Autonomic Neuroscience: Basic and Clinical 153 (2010) 99–105

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

journal homepage: www.elsevier.com/locate/autneu

Peripheral relays in stress-induced activation of visceral afferents in the gut

René M. van den Wijngaard [a,*], Tamira K. Klooker [a], Wouter J. de Jonge [a], Guy E. Boeckxstaens [a,b]

[a] Division of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, The Netherlands
[b] Division of Gastroenterology, University Hospital Gasthuisberg, Catholic University of Leuven, Leuven, Belgium

ARTICLE INFO

Article history:
Received 5 April 2009
Received in revised form 6 July 2009
Accepted 7 July 2009

Keywords:
Stress
Visceral afferents
Gut
Mast cells
CRF
Barrier dysfunction
TLR
TRPV1

ABSTRACT

Multiple organs are targeted by the stress response, but the focus of this article is on stress-induced activation of visceral afferents in the gut. During recent years it became apparent that mast cells are pivotal in this response. Peripheral corticotrophin releasing factor (CRF) induces their degranulation whereupon mast cell mediators activate visceral afferents. In addition, these mediators are responsible for gut barrier dysfunction and subsequent influx of luminal antigens and bacteria. Some research groups have begun to investigate the possible importance of barrier dysfunction for enhanced visceral sensitivity. After reviewing the current knowledge on CRF-induced mast cell degranulation we will discuss these groundbreaking papers in a more elaborate way. They form the basis for a hypothesis in which not only CRF-induced but also antigen-mediated mast cell degranulation is relevant to stress-related afferent activation. Part of this hypothesis is certainly speculative and needs further investigation. At the end of this article we sum up some of the unanswered questions raised by others and during this review.

1. Introduction

Homeostasis is the tendency to remain in a state of equilibrium. It refers to those systems that are essential to life and, therefore, should be maintained over a narrow range (e.g. body temperature and PH). Stress is defined as a threat, real or perceived, to homeostasis and systems that respond to stress are typically those that actively help maintain the internal equilibrium. Most relevant and broadly investigated are the hypothalamic-pituitary-adrenocortical (HPA)- and the sympathetic-adrenal-medullary-(SAM) axis. Their activation leads to increased release of hormones capable of regulating functions essential for survival and their activity can vary over a broad range without causing death. Corticotrophin releasing factor (CRF), cortisol, adrenaline and noradrenalin are some of the key hormones of the stress response. They have the capacity to interact with target cells that, subsequently, release mediators which activate and/or modulate sensory neuron expressed receptors and ion channels. Thus, although stress begins in the brain, the stress response also affects peripheral organs and sensory systems. When discussing stress and visceral afferents one should explain 1) how the brain perceives stress, 2) how it subsequently activates the HPA and SAM axis, 3) how this leads to activation of peripheral cell types and, finally, 4) how this leads to the generation of action potentials in visceral sensory neurons. Because it is impossible to discuss all 4 themes in a concise review such as this, we will restrict ourselves to some of the peripheral cell types and molecular mechanisms involved.

Multiple organs will be targeted by the stress response, but our focus will be on stress-induced changes in the gut and more specifically those that may ultimately lead to acute and/or prolonged activation of local visceral afferents. Although CRF was initially described as a central stress hormone, we will discuss evidence that peripheral-CRF also plays a major role. Indications are that it induces degranulation of peripheral mast cells in the gut which, subsequently, leads to afferent activation. Other means of mast cell activation may also be relevant in prolonged post-stress activation of afferents: stress-induced gut barrier dysfunction and subsequent antigen influx may lead to antigen-mediated activation of these cells. Although few in number at present, there are some papers to support this idea. They will receive special attention because they pave the way for new avenues in our ways of thinking about stress and afferent activation. In addition, some of the shortcomings in our current knowledge are pointed out. We certainly do not mean to give a complete overview of the current status of the field and apologize to those whose original and important contributions are not mentioned here.

2. Stress and peripheral CRF

There are intimate contacts between gut mucosal mast cells and peptidergic nerves (Stead et al., 1989; Stead et al., 1987) and (bidirectional) functional communications are known to take place (Coldwell et al., 2007; De Jonge et al., 2004; Jiang et al., 2000; Rijnierse et al., 2007). Such interactions also seem relevant in stress-induced

* Corresponding author. Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.
E-mail address: R.vandenWijngaard@AMC.UVA.NL (R.M. van den Wijngaard).

1566-0702/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.autneu.2009.07.004

activation of visceral afferents. Regarding the initial trigger for mast cell activation CRF-induced degranulation presently stands out as one of the most investigated mechanism responsible for stress-related responses. Early investigations by the group of Bueno (Toulouse, France) showed that partial restraint stress induces enhanced sensitivity to colorectal distension in male Wistar rats (Gue et al., 1997). This response, also known as visceral hypersensitivity, could be mimicked by intracerebroventricular (icv) CRF-administration and blocked by icv pretreatment with CRF-receptor antagonist. Intraperitoneal doxantrazole (a mast cell stabilizing compound) also prevented CRF- and restraint-induced visceral hypersensitivity. More recent observations suggest that stress-induced hypersensitivity is not only mediated by central- but also by peripheral-CRF. When sustained visceral hypersensitivity was induced by repeated water avoidance stress (WA) for 10 consecutive days (1 h/day), daily, pre-WA, subcutaneous injection of astressin, a non-selective CRF-receptor antagonist, inhibited this abnormal pain response (Larauche et al., 2008). Astressin is not supposed to cross the blood brain barrier (BBB), thus making the case for peripheral CRF. Some caution is needed however because stress is known to compromise the BBB (Esposito et al., 2002; Esposito et al., 2001). Nevertheless, there is also other convincing evidence for a role of peripheral CRF. Nijsen et al. (2005) showed that i.p. injection of CRF induces duodenal hyper sensitivity to distension 30 min post-administration. The use of radioactive labeled CRF (i.p.) showed that, at the dosage used, CRF did not cross the BBB within 30 min from administration. In the same paper (Nijsen et al., 2005) and others (Stengel and Tache, 2009) the role of the two different CRF receptors (CRF-R_1 and CRF-R_2) was further elucidated. Importantly, these receptors may have divergent roles in the modulation of visceral pain. Activation of CRF-R_1 is nociceptive, whereas specific activation of CRF-R_2 has anti-nociceptive effects. CRF shows highest affinity for CRF-R_1, but in addition to CRF, three other related peptides are known; urocortin 1, 2 and 3. Like CRF, urocortin 1 interacts with both receptors whereas urocortins 2 and 3 preferentially bind CRF-R_2. In search of a physiological explanation for this complexity, it was hypothesized that activation of CRF-R_1 is important for the onset of visceral pain, whereas CRF-R_2 plays a role in the recovery phase.

3. The source of peripheral CRF

Peripheral CRF is important in stress-induced visceral hypersensitivity (Larauche et al., 2008; Nijsen et al., 2005; Stengel and Tache, 2009). Surprisingly little however is known about its cellular localization. In rat duodenum (Wolter, 1984) and guinea-pig colon (Liu et al., 2006) CRF was detected within the enteric nervous system (ENS). In an abstract presented at DDW 2008 (Yuan et al., 2008), the Tache group (Los Angeles, USA) confirmed CRF-immunoreactivity in colonic rat myenteric neurons and showed co-localization with tryptophan 5-hydroxylase (TPH) in crypts, which is indicative of enterochromaffin cells. When Kawahito et al. investigated CRF expression in normal and inflamed human colon, CRF expression was mainly confined to enterochromaffin cells (Kawahito et al., 1994) whereas inflammation was also associated with CRF expressing macrophages and other, undefined, inflammatory cells (Kawahito et al., 1995). They did not observe CRF immuno-reactivity in mucosal or submucosal nerve fibers or plexus. These limited data may suggest that enterochromaffin cells are the main CRF releasing cell type, but the exact trigger for CRF-release is unclear. Although it was shown that noradrenaline induces the release of serotonin from isolated gut enterochromaffin cells (Schafermeyer et al., 2004), this does not necessarily imply the simultaneous release of CRF. Moreover, when Schwetz et al. (2004) induced a chemical sympathectomy in rats, this procedure did not influence the course of stress-induced visceral hypersensitivity, although hypersensitivity was CRF-dependent indeed. Thus, further investigations are needed to elucidate which cell type is responsible for the peripheral release of CRF and how its release is being triggered.

4. CRF-receptors involved in mast cell activation and subsequent phenotypical changes

Mast cell stabilizers as well as mast cell deficient mouse and rat strains have been used to emphasize the importance of the stress/peripheral-CRF/mast cell axis. CRF-induced mast cell degranulation was not only shown to induce visceral hypersensitivity, but was also directly linked to stress-induced and CRF-dependent mucin release from colon (Castagliuolo et al., 1996; Castagliuolo et al., 1998). Furthermore there is extensive literature to show that acute as well as chronic stress induces mast cell dependent colonic permeability changes in rodents (Barreau et al., 2007; Demaude et al., 2006; Santos et al., 2001; Soderholm et al., 2002). CRF-receptor subtypes involved were investigated in the maternal deprivation model (Barreau et al., 2004; Barreau et al., 2007). When Wistar rat pups were subjected to the separation protocol they showed permanent alterations of gut mucosal barrier function at adult age. Barrier dysfunction was abolished by *in vivo* mast cell stabilization (Barreau et al., 2004) and selective CRF-R_1 antagonism (Barreau et al., 2007). Similarly, when i.p. CRF was administered to normal control rats, the increase in paracellular permeability was abolished by pre-CRF mast cell stabilization. In contrast to these findings, using the same model but another rat strain (Sprague Dawley), others showed that CRF-induced barrier-dysfunction may occur due to CRF-R_2 expressed on cholinergic submucosal plexus enteric nerves (Gareau et al., 2007). CRF-receptor subtype activation in human mast cells was recently investigated by Wallon et al. (2008) who also investigated barrier dysfunction. Colonic biopsies of normal controls were mounted in Ussing chambers and subjected to CRF. Increased flux of horseradish peroxidase was inhibited by mast cell stabilization and the non-selective CRF-receptor antagonist α-helical CRH (9–41). Selective CRF-R_1 and CRF-R_2 antagonists only resulted in partial inhibition of macromolecular permeability, suggesting that both receptors play a role. Most interestingly, in situ evaluations showed the presence of CRF-R_1 and -R_2 in the lamina propria exclusively on mast cells. In an earlier report on CRF receptor localization in human colonic mucosa, these receptors were shown to be expressed by lamina propria mononuclear cells that were not characterized further (Muramatsu et al., 2000).

5. Which mediators are released upon CRF-induced activation of mast cells?

Mast cells are known to express a wide range of mediators that can either be preformed and granule-associated, or newly generated such as lipid mediators, cytokines and chemokines. Several of these mediators (e.g. histamine (Barbara et al., 2007; Cenac et al., 2007), tryptase (Cenac et al., 2007), prostaglandin E_2 (Gold et al., 2002), nerve growth factor (NGF) (Ji et al., 2002)) can activate or sensitize sensory afferents. Which mediators are actually released during stress is uncertain, mainly because different mast cell activating mechanisms give rise to different patterns of mediator release. In that respect, Fc- and complement-receptor mediated mast cell activation leads to degranulation-associated release of histamine and proteases, whereas Toll-like receptor (TLR)-mediated activation occurs in the absence of 'classical' degranulation and histamine/protease release (Marshall, 2004). Such 'piecemeal' type of degranulation (loss of granular density without fusion of intergranular membranes as defined by electron microscopy) was also shown in the majority of colonic mast cells in a chronic stress model in rats (Santos et al., 2001). Which mediator-release profile was associated with this type of degranulation was not investigated. The group of Theoharides (Boston, USA) used the human leukemic mast cell (HMC-1) line and