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Role of neuronal nitric oxide synthase in the regulation of the neuroendocrine stress response in rodents: insights from mutant mice

Review Article

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Summary. Nitric oxide (NO) is a free radical gas synthesised from arginine and oxygen by enzymes of the family of the nitric oxide synthase. In particular, the neuronal nitric oxide synthase (nNOS) is highly expressed by cells of the hypothalamic paraventricular nucleus, where the sympathoadrenal system, the hypothalamic-pituitary-adrenal axis and the hypothalamic-neurohypophyseal system originate. These structures are deputed to regulate the neuroendocrine stress response. In the past years, evidence has been accumulated to suggest that NO of nNOS origin plays a significant role in modulating the activity of the above mentioned systems under acute stressor exposure. The availability of nNOS knock-out mice allowed to investigate not only the physiological consequences of a constitutive lack of NO of nNOS origin at the hormonal and molecular level, but also to examine possible behavioural alterations. In this review, we shall discuss and confront the current trends of research in this area, especially focusing on the latest findings gained from genetically modified mice.

Keywords: Neuronal nitric oxide synthase – Hypothalamus – Neuroendocrine stress response – Knock-out mice – Catecholamine – Glucocorticoids

Introduction

The concept of stress

Living organisms survive by maintaining a stable and harmonious internal environment in spite of dynamic inner or external disturbances. The so called ''steady state'' is accomplished by a multitude of counteracting and re-establishing forces, which oppose changes and efficiently guarantee an internal equilibrium, or *homeostasis* (for review, see Koolhaas et al., 1999). Walter Cannon coined in the 1930s the term ''homeostasis'' from the Greek words for ''same'' and ''steady'' to designate the natural tendency of living systems to maintaining a state of equilibrium, which he extended from physical to emotional parameters for human beings. He also described as ''fight or flight reaction'' the collection of responses which are evoked in an organism by an acute challenge or a threat, be they real or ''just'' perceived, to defend the stability of the internal environment. In 1936 Hans Selye integrated and further developed the ''fight and flight reaction'' into a broader theory, which he elaborated to describe the concept of *stress*. The collection of biological changes that occur in the organism when it undergoes severe and prolonged stress was named by Selye more specifically ''General Adaptive Syndrome'', since he believed the physiological alterations to be general and independent from the detailed nature of the stressor (Selye, 1955, 1998). According to his theory, the body adaptive response to stressors consists of three different stages. The first is the stage of alarm-reaction, in which the organism recognises the stressor as a potential threat and prepares for action. Upon the resulting interpretation of the threat as fear or anger, heartbeats fasten and the respiration quickens, endocrine glands become active and in particular the adrenal glands become increasingly enlarged to release large amounts of steroids into the bloodstream, aimed at mobilising the bodily reserves of energy. In this initial phase the body exhibits a ''fight or flight'' reaction, as it prepares itself for either conflict or flight. Cessation of the stressor usual terminates the alarm response and the organism restores its normal state. If the stressor persists, a second phase of resistance-adaptation follows, during which the organism

seems apparently to adapt to the stressor by establishing a new equilibrium. The adrenal glands stop secreting steroids and regain their usual size, blood sugar and salts concentrations return to basal levels. The individual tries to moderate the consequences of a prolonged stressor exposure by achieving a state of resistance, which may be long-lasting. In some cases a continuous, mild stimulation can be beneficial, as it is for instance physical exercise for cardiovascular tone, but in case of inappropriate compensatory mechanisms or overwhelming stimuli, the effect may be deleterious and the body adaptive responses begin to fail to keep control over the stressor. Ultimately, if the stimulus is not withdrawn or reduced, the third and final stage of exhaustion occurs. Serious illnesses, which typically include gastrointestinal and cardiovascular disorders, severe immunosuppression, anxiety and depression, or even death may ensue at this point, depending upon the type of the stressor, the length of exposure and the individual coping abilities. Although Selye's ideas have been widely accepted, his definition of ''stress'' and ''stressor'' have been criticised by other researches (Lazarus et al., 1985) and modified definitions were proposed (Chrousos and Gold, 1992). In the present work, we consider stress as the status of threatened homeostasis that arises when an animal interprets a stimulus as ''challenging'' or ''disturbing''. The emotional evaluation of a stimulus as potentially threatening is a highly subjective event that occurs at the level of the limbic system and depends on several factors, such as the genetic background, earlier conditioning and individual past life experiences. Therefore, a stimulus becomes a stressor only if the individual perceives it as such. Under laboratory conditions, several experimental stressor paradigms have been established to examine the activity of the sympatho-adrenal system (SAS), the hypothalamic-pituitary-adrenal (HPA) axis and also the hypothalamic-neurohypophysial system (HNS), which has been postulated to be involved in coordinating the neuroendocrine response (Engelmann and Ludwig, 2004). However, according to our definition of stress, stressor paradigms that implicate the activation of somato-visceral afferent pathways without requiring an emotional evaluation at the level of the forebrain, such as haemorrhage or hypoxia applied under anaesthesia, are not appropriate tools to investigate the modulation of the systems deputed to coordinate the neuroendocrine stress response. Hence, we employed in previous studies (Orlando et al., 2007, 2008) an experimental approach, namely forced swimming, which has a strong emotional components due to the fact that the animal cannot escape from the water, resembles naturally occurring stressors, and activates brain regions deputed to control the stress response (Engelmann et al., 1998; Wotjak et al., 1998, 2001; Salchner et al., 2004; Drugan et al., 2005).

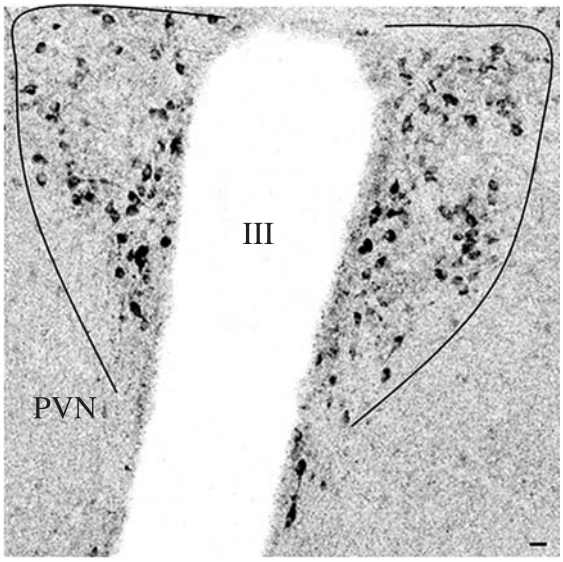

Fig. 1. nNOS-immunoreactive cell bodies are abundant in the PVN of the mouse. *PVN* Paraventricular nucleus; *III* third ventricle; scale bar: 20 μm

In this review, we sought to summarise findings supporting a role for nitric oxide (NO) of neuronal nitric oxide synthase (nNOS) origin on the regulation of the systems that coordinate the neuroendocrine stress response with particular respect to results obtained from studies with mutant mice. For a more general overview on the expression and the role the other NOS subtypes play in the stress axis, we invite the reader to refer to more specialised literature (Lopez-Figueroa et al., 1998; Bredt, 1999; Riedel, 2000; McLeod et al., 2001; Givalois et al., 2002).

Nitric oxide

As mentioned above, the neuroendocrine stress response is modulated by a complex interplay of neurotransmitters, among which the endogenously produced gas NO has attracted considerable attention as a significant factor (for review see Wolf, 1997; Carrasco and Van de Kar, 2003). NO is a highly diffusible free radical gas that is produced on demand through an oxidative reaction catalysed by nNOS from L-arginine and oxygen to produce citrulline and NO (Alderton et al., 2001). To date, three subtypes of NOS have been described: the inducible NOS (iNOS), which may be induced in macrophages, hepatocytes, microglia and other cell types (Bandaletova et al., 1993) upon stimulation with lipopolysaccharides and cytokines (Xie et al., 1992), the endothelial NOS (eNOS),

which is mainly found in the endothelium of blood vessels (Marsden et al., 1993), but also in human neuronal cells (Abe et al., 1997), human and rat astrocytes (Colasanti et al., 1998), T-cells and bone marrow cells (Reiling et al., 1996; Helfrich et al., 1997) and other cell types (Xue et al., 1994; Wang et al., 1996), and the neuronal NOS (nNOS; Bredt et al., 1990), which is expressed in neurones and astrocytes (Arbones et al., 1996; Cork et al., 1998), cardiac and skeletal myocytes (Kobzik et al., 1994; Xu et al., 1999), rat *penile corpora cavernosa*, urethra (Magee et al., 1996) and human prostate (Waldkirch et al., 2007), the adrenal medulla (Oset-Gasque et al., 1994) as well as in other tissues (Asano et al., 1994; Shimizu et al., 1997). In neurones, nNOS is located mainly at the post-synaptic terminal, where it is activated by increased intracellular levels of calcium upon glutamate-driven opening of the ionotropic N-methyl-D-aspartate receptor. Due to its subcellular location, nNOS is thought to participate to the anchoring of the post-synaptic density to the cytoskeleton (Valtschanoff and Weinberg, 2001).

Because of its diffusible nature and its ability to freely cross cell membranes, NO can act in an autocrine and paracrine manner also on targets relatively distant from its source of production (Wood and Garthwaite, 1994). It activates primarily two pathways: the guanylyl cyclase pathway, which leads to the production of cyclic guanosine-3′,5′-monophosphate (cGMP), and the cyclooxygenase pathway. The increase in cGMP provokes a rise in intracellular free calcium concentrations with subsequent activation of phospholipase A_2, which catalyses the conversion of membrane phospholipids into arachidonate. Arachidonate is used by cyclooxyenase as substrate to synthesise prostaglandin G_2, which activates adenylate cyclase leading to the production of cyclic adenosine-3′,5′-monophosphate (cAMP). cAMP activates protein kinase A, which ultimately leads to secretory granules extrusion (Ignarro, 1991; Canteros et al., 1995; Rettori et al., 1997; Mohn et al., 2005). Moreover, cGMP may also activate the protein kinase G_1, which is mostly expressed in cerebellum, smooth muscle cells, platelets (Butt et al., 1993) and adrenal glands (Walter, 1981) and is involved in the control of intracellular calcium (Lau et al., 2003), and G_2, which is predominantly expressed in intestine, brain and kidney (Vaandrager and de Jonge, 1996) and controls the flux of anions, such as chloride (French et al., 1995).

The sympatho-adrenal system

The neuroendocrine stress response follows a determined time course. Within a few seconds from stressor onset, the activation of the SAS leads to the release of catecholamine into the bloodstream to modulate the ''fight or flight'' reaction. In laboratory rodents, a subpopulation of oxytocin (OXT)-containing parvocellular neurones of the hypothalamic paraventricular nucleus (PVN; Swanson and Sawchenko, 1980) regulates the activity of the SAS by projecting to the brain medulla, where they synapse to neurones whose terminals descend down the spinal cord (Swanson, 1987). Via the greater (major supply) and lesser thoracic splanchnic nerve, the hypothalamus exerts a direct control over catecholamine secretion by modulating the preganglionic sympathetic innervation of the adrenal medulla. The synaptic nature of this transmission renders the ''fight or flight'' response an immediate reaction. The secretion of norepinephrine (NE) and epinephrine (E) into the bloodstream in response to stressor exposure increases heartbeats and blood pressure, leads to elevated perfusion of skeletal muscle, brain, and liver, and triggers the release of glucose into the blood. Yet, it is worth mentioning that, although the adrenal glands contribution is not negligible, most of NE secreted into the blood upon high demand is released by sympathetic terminals (Benedict et al., 1978). Catecholamine are produced by four sequential enzymes: tyrosine hydroxylase (TH), which is the rate-limiting enzyme, aromatic L-amino acid decarboxylase, dopamine beta-hydroxylase, and phenylethanolamine

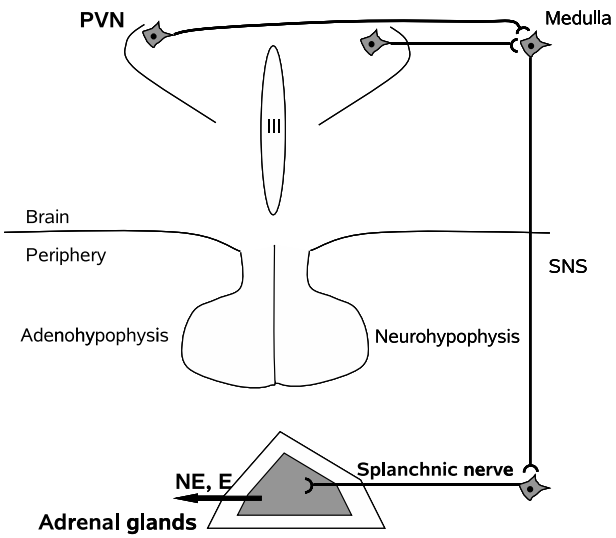

Fig. 2. Schematic representation of the SAS. The synaptic pathways originating in the PVN lead to catecholamine (NE and E) exocytosis from the adrenal medulla into the blood (*arrow*). PVN neurones synaptically contact a population of neurones in the medulla, which in turn project to sympathetic pre-ganglionic neurones of the spinal cord that, through the splanchnic nerve, directly relay the information to chromaffin cells. *PVN* Paraventricular nucleus; *III* third ventricle; *SNS* sympathetic nervous system; *NE* norepinephrine; *E* epinephrine

N-methyltransferase (PNMT). The final biosynthetic step, in which NE is N-methylated to form E, occurs only in epinephrinergic chromaffin cells of the adrenal medulla and neurones of the central nervous system. Plasma catecholamine levels have been reported to increase in response to forced swimming and chronic isolation (Itoh et al., 2006; for review see Nankova and Sabban, 1999).

Modulation of the sympatho-adrenal system activity: the role of nitric oxide of nNOS origin

The adrenal medulla is a tissue of ectodermal origin, as are neurones. Thus, it came not as a surprise that high levels of nNOS have been demonstrated in chromaffin cells (Oset-Gasque et al., 1994; Schwarz et al., 1998) and in fibres closely associated with them (Afework et al., 1994; Heym et al., 1994; Tanaka and Chiba, 1996). Data derived from pharmacological studies suggest that NO inhibits catecholamine release evoked by depolarising stimuli like acetylcholine, nicotine and high KCl. NO acts by elevating intracellular cGMP and activating protein kinase G_1, which selectively inhibits voltage-dependent Ca^{++} influx and therefore reduces catecholamine exocytosis (Oset-Gasque et al., 1994; Uchiyama et al., 1994; Rodriguez-Pascual et al., 1996; Schwarz et al., 1998). These data suggest that NO controls catecholamine secretion under conditions of high levels of stimulation. On the other hand, the role of NO on basal catecholamine secretion is still under discussion. Some investigators have shown a stimulatory effect (Oset-Gasque et al., 1994; Uchiyama et al., 1994), while some have reported an inhibitory action (Rodriguez-Pascual et al., 1995; Ward et al., 1996) or no effect (Marley et al., 1995) of NO on intracellular Ca^{++} concentration and catecholamine exocytosis under resting conditions. Furthermore, some investigators (Kim et al., 2003) reported a long-term up-regulation by NO of the genes encoding for the catecholamine biosynthetic enzymes. These contradictory results might be due to the use of dissociated chromaffin cell cultures, which contain different proportions of adrenergic/noradrenergic cells according to the method of separation. NOS is unevenly distributed among chromaffin cells, with noradrenergic cells being the main NOS-immunoreactive subpopulation of the adrenal medulla (Dun et al., 1993; Heym et al., 1994). Thus, the average response, for instance in terms of calcium influx, observed in a mixed population following pharmacological stimulation might be significantly affected by the proportion of noradrenergic versus adrenergic cells present in culture.

The use of nNOS knock-out (KO) mice is a valid experimental alternative approach that allows to circumvent the limitations due to the use of pharmacological compounds. The activity of the sympathetic nervous system, measured in terms of NE and E plasma values, appeared normal in mutant mice under resting conditions. If exposed to a 10 min-forced swimming session, KO mice failed to show an increase in E plasma levels 15 min after stressor onset, as observed in wild type (WT) animals (Orlando et al., 2008). The reduced E secretion into the blood after forced swimming may suggest a change in E synthesis, which is then reflected by a lower amount of E stored in vesicles ready to be released upon stimulation. Previous reports have shown that the activity of catecholamine biosynthetic enzymes can be regulated through phosphorylation by different protein kinases (Zigmond et al., 1989). Indeed, nNOS KO animals showed significantly reduced TH and PNMT protein levels if compared to WT (Orlando et al., 2008), which is in agreement with other studies reporting up-regulated transcript levels of these enzymes upon NO activation (Kim and Rivier, 2000). The fact that plasma E levels in KO mice were normal under resting conditions may indicate that the biosynthesis is sufficient to maintain basal levels, but become inadequate in case of higher demand. Therefore, these findings suggest that a constitutive lack of NO of nNOS origin affects the capability of the adrenal glands to mount an adequate E response to acute stressor exposure.

Unlike E, NE plasma levels after stressor exposure were not significantly altered in nNOS KO mice. It is not clear yet whether this is due to a different subcellular localisation of NOS in the adrenal medulla, with noradrenergic cells specialised in producing NO (Dun et al., 1993; Heym et al., 1994), whereas adrenergic cells represent its main target (Oset-Gasque et al., 1998).

The hypothalamic-pituitary-adrenal axis

Stress-related sensory information are conveyed from the periphery to a population of parvocellular neurosecretory neurones that reside within the PVN and comprise the central nervous portion of the HPA-axis. Corticotropin-releasing hormone (CRH; Vale et al., 1981) is secreted from these neurones into the portal blood that supplies the anterior pituitary within 1 min from stressor onset (Plotsky et al., 1987). CRH acts on corticotrope cells of the anterior pituitary to stimulate basal and stress-driven release of adrenocorticotropic hormone (ACTH) into the bloodstream. ACTH ultimately promotes the secretion of glucocorticoids (corticosterone in rodents

and cortisol in humans) from the adrenal cortex (for reviews, see Angelucci, 2000; Korte, 2001; Makara and Haller, 2001). In case of chronic, prolonged stress, ACTH secretion into the blood is robustly enhanced by the synergistic action of arginine-vasopressin (AVP), which is co-released with CRH by parvocellular neurones into the portal vasculature in particular under chronic stress conditions (Antoni, 1993; Makino et al., 1995; Ma and Lightman, 1998; Aguilera and Rabadan-Diehl, 2000). ACTH binds to high affinity receptors located on the surface of adrenal cortical cells and triggers the secretion of Cort, which is quickly secreted into the blood upon production. Since the adrenal cortex synthesizes Cort to preserve basal serum levels only for a few minutes, the effect of ACTH on Cort production can be observed in the main circulation within minutes from its stimulation.

It has been suggested that the activity of the adrenal cortex can be modulated by splanchnic nerve stimulation (Ehrhart-Bornstein et al., 1995), as the integrity of the sympathetic innervation is required to ensure normal basal levels of circulating glucocorticoids (Ottenweller and Meier, 1982; Edwards and Jones, 1987; Dijkstra et al., 1996). The mechanism by which the SAS promotes glucocorticoid secretion into the blood is still elusive, but it is reasonable to infer that the close anatomical localisation of chromaffin and cortical cells in the adrenal gland promote an intensive cross-talk between the two systems (Bornstein et al., 1990, 1991, 1994).

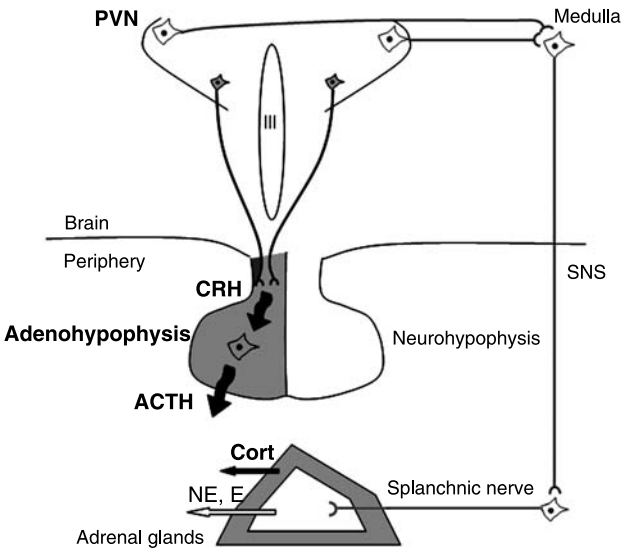

Fig. 3. Schematic representation of the SAS and the HPA-axis. CRH-containing neurones of the PVN project to the adenohypophysis, where they release CRH into the portal blood. Upon CRH stimulation, corticotropes secrete ACTH into the general circulation, which in turn elicits Cort release from the adrenal cortex: *CRH* corticotrophin-releasing hormone; *ACTH* adrenocorticotropin; *Cort* corticosterone; arrows represent neurohormones released into the portal blood and into the peripheral blood. See Fig. 2 for more details

Modulation of the HPA-axis activity: the role of nitric oxide

nNOS is widely present in the HPA-axis and in closely related anatomical structures. At the hypothalamic level, nNOS immunoreactivity can be found in parvocellular neurosecretory and medullary-projecting preautonomic neurones (Arevalo et al., 1992; Bhat et al., 1996; Ceccatelli et al., 1996; Nylen et al., 2001), whereas at the level of the anterior pituitary nNOS is present in folliculo-stellate cells and gonadotrophs, but not in corticotrophs (Ceccatelli et al., 1993; Wang et al., 1997). Interestingly, nNOS mRNA and immunoreactivity have been detected also in the adrenal cortex (Tsuchiya et al., 1996).

The influence of NO of nNOS origin on the activity of the HPA-axis under basal conditions and following defined stressor exposure is still a matter of debate. Although the involvement of NO in the modulation of both CRH and ACTH secretion may be conceivable due to the subcellular localisation of nNOS in the PVN of rodents (Torres et al., 1993; Siaud et al., 1994; Hatakeyama et al., 1996), earlier investigations (Costa et al., 1993; Rivier and Shen, 1994; Hashimoto et al., 1995; Giordano et al., 1996; Lee et al., 1999; Riedel, 2000) addressing this issue yielded contradictory results, most likely because of the use of different experimental approaches (i.e., in vivo versus in vitro experiments or peripheral versus central pharmacological administrations). Interestingly, CRH mRNA levels in nNOS KO and WT mice were similar. Furthermore, also plasma ACTH levels, under basal conditions and in response to forced swimming, are unchanged in mutant mice if compared to WT (Orlando et al., 2008). On one hand, these results suggest that nNOS gene disruption does not significantly impair basal CRH gene expression. On the other hand, unaltered ACTH plasma values in response to defined stressor exposure might derive from complementary effects of NO at the level of the anterior pituitary, where NO has been reported to exert a stimulatory role (Brunetti et al., 1993), and at the level of the median eminence, where others have suggested an inhibitory influence (Rivier and Shen, 1994).

The effect of nNOS gene inactivation on baseline Cort secretion is also a matter of discussion. An earlier investigation (Bilbo et al., 2003) showed significantly higher basal Cort plasma levels in nNOS KO mice single-housed at weaning, whereas in our hands mutant mice, which were single-housed a week before the experiments, displayed Cort plasma levels indistinguishable from those seen in WT (Orlando et al., 2008). This incongruence may be due to the different husbandry conditions, which can considerably affect glucocorticoid release. After forced swimming Cort plasma levels rose similarly in both genotypes, reaching a peak at 15 min and returning close to basal levels at 60 min. This indicates that nNOS gene inactivation does not affect the HPA-axis peripheral activity either under resting conditions or upon acute stressor exposure.

The hypothalamic-neurohypophyseal system

The HNS governs body fluid homeostasis, reproduction and mating behaviour via the release of AVP and OXT into the bloodstream (for review, see Cunningham and Sawchenko, 1991). It is composed of magnocellular neurones located in the PVN and in the hypothalamic supraoptic nucleus (SON). The axons of these neurones project to the neurohypophysis, where AVP and OXT are released into the blood. Both AVP and OXT may also be released from somata and dendrites of magnocellular neurones into the extracellular space of the PVN and the SON (Di Scala-Guenot et al., 1987; Pow and Morris, 1989; Hattori et al., 1990, 1992; Landgraf and Ludwig, 1991; Ludwig et al., 1994) not only during thirst (Ludwig et al., 1996), suckling and parturition (Neumann et al., 1993), but also in response to defined stressor exposure, including forced swimming (Wotjak et al., 1998).

It was originally believed that ACTH secretion in response to stressor exposure could be modulated by the HNS through the release of AVP of magnocellular origin into the main circulation (Bargmann, 1949; Bargmann and Scharrer, 1951; McCann and Brobeck, 1954; Mirsky et al., 1954; Martini and Monpurgo, 1955). However, after it was demonstrated (Vale et al., 1981) that hypothalamic parvocellular neurones secrete CRH, the most potent secretagogue of ACTH, this theory was abandoned. The role of the HNS with regard to its function in modulating the HPA-axis and in processing stress-related information was revaluated in recent years (for review see Engelmann et al., 2004), when increasing evidence indicated that the HPA-axis and the HNS closely interact with each other (Holmes et al., 1986; Wotjak et al., 1996, 2001; Engelmann et al., 2004) to orchestrate the neuroendocrine stress response. For instance, electrical stimulation of the SON (Makara et al., 1982) triggered a significant augment in Cort plasma levels. Moreover, animal models characterised by a disrupted magnocellular AVP tone showed a marked hypo-activity of the HPA-axis (Conte-Devolx et al., 1982; Dohanics et al., 1991).

AVP and OXT have been shown to act at the level of the pituitary to promote ACTH release from the adenohypophysis (Schlosser et al., 1994). In contrast, their action at the level of the hypothalamus seems to be a counterbalancing mechanism aimed at preventing an overshooting of the HPA-axis. In fact, AVP released from somata and dendrites within the SON and the PVN inhibits ACTH secretion and the activation of magnocellular neurones (Hermes et al., 2000; Wotjak et al., 2002; Hirasawa et al., 2003). Similarly, intra-PVN released OXT reduces ACTH and Cort secretion (Neumann et al., 2000a, b; for review, see Neumann, 2002). Conversely, it remains controversial the effect that intra-SON released OXT may have on magnocellular neurones, with reports of auto-inhibitory and auto-excitatory actions (Brussaard et al., 1996; Pittman et al., 2000; Kombian et al., 2002; Landgraf and Neumann, 2004). In any case, there is substantially evidence that the HNS participates in the regulation of the neuroendocrine stress response.

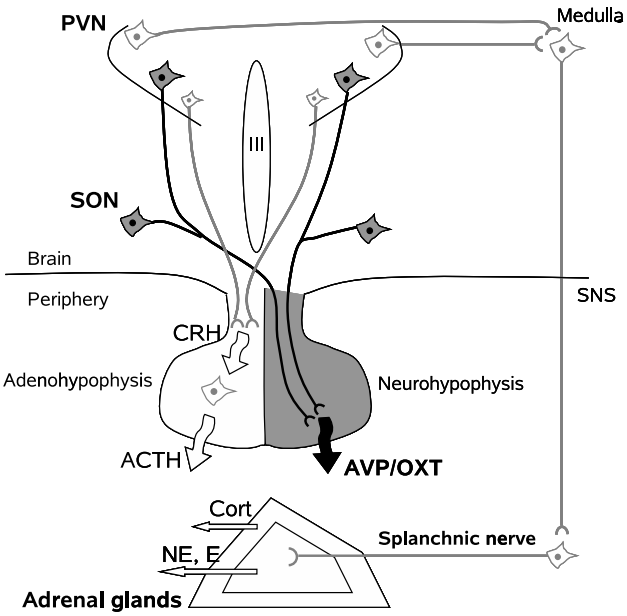

Fig. 4. Schematic representation of the SAS, the HPA-axis and the HNS with the focus on the latter endocrine system. Magnocellular neurones of the PVN and the SON project to the neurohypophysis and release AVP and OXT from their axon terminals into the peripheral blood (*black arrow*). *AVP* Vasopressin; *OXT* oxytocin; *SON* supraoptic nucleus. See Figs. 2 and 3 for more details

Modulation of the HNS activity: the role of nitric oxide

Previous studies suggested a role for nNOS on the correct postnatal development of vasopressinergic and oxytocinergic cells of the hypothalamus (Yuan et al., 2006). In fact, the time course of nNOS expression in hypothalamic structures coincides with the maturation of vasopressinergic and oxytocinergic neurones. However, studies on nNOS KO mice showed that nNOS gene inactivation had no impact on the number of AVP- and OXT-immunopositive cells either in the PVN or in the SON (Bernstein et al., 1998). Interestingly, in situ hybridisation studies revealed that in nNOS KO mice the intensity of the hybridisation signal for AVP mRNA in the neurones of the PVN was remarkably reduced (Orlando et al., 2008). This observation would fit with results of previous investigations reporting that, in the intact rat, AVP transcriptional activity at the level of the PVN is promoted by NO donors (Lee et al., 1999). On contrast to the PVN, the two studies investigating AVP mRNA expression in the SON of KO mice reported contradictory results: Nomura et al. (2005) showed unchanged basal AVP mRNA levels in KO mice, whereas Orlando et al. (2007) reported that AVP gene transcription was up-regulated in mutant mice if compared to WT. The use of oligonucleotides instead of cRNA probes may explain the opposite observations. The data reported so far imply that NO of nNOS origin acts at hypothalamic level on AVP-producing magnocellular neurones of both the PVN and the SON, but with opposite effects: an inhibitory action at the level of the SON, and a stimulatory action at the level of the PVN.

Unlike AVP, OXT mRNA levels in the SON and the PVN of nNOS KO mice did not significantly differ from WT (Orlando et al., 2007, 2008). Therefore, NO of nNOS origin seems to act specifically on AVP-producing rather than on OXT-producing magnocellular neurones.

Baseline AVP and OXT plasma levels were unaffected by nNOS gene inactivation. However, the peripheral release of AVP and OXT in response to forced swimming was found to be altered in mutant mice. In fact, AVP and OXT plasma levels 15 min after the beginning of a 10 min-swimming session were significantly reduced if compared to basal values and to the corresponding values in WT (Orlando et al., 2007). This might be due to the absence of NO-mediated inhibition of monoamines transporters on magnocellular neurones (Pogun et al., 1994; Kaye et al., 1997; Kiss and Vizi, 2001), which may then lead to a reduced glutamatergic and noradrenergic stimulation of vasopressinergic cells during forced swimming. In addition, mutant mice showed in the SON an increased AVP mRNA content (Orlando et al., 2007), which might indicate a stronger autocrine negative feedback of AVP on SON neurones themselves upon somato-dendritic release (Ludwig and Leng, 1998; Kombian et al., 2000).

OXT plasma concentration was robustly increased 60 min after stressor onset in KO animals (Orlando et al., 2007). These results suggest that, under acute stress conditions, NO may inhibit a retarded release of OXT into the blood. This latter assumption is in accordance with data obtained under pharmacological manipulations, which showed a selective facilitative effect of NO synthase inhibitors on OXT release versus AVP in dehydrated animals (Summy-Long et al., 1993) and in response to salt-loading (Ventura et al., 2005).

Conclusions and clinical implications

NO of nNOS origin seems to play an important role in the regulation of the endocrine stress response. Although nNOS KO mice display a fairly normal phenotype under resting conditions (Huang et al., 1993; Bernstein et al., 1998; Orlando, 2007, 2008), the constitutional absence of nNOS affects the neuroendocrine response to acute stressor exposure. NO seems to collaborate in maintaining constant AVP, OXT and E plasma profile release under conditions of stress, as KO animals revealed anomalous AVP, OXT and E blood levels in response to forced swimming. On the other hand, nNOS gene inactivation influences preferentially AVP vs OXT mRNA expression at the level of the hypothalamus. Interestingly, a similar behavioural response in WT and nNOS KO mice was observed during forced swimming. Upon first view, this could indicate that a congenital absence of nNOS results primarily in an altered endocrine response. However, other studies suggested an altered emotional response and impaired spatial navigation in the Morris water maze in nNOS KO animals (Weitzdoerfer et al., 2004). The latter is of particular interest with respect to the substantial impact of the Morris water maze learning on endocrine parameters (Engelmann et al., 2006). Thus, further studies are required, e.g., using conditioned nNOS KO models to allow to separate more clearly emotional vs endocrine control of NO. A deeper knowledge of the cellular and molecular mechanisms underlying the action of NO on neuronal activation under stress conditions may be of help in broadening our understanding of the pathogenesis of mood disorders, such as depression, anxiety or anorexia nervosa (Gold et al., 1986; Kaye et al., 1987; for review, see: Yehuda et al., 1991; Scott and Dinan, 1998; Holsboer, 1999; Strohle and Holsboer, 2003; Shelton, 2004).

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