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Abdominal surgery activates nesfatin-1 immunoreactive brain nuclei in rats

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Abstract

Abdominal surgery-induced postoperative gastric ileus is well established to induce Fos expression in specific brain nuclei in rats within 2-h after surgery. However, the phenotype of activated neurons has not been thoroughly characterized. Nesfatin-1 was recently discovered in the rat hypothalamus as a new anorexigenic peptide that also inhibits gastric emptying and is widely distributed in rat brain autonomic nuclei suggesting an involvement in stress responses. Therefore, we investigated whether abdominal surgery activates nesfatin-1-immunoreactive (ir) neurons in the rat brain. Two hours after abdominal surgery with cecal palpation under short isoflurane anesthesia or anesthesia alone, rats were transcardially perfused and brains processed for double immunohistochemical labeling of Fos and nesfatin-1. Abdominal surgery, compared to anesthesia alone, induced Fos expression in neurons of the supraoptic nucleus (SON), paraventricular nucleus (PVN), locus coeruleus (LC), Edinger-Westphal nucleus (EW), rostral raphe pallidus (rRPa), nucleus of the solitary tract (NTS) and ventrolateral medulla (VLM). Double Fos/nesfatin-1 labeling showed that of the activated cells, 99% were nesfatin-1-immunoreactive in the SON, 91% in the LC, 82% in the rRPa, 74% in the EW and VLM, 71% in the anterior parvicellular PVN, 47% in the lateral magnocellular PVN, 41% in the medial magnocellular PVN, 14 % in the NTS and 9% in the medial parvicellular PVN. These data established nesfatin-1 immunoreactive neurons in specific hypothalamic and pontine nuclei as part of the neuronal response to abdominal surgery and suggest a possible implication of nesfatin-1 in the alterations of food intake and gastric transit associated with such a stressor.

Keywords

Abdominal surgery; Fos; hypothalamus; NUCB2; postoperative ileus; rat brain

1. Introduction

Abdominal surgery in animals is a model to study postoperative ileus (POI), a condition which occurs after gastrointestinal tract surgery in the clinical setting and characterized by

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the suppression of effective transit of bowel contents including delayed gastric emptying [66]. Abdominal surgery consisting of laparotomy with manipulation of the bowel [6, 62] activates endocrine markers of the stress response in experimental animals and humans [11, 40, 42]. We have previously reported that brains of rats which have undergone abdominal surgery display neuronal activation in specific nuclei known to be responsive to various stressors including the hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei as well as catecholaminergic areas of the hindbrain such as the locus coeruleus (LC), nucleus of the solitary tract (NTS) and ventrolateral medulla (VLM) [6, 7].

Nesfatin-1, the 82-amino acid cleavage product of its precursor nucleobindin2 protein (NUCB2) has recently been identified in the rat hypothalamus and shown to reduce food intake in rodents upon brain ventricle or peripheral injection [43, 53, 57]. Nesfatin-1 mRNA and immunoreactivity have been localized in various brain nuclei [9, 21, 27, 36, 43] including prominent expression in autonomic nuclei of the hypothalamus and brainstem [27]. Moreover, we recently observed that injection of nesfatin-1 into the lateral brain ventricle in addition to curtailing the feeding response during the dark phase, delays gastric emptying in rats [59]. Since postoperative gastric ileus is a well established phenomenon associated with abdominal surgery in experimental animals and humans [40, 49] this points towards a possible recruitment of nesfatin-1 neurons in response to and mediation of POI.

Therefore, in the present study, we investigated whether nesfatin-1-immunoreactive (ir) neurons in autonomic brain [27] regions are activated by abdominal surgery with cecal palpation performed in rats under short anesthesia as in our previous studies [6, 7].

2. Methods

2.1 Animals

Adult male Sprague-Dawley rats (Harlan, San Diego, CA, USA, body weight: 280–350 g) were housed 4 animals/cage under conditions of controlled illumination (12:12 h light/dark cycle, lights on/off: 6.00 h/18.00 h) and temperature (22 ± 2 °C). Animals were fed with a standard rodent diet (Prolab RMH 2500; LabDiet, PMI Nutrition, Brentwood, MO, USA) and tap water *ad libitum*. Animal care and experimental procedures followed institutional ethic guidelines and conformed to the requirements of the federal authority for animal research conduct. All procedures were approved by the Animal Research Committees at Veterans Affairs Greater Los Angeles Healthcare System (animal protocol # 05058-02).

2.2 Procedures

2.2.1 Abdominal surgery—Rats (n=6) were separated in single cages and fasted for 20 h with free access to tap water prior to the experiment. Between 9.00 h and 11.00 h, rats were exposed to isoflurane (4.5% vapor concentration in oxygen; VSS, Rockmart, GA, USA) and abdominal surgery was performed as described before [6, 7]. After a median laparotomy (2–3 cm), the cecum was exteriorized, placed in saline-soaked gauze and gently manipulated between two fingers for 1 min. Thereafter, the cecum was replaced into the abdominal cavity and the peritoneum, muscle and skin were sutured. Anesthesia and surgery lasted for approximately 10 min and animals woke up within 2–3 min after removal of isoflurane. The control group (n=6) consisted of rats undergoing exposure to anesthesia alone for 10 min. After anesthesia, animals were placed singly in cages without access to food or water until euthanasia.

2.2.2 Fos and nesfatin-1 double immunohistochemistry—Two hours after the end of isoflurane anesthesia, surgically manipulated rats and their controls (anesthesia alone) were deeply anesthetized with sodium pentobarbital (70 mg/kg, intraperitoneally, Nembutal,

Abbott Lab., Chicago, IL, USA) and transcardially perfused as described before [35]. After thoracotomy a cannula was inserted into the ascending aorta *via* the left heart ventricle. Perfusion consisted of a one minute flush with sodium chloride (0.9% NaCl) and 500 ml of fixative (4% paraformaldehyde, 14% saturated picric acid in 0.1 M phosphate buffer). Brains were removed and post-fixed overnight, then cryoprotected in 10% sucrose for 24 h and snap-frozen in dry ice-cooled 2-methylbutane. Coronal sections (25 μm) of the whole brain were cut by cryostat (Microm International GmbH, Walldorf, Germany). Every third brain section was incubated using the free floating technique. Each incubation step was followed by a 3×15 min washing step in PBS (Sigma-Aldrich, San Louis, MO). The first primary antibody was rabbit anti-c-Fos serum (1:10,000, Oncogene, Cat. No. PC#38; Cambridge, MA) overnight at 4 °C followed by the secondary biotinylated goat anti-rabbit IgG Fab fragment (1:1,000, Jackson ImmunoResearch Laboratories Inc, Cat. No. 111-067-003; West Grove, PA) for 2 h and avidin-biotin-peroxidase complex (ABC, Vector, Vermont, CA, USA) method as previously described [64]. Fos staining was visualized with 3,3′-diaminobenzidine tetrachloride (DAB) and nickel ammonium sulfate. The sections were thereafter incubated in rabbit anti-rat nesfatin-1 antibody (1:10,000, Phoenix Pharmaceuticals, Cat. No. H-003-22; Burlingame, CA, USA) at 4 °C overnight followed by 2 h incubation with biotinylated goat anti-rabbit IgG (1:1,000 Jackson ImmunoResearch, Cat. No. 111-065-144), ABC and visualization by DAB. Sections were mounted on Fisher Super Frost Plus slides, air-dried for 24 h and completely dehydrated through a gradient of ethanol and xylene before coverslipping. Cells with dark blue nuclear staining were Fos-ir and cells with strong brown cytoplasmic staining were nesfatin-1-ir.

The anti-nesfatin-1 antibody was raised in rabbit against rat nesfatin-1 corresponding to rat NUCB2 amino acid residues 1-82 (Phoenix Pharmaceuticals). To assess the specificity of nesfatin-1 immunostaining, anti-rat nesfatin-1 antibody (1ml, 1:10,000) was preabsorbed with rat nesfatin-1 peptide (10 μg, Phoenix Pharmaceuticals) as described before [58] and immunostaining performed as described above.

2.2.3 Cell counting and statistical analysis—Fos and nesfatin-1-ir cells were observed by light microscopy (Axioscop II, Carl Zeiss, Jena, Germany). For quantitative assessment, the number of immunoreactive cells was counted unilaterally in several sections of selected brain nuclei identified with the Paxinos and Watson's atlas [46] using the following coordinates (in mm from bregma): SON (−0.6 to −1.56, 12 sections), anterior parvicellular (ap)PVN (−1.08 to −1.32, 4 sections), medial parvicellular (mp)PVN, (−1.72 to −1.92, 3 sections), medial magnocellular (mm)PVN (−1.44 to −1.72, 3 sections), lateral magnocellular (lm)PVN (−1.72 to −1.92, 3 sections), LC (−9.48 to −10.08, 7 sections), Edinger-Westphal nucleus (EW) (−6.12 to −6.84, 10 sections), rostral raphe pallidus (rRPa) (−13.8 to −13.2; 8 sections), VLM, also named A1/C1 (−13.38 to −14.28, 12 sections) and NTS at the level of the area postrema (−13.68 to −14.28, 8 sections). Images were acquired by a digital camera (Hamamatsu, Bridgewater, NJ) using the image acquisition system SimplePCI (Hamamatsu Corporation, Sewickley, PA, USA).

The average number of singly or doubly labeled Fos-ir and nesfatin-1-ir cells/section derived from the total number of sections analyzed for each nucleus was determined for each animal and used to calculate the mean value/group. In dual-immunolabeling, positive cells were identified as those expressing both brown cytoplasmic reaction products and black nuclear staining. The investigator was blinded to the treatment. Data were analyzed by analysis of variance (ANOVA) followed by all pair wise multiple comparison procedures (Tukey post hoc test). Differences were considered significant when $p < 0.05$. Data are expressed as mean ± SEM.

3. Results

3.1 Abdominal surgery activates nesfatin-1-immunoreactive neurons in the forebrain

Nesfatin-1 immunoreactivity was detected in the rat supraoptic nucleus (SON, Fig. 1) and paraventricular nucleus (PVN, Fig. 2) of the hypothalamus among forebrain nuclei as previously reported [9, 21, 27, 43]. Quantitative assessment showed that the number of nesfatin-1 immunoreactive cells did not differ between control and surgically manipulated rats as monitored 2 h later ($p > 0.05$; Figs. 1C and 3). With regard to the induction of Fos protein expression, control rats exposed to brief anesthesia alone, displayed a low number of Fos-positive cells (<8 cells/nucleus) in the SON (Fig. 1C) and PVN except for the medial parvicellular subnucleus of the PVN (<17) as assessed 2 h after the end of anesthesia (Fig. 3). Abdominal surgery increased the number of Fos-ir neurons compared to control animals in the mpPVN (104.8 ± 11.7 *vs*. 16.8 ± 4.5; $p < 0.001$; Figs. 2E-F and 3B), mmPVN (77.4 ± 6.3 vs. 7.1 ± 2.2; $p < 0.001$; Figs. 2C-D and 3C), SON (62.2 ± 12.8 *vs*. 2.1 ± 0.7; $p < 0.001$; Fig. 1A-C) and to a smaller extent in the lmPVN (36.8 ± 3.3 *vs*. 7.3 ± 1.7; $p < 0.001$; Fig. 2E-F and 3D) and apPVN (20.2 ± 1.8 *vs*. 2.4 ± 0.8; $p < 0.001$; Figs. 2A-B and 3A). Abdominal surgery significantly increased the number of double labeled cells in the SON (61.7 ± 12.8 *vs*. 1.7 ± 0.6, $p < 0.001$; Fig. 1C), apPVN (14.3 ± 0.5 *vs*. 0.4 ± 0.3, $p < 0.001$; Fig. 3A), lmPVN (17.3 ± 2.0 *vs*. 1.7 ± 0.5, $p < 0.001$; Fig. 3D), mmPVN (31.9 ± 5.1 *vs*. 1.8 ± 0.8, $p < 0.001$; Fig. 3C) and in the mpPVN (9.7 ± 1.5 *vs*. 1.2 ± 0.8, $p < 0.001$; Fig. 3B) compared to controls. Of the activated neurons, 99% were nesfatin-1-ir in the SON (Fig. 1C), 71% in the apPVN (Fig. 3A), 47% in the lmPVN (Fig. 3D), 41% in the mmPVN (Fig. 3C) and 9% in the mpPVN (Fig. 3B).

2.2 Abdominal surgery activates nesfatin-1-immunoreactive neurons in the midbrain and hindbrain

Besides the hypothalamic and limbic brain nuclei specified above, nesfatin-1 immunoreactivity was also observed in nuclei of the midbrain and hindbrain, namely the EW, LC, NTS, VLM and rRPa in control rats consistent with earlier reports [9, 21, 27, 43]. The number of nesfatin-1-positive neurons did not vary 2 h after abdominal surgery ($p > 0.05$; Figs. 4C, 4F, 5C, 5F, 5I).

In fasted control rats exposed to brief isoflurane anesthesia alone the number of Fos-positive neurons was low (<6 cells/nucleus) in most hindbrain nuclei (LC, VLM, rRPa) except in the EW (Figs. 4D, 4F) and NTS (Figs. 5D, 5F) which displayed 15 Fos-positive cells/nucleus. Abdominal surgery increased the number of Fos-ir neurons in the NTS at the level of the area postrema (40.8 ± 5.6 *vs*. 14.9 ± 3.0; $p = 0.002$; Fig. 5D-F), EW (26.7 ± 2.3 *vs*. 15.0 ± 1.2; $p = 0.001$; Fig. 4D-F), LC (15.6 ± 4.4 *vs*. 2.8 ± 0.6; $p < 0.05$; Figs. 4A-C), VLM/A1/C1 (15.1 ± 1.4 *vs*. 5.2 ± 0.9; $p < 0.001$; Fig. 5G-I) and rRPa (6.1 ± 1.0 *vs*. 2.4 ± 0.7; $p < 0.05$; Fig. 5A-C) compared to controls. Abdominal surgery significantly increased the number of double labeled cells in the LC (14.2 ± 4.1 *vs*. 2.2 ± 0.5, $p < 0.05$; Fig. 4C), rRPa (5.0 ± 1.1 *vs*. 1.6 ± 0.4, $p < 0.05$; Fig. 5C), EW (19.8 ± 2.1 *vs*. 9.2 ± 1.2, $p < 0.01$; Fig. 4F), VLM (11.1 ± 2.1 *vs*. 4.0 ± 0.7, $p < 0.01$; Fig. 5I) and NTS (5.6 ± 1.0 *vs*. 1.0 ± 0.4, $p < 0.01$; Fig. 5F) compared to controls. Of these Fos-positive neurons, 91% were nesfatin-1-immunoreactive in the LC (Fig. 4C), 82% in the rRPa (Fig. 5C), 74% in the EW (Fig. 4F) and VLM (Fig. 5I) and 14 % in the NTS (Fig. 5F).

Following pre-absorption of the anti-nesfatin-1 antibody as we described before [58], no immunostaining could be detected (data not shown).

4. Discussion

Abdominal surgery consisting of laparotomy and 1-min cecal palpation performed under short isoflurane anesthesia in rats induced a distinct pattern of Fos expression in hypothalamic and brainstem nuclei as monitored in conscious rats 2 h after the end of abdominal surgery compared with controls exposed to anesthesia alone. Fos expression occurred namely in the PVN, SON, LC, VLM, and NTS as previously reported [2, 6, 67] as well in other nuclei such as EW and rRPa not previously investigated. In addition, we showed that of the activated neurons 99% in the SON, 91% in the LC, 82% in the rRPa, 74% in the EW and VLM, 71% in the apPVN, 47% in the lmPVN, 41% in the mmPVN, 14% in the NTS and 9% in the mpPVN are also nesfatin-1-ir. These data identify a novel phenotype of neurons activated by abdominal surgery and also establish nesfatin-1-immunoreactive neurons in specific brain nuclei as part of the neuroanatomical substrata activated by a visceral stressor. The specificity of the nesfatin-1 antibody was shown by the lack of nesfatin-1 immunolabeling in these brain nuclei when the antibody was pre-absorbed with synthetic nesfatin-1 peptide. It is to note, however, that the antibody also recognizes the full length NUCB2, but does not cross-react with nesfatin-2 and nesfatin-3 [9, 21]. In our previous studies, this antibody stained a 10 kDa band by Western Blot representing nesfatin-1 and also a ~50 kDa band corresponding to NUCB2, which contains the epitope of nesfatin-1 [58]. Therefore, the activated neurons are likely to reflect nesfatin-1/NUCB2 immunoreactivity consistent with the expression of the gene encoding NUCB2 that mirrors the distribution of cell bodies exhibiting nesfatin-1 immunolabeling in various brain nuclei [21, 27, 43]. We previously reported that restraint also activates nesftain-1 neurons in the rat brain [26]. Both, abdominal surgery lasting 5-10 min and 30 min restraint bear similarity in the pattern of Fos/nesfatin-1double labeled neurons and the percentage of nesfatin-1 activated neurons in specific nuclei such as the SON, mpPVN, LC and VLM. However, there are also divergences as only abdominal surgery results in a prominent activation of nesfatin-1-ir neurons in the EW, and in other nuclei (rRPa, mmPVN, apPVN and lmPVN) the percentage of nesfatin-1/Fos positive neurons was 1.7 to 2.7-fold higher after abdominal surgery than restraint [26] (present study). As in both studies brains were processed 2 hours after the initiation of the stress, the variation in percentage of Fos expression within nesfatin-1 neurons is likely to reflect a stressor-related difference and not the time at which Fos expression was monitored.

Numerous nesfatin-1-ir neurons can be found in magnocellular neurons of the hypothalamus including the magnocellular division of the PVN and SON consistent with previous reports [9, 21, 27, 36]. Abdominal surgery increased the number of Fos-positive neurons in the SON, lmPVN and mmPVN as previously established [6] and of those 99% were nesfatin-1-immunoreactive in the SON, 47% in the lmPVN and 41% in the mmPVN. Hypothalamic magnocellular neurons activated by abdominal surgery were found previously to encompass a population of arginine vasopressin neurons in the SON and in the PVN both, arginine vasopressin and more prominently oxytocin neurons [6]. Convergent reports showed a robust expression of nesfatin-1 in arginine vasopressin- and more so in oxytocin-containing neurons in the magnocellular neurons of the hypothalamus [9, 21, 36]. Taken together, these data suggest that subpopulations of nesfatin-1 neurons in the SON as well as in the lmPVN and mmPVN activated by abdominal surgery also co-express oxytocin and/or arginine vasopressin. These activated magnocellular hypothalamic nesfatin-1-oxytocin neurons may play a role to induce gastric ileus through separate or common pathways in the acute post-operative period. We showed that nesfatin-1 injected into the lateral brain ventricle delays gastric emptying in rats [57]. In addition, nesfatin-1 modulates the activity of PVN oxytocin neurons [48]. Anatomical and functional studies established that oxytocinergic neurons of the PVN project to gastric subregions of the medial part of the NTS [50] and activation of PVN neurons releases oxytocin in the dorsal vagal complex [39] leading to delayed gastric

emptying [20, 51]. Moreover, the major projections of oxytocinergic and vasopressinergic magnocellular hypothalamic neurons are to the posterior pituitary where the neuronal hormones are released into the systemic circulation during stress [33]. Nesfatin-1 was detected in the systemic circulation [57], but whether activation of nesfatin-1 neurons in the magnocellular nuclei of the hypothalamus by abdominal surgery translates into changes in circulating levels will require further investigations.

In the present study we found a similar number (~40) of nesfatin-1-labeled neurons in the anterior and medial parvicellular subnucleus of the PVN, which did not change after abdominal surgery. However, the number of activated neurons as assessed by Fos immunohistochemistry was 5-fold higher in the mpPVN compared to the apPVN. This could be, at least in part, attributed to the larger area of the mpPVN compared to the apPVN. However, a high proportion of nesfatin-1-ir neurons in the apPVN (71%) was activated by abdominal surgery whereas in the mpPVN only 9% of nesfatin-1-immunopositive neurons were Fos-ir. This divergence could be explained by the more heterogeneous pattern of neurons in the mpPVN that besides corticotropin-releasing factor (CRF) and nesfatin-1 contains neurons synthesizing thyrotropin-releasing hormone, growth hormone-releasing factor or proenkephalin which have all been shown to be activated after a stressor [63] whereas in the apPVN many neurons contain CRF [52]. Previous functional studies indicate that the PVN, in particular CRF-producing neurons in the parvicellular part [52], play a pivotal role in the induction of delayed gastric emptying occurring within 2-h post surgery [2-4, 6, 7, 40]. Recently abdominal surgery was reported to induce strong Fos activation in CRF-containing neurons of the parvicellular PVN as shown by double labeling of Fos and CRF mRNA expression in rats [28]. In addition, gastric electrical stimulation, known to improve ileus-related symptoms in patients [55], is associated with decreased activity of CRF neurons in the PVN in rats [28]. We could recently show that the food intake-reducing effect after intracerebroventricular (icv) injection of nesfatin-1 is mediated by a CRF_2 receptor-dependent pathway as shown by the complete blockade of nesfatin-1's action using the selective CRF_2 receptor antagonist astressin$_2$-B [59]. Therefore, the activation of nesfatin-1-ir neurons in the parvicellular part of the PVN may contribute to the postoperative delayed gastric emptying with CRF being involved in the downstream signaling.

The CRF system in the PVN and the hindbrain sympathetic nervous system are anatomically and functionally interrelated and regulate each other's activity. This is exemplified by the connections between the CRF-containing neurons in the PVN and catecholaminergic and serotonergic neurons in the VLM, raphe nuclei and NTS that project to the PVN and contribute excitatory input to CRF neurons [14]. We and others demonstrated the inhibitory role of splanchnic afferent C-fibers on gastric motility after abdominal surgery by the use of capsaicin injected peripherally or locally applied on the celiac and mesenteric ganglia in rats [7, 30, 47]. Previous reports pointed to the efferent limb of the reflex involving sympathetic hyperactivity to the intestine [23] and evidence suggests a role of sympathetic activity in the inhibition of bowel movement since intestinal catecholamine stores are depleted more rapidly after laparotomy [17, 34]. In the present study neuronal activation was detected in the NTS, VLM and rRPa which underlines the somatovisceral input to catecholaminergic and serotonergic regions arising from the spinosolitary tract and originating from dorsal horn neurons and the caudal trigeminal nucleus after abdominal surgery [7, 41] (Fig. 6). Of these neurons 14% in the NTS, 74% in the VLM and 82% in the rRPa showed also double labeling with nesfatin-1 suggesting an interrelation of nesfatin-1 with the catecholaminergic and serotoninergic systems.

The A1 region (VLM) has been shown to project to the LC (A6) [14, 22] and there is a smaller projection from the LC to the PVN (Fig. 6) [31]. Electrophysiological studies have shown that icv injection of CRF as well as microinjection around the LC region act on

noradrenergic LC neurons [15, 44, 60] and LC neurons increase their activity dramatically in response to painful stimuli [22, 45]. Various stressors, for example restraint, tail shock, auditory and hypotensive stress increase extracellular norepinephrine levels in LC terminal regions [1, 10] and elevated levels of Fos mRNA and protein in the LC are induced in response to abdominal surgery, restraint, shock, hypotension, swim force, immune challenge, water avoidance stress and social stress [5, 6, 8, 12, 13, 18, 19, 24, 29, 32, 54] (present study). Taken together, these data suggest that the activation of nesfatin-1-ir neurons in the LC combined with LC-arising projections to CRF-containing neurons in the PVN which are also activated by abdominal surgery [6] (present study) and release of brain CRF contribute to the inhibition of gastric motor function and might be part of the factors leading to the development of postoperative ileus occurring after abdominal surgery (Fig. 6).

In this study we showed for the first time that neuronal activation is induced in the EW upon abdominal surgery and 74% of the Fos-ir neurons of the EW were positive for nesfatin-1. These data indicate that the EW responds not only to environmental changes as previously reported [25, 37] but also to somatosensory-visceral input (laparotomy plus cecal palpation). It is of interest that NUCB2/nesfatin-1 immunoreactivity overlaps with another member of the CRF family, urocortin 1 (Ucn 1) highly expressed in the EW nucleus [21, 38, 61, 65]. CRF, Ucn 1 and nesfatin-1 reduce food intake and gastric emptying after central as well as peripheral injection [16, 43, 53, 56] and we could recently show that central nesfatin-1 exerts its anorexigenic effect *via* a CRF_2 receptor-dependent pathway [60]. This may contribute to the anorexia and delayed emptying accompanying POI and further corroborates the hypothesis that Ucn 1 and nesfatin-1 share similarities in their functions and seem to use similar if not the same pathways.

Taken together, abdominal surgery activates a large proportion of nesfatin-1-ir neurons in the forebrain more prominently in magnocellular neuroendocrine hypothalamic (SON and PVN) and anterior parvicellular PVN neurons, in nuclei of the catecholaminergic (LC, NTS and VLM) and serotonergic (rRPa) systems as well as in the EW nucleus where Ucn 1 is prominently expressed. This activation suggests a possible role of brain nesfatin-1 in the functional alterations induced by abdominal surgery including reduced gastric transit and food intake that will need to be further assessed.

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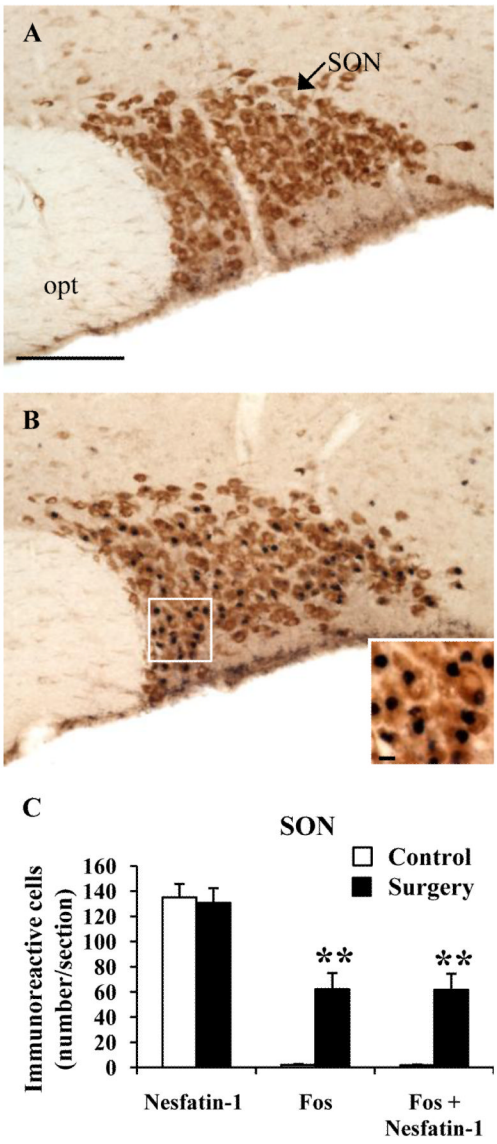

Fig. 1.

Abdominal surgery induces Fos expression in nesfatin-1 immunopositive neurons in the rat supraoptic nucleus (SON). Fasted rats were exposed to 7-10 min anesthesia alone (control, A) or combined with abdominal surgery and cecal palpation (surgery, B) and euthanized 120 min later. Brains were processed for immunohistochemical detection of Fos (dark blue) and nesfatin-1 (brown) in the SON (A-B). The insert in B shows a higher magnification of activated neurons co-localizing with nesfatin-1 in the SON. Scale bars: 100 μm in A and B and 10 μm in the insert. Unilateral cell count/section in the SON after abdominal surgery (C). Data are mean ± SEM of 6 rats/group. ** $p < 0.001$. opt = optic tract.

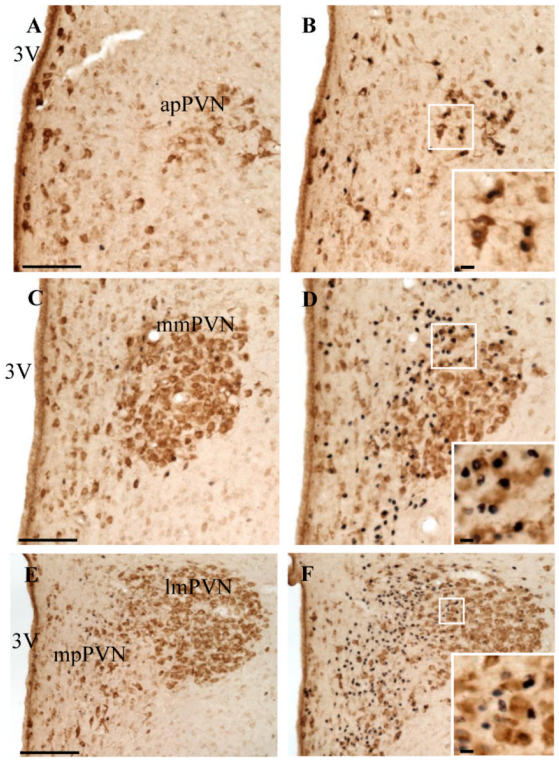

Fig. 2.

Abdominal surgery induces Fos expression in nesfatin-1 immunoreactive neurons in the rat paraventricular nucleus of the hypothalamus (PVN). Double immunohistochemical staining for Fos (dark blue) and nesfatin-1 (brown) in the anterior parvicellular part of the paraventricular nucleus (apPVN, A-B), medial magnocellular part of the PVN (mmPVN, C-D), lateral magnocellular part of the PVN (lmPVN, E-F) and medial parvicellular part of the PVN (mpPVN, E-F) in control rats (A, C, E) and 120 min after abdominal surgery (B, D, F). The inserts in B, D and F show higher magnification of activated neurons co-localizing with nesfatin-1 in the apPVN (insert B), mmPVN (insert D) and lmPVN (insert F). Scale bar: 100 μm in A-F and 10 μm in the inserts. 3V = third ventricle.

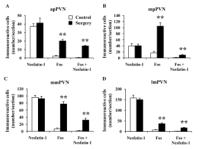

Fig. 3.

Abdominal surgery induces Fos expression in nesfatin-1 immunoreactive neurons in the rat paraventricular nucleus of the hypothalamus (PVN). Cell counting per section (unilateral) in the apPVN (A), mpPVN (B), mmPVN (C) and lmPVN (D) in control rats and 120 min after abdominal surgery. Data are mean ± SEM of 6 rats/group. ** $p < 0.001$.

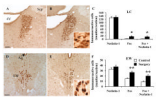

Fig. 4.

Abdominal surgery induces Fos expression in nesfatin-1 immunoreactive neurons in the rat locus coeruleus (LC) and Edinger-Westphal (EW) nuclei. Double immunohistochemical staining for Fos (dark blue) and nesfatin-1 (brown) in the LC (A−B) and EW (D−E) in control rats (A+D) and 120 min after abdominal surgery (B+E). The inserts show a higher magnification of activated neurons co-localizing with nesfatin-1 in the LC (insert B) and EW (insert E). Scale bar: 100 μm in A, B, D, E and 10 μm in the inserts. Unilateral cell count/section in the LC (C) and EW (F). Data are mean ± SEM of 6 rats/group. * $p < 0.05$; ** $p < 0.002$. 4V = fourth ventricle, Aq = aqueduct, scp = superior cerebellar peduncle.

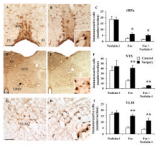

Fig. 5.

Abdominal surgery induces Fos expression in nesfatin-1 immunopositive neurons in the rat rostral raphe pallidus (rRPa), nucleus of the solitary tract (NTS) and ventrolateral medulla (VLM). Double immunohistochemical staining for Fos (dark blue) and nesfatin-1 (brown) 120 min after abdominal surgery in the rRPa (B), NTS (E), VLM (H) and in control rats (A, D, G). The inserts in B, E and H show higher magnification of activated neurons co-localizing with nesfatin-1. Scale bar: 100 μm in A, B, D E, G, H and 10 μm in the inserts. Cell count per section (unilateral) in the rRPa (C), NTS at the level of the area postrema (F) and VLM (I). Data are mean ± SEM of 6 rats/group. * $p < 0.05$; ** $p < 0.01$. AP = area postrema; CC = central canal; DMN = dorsal motor nucleus of vagus; py = pyramidal tract.

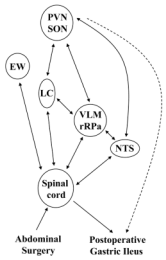

Fig. 6.

Hypothetical scheme of neuronal activation and projections in nesfatin-1-immunoreactive brain nuclei involved in abdominal surgery and postoperative ileus. Activated nesfatin-1 neurons in these brain nuclei in response to abdominal surgery could underlie a neuronal circuitry by which postsurgical alterations of gastric transit and reduction in food intake are mediated. EW = Edinger-Westphal nucleus; LC = locus coeruleus; NTS = nucleus of the solitary tract; PVN = paraventricular nucleus; rRPa = rostral raphe pallidus; SON = supraoptic nucleus; VLM = ventrolateral medulla; solid line, neuronal projections; dashed line, neurohumoral pathway.