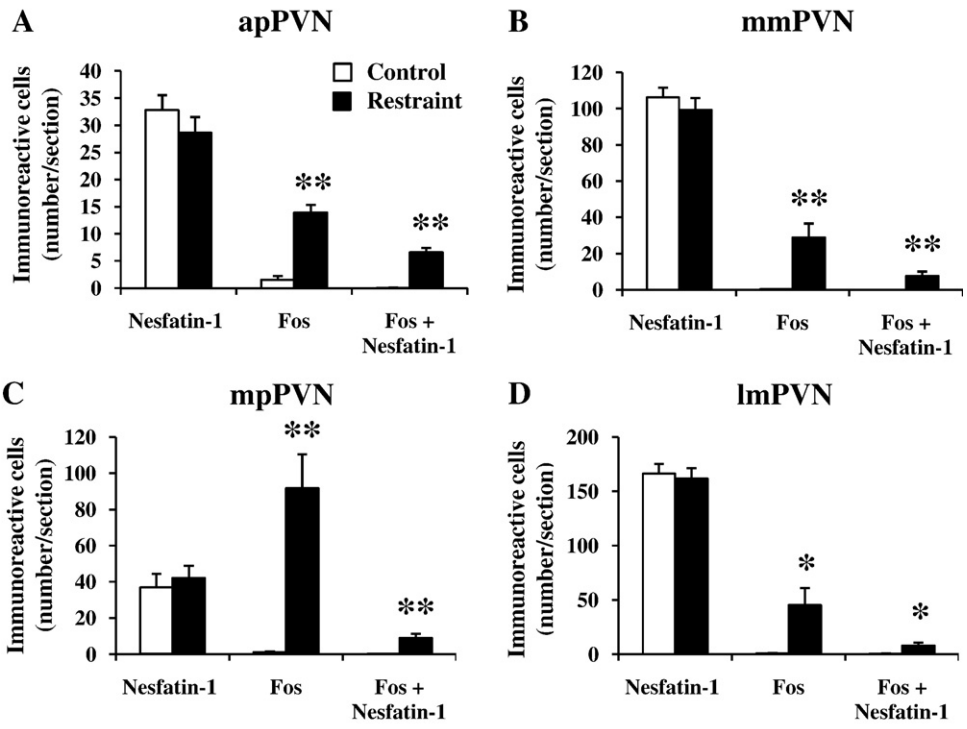

Fig. 1 – Restraint stress induced Fos expression in a small population of nesfatin-1-positive neurons in the paraventricular nucleus of the hypothalamus in conscious rats. Unilateral cell count/section in (A) the anterior parvicellular part of the paraventricular nucleus (apPVN), (B) medial magnocellular part of the PVN (mmPVN), (C) medial parvicellular part of the PVN (mpPVN) and (D) lateral magnocellular part of the PVN (lmPVN) 90 min after a 30-min restraint exposure. Data are mean ± SEM of 6 rats/group. *$p<0.05$; **$p<0.01$ compared with the respective control.