MINIREVIEW

dedicated to Prof. V. Schreiber

Stressor-Specific Activation of the Hypothalamic-Pituitary-Adrenocortical Axis

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Summary

New information has accrued from *in vivo* microdialysis studies about stress-related changes in norepinephrine concentrations in extracellular fluid of the paraventricular nucleus (PVN) and the activation of the hypothalamic-pituitary-adrenocortical (HPA) axis. Our data on the effects of lower brainstem hemisections show that paraventricular noradrenergic terminals are derived mainly from medullary A1 and A2 catecholaminergic cells. The activation of these cells contributes importantly to stress-induced noradrenergic activation in the paraventricular nucleus of conscious animals. The results from brainstem hemisection experiments also indicate that baseline levels and immobilization-induced increments in corticotropin-releasing hormone (CRH) mRNA expression in the PVN depend on ipsilaterally ascending medullary tract. Thus, the prevalent concept that stress-induced noradrenergic activation of the HPA axis depends mainly on activation of locus ceruleus noradrenergic neurons requires re-evaluation. Our new stress concepts favor stressor-specific activation of the HPA axis. The present data also suggest the existence of stressor-specific central pathways that differentially participate in the regulation of sympathoneuronal and adrenomedullary outflows as well as of the activity of the HPA axis. Furthermore, the results are inconsistent with a founding tenet of Selye's stress theory, the doctrine of nonspecificity, which defines stress as the nonspecific response of the body to any demand. We expect that future studies in this area will focus on further examination of the notion of stressor-specific patterns of central neurotransmitter release and elucidate the genetic bases of these patterns.

Selye's doctrine of nonspecificity

Despite the popularity of stress and stress-related diseases, no scientifically accepted definition of stress exists. This results in the view that stress can be virtually anything and contributes to virtually any disease in humans.

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Hans Selye deserves much of the credit for introducing the term stress and for popularizing the concept of stress in the scientific and medical literature. He defined stress as "the nonspecific response of the body to any demand upon it" (Selye 1950). The starting point for the elaboration of his stress theory was his article, published in *Nature* in 1936, describing a pathological triad (adrenal enlargement, gastrointestinal ulceration and thymolymphatic involution) that would result from exposure to any stressor (Selye 1936). However, it has to be emphasized that Selye's stress theory does not deny the existence of stressor-specific response patterns, only that they would not constitute stress, the shared nonspecific component.

Numerous previous and recent studies suggested differential responses of stress effector systems to various stressors, providing evidence against Selye's doctrine of nonspecificity (Roberston *et al.* 1979, Lachuer *et al.* 1991, Goldstein *et al.* 1992, Mitrakou *et al.* 1992). Currently, many scientists view stress as the occasion when the hypothalamic-pituitary-adrenocortical axis, represented mainly by elevated adrenocorticotropin (ACTH) levels, is activated (Ganong 1995). Other investigators suggest that activation of other systems with or without an elevation in ACTH may reflect stress-induced disturbed homeostasis (Vigaš 1985, Pacák *et al.* 1995a). Using an *in vivo* microdialysis technique, we investigated the effects of various stressors on norepinephrine release in the hypothalamic paraventricular nucleus and subsequent stressor-specific activation of the hypothalamic-pituitary axis. Based on our findings of stressor-specific neuroendocrine responses, we tested Selye's doctrine of nonspecificity of stress responses. Using some simplifying assumptions, our results refute the existence of a unitary stress syndrome.

Hypothalamic paraventricular nucleus

There is general consensus that the paraventricular nucleus of the hypothalamus is a focal point in the complex of interacting systems regulating stress responses. The rat paraventricular nucleus consisting of about 20 000 neurons contains more than 20 neuropeptides and putative neurotransmitters (Swanson *et al.* 1986, Palkovits 1988, Meister *et al.* 1990, Kiss *et al.* 1991). This nucleus is located in a triangular area in the midportion of the hypothalamus between the third ventricle and the fornix. It is divided into magnocellular, mediocellular, and parvicellular subnuclei. Among many neuropeptides and neurotransmitters located in the paraventricular nucleus, CRH (corticotropin-releasing hormone), vasopressin and oxytocin appear to play prominent roles in the responses to various stressors. From the medial parvicellular subdivision of the paraventricular nucleus, CRH neurons project to the median eminence where CRH enters the portal system and is released in the anterior pituitary where it stimulates ACTH release. CRH neurons also project to medullary and spinal cord autonomic centers where they participate in the regulation of peripheral sympathoneuronal outflow (Swanson *et al.* 1982). Magnocellular neurons in the paraventricular nucleus synthesize vasopressin and oxytocin that also participate in the regulation of the hypothalamic-pituitary-adrenocortical axis (Sawchenko *et al.* 1984, Kiss et al 1984, 1991, Lightman and Young 1987, Herman *et al.* 1989). The cerebral cortex provides indirect input to CRH-synthesizing cells in the hypothalamic paraventricular nucleus *via* the hippocampus and amygdala through the bed nucleus of the stria terminalis.

The paraventricular nucleus receives catecholaminergic and non-catecholaminergic innervation from several brain regions (for review see Pacák *et al.* 1995a). Catecholaminergic innervation of the paraventricular nucleus is derived mainly from cells in the ventrolateral and the dorsomedial medulla oblongata and from the locus ceruleus (Cunninghan and Sawchenko 1982, Sawchenko and Swanson 1982). The ventrolateral medulla oblongata contains three groups of cells that express catecholamine synthesizing enzymes: A1 and A5 noradrenergic cells, and C1 adrenergic cells (Armstrong *et al.* 1982, Kalia *et al.* 1985). Noradrenergic (A2) and adrenergic (C2) cells in the dorsomedial medulla oblongata are localized mainly in the nucleus of the solitary tract (Palkovits *et al.* 1992). From these areas two noradrenergic pathways, the ventral and dorsal noradrenergic bundles, ascend to the hypothalamus. Recently, using an *in vivo* microdialysis technique in the paraventricular nucleus with brainstem hemisections, we clarified the participation of brainstem and pontine catecholaminergic areas in norepinephrine release in the paraventricular nucleus under basal and stress conditions (Pacák *et al.* 1993). Ipsilateral brainstem hemisections between the A1 and A2 regions and the locus ceruleus (the ventral noradrenergic bundle transected) reduced basal extracellular norepinephrine levels in the paraventricular nucleus by 57 %, indicating important

noradrenergic innervation of the paraventricular nucleus from the lower brainstem but not from the locus ceruleus. Contralateral to the hemisection, baseline norepinephrine levels were decreased by only 26 %, suggesting that a portion of the ascending noradrenergic fibers may decussate below the level of the pons.

Central noradrenergic regulation of the hypothalamic-pituitary-adrenocortical axis

Abundant evidence indicates the participation of brain catecholamines in the regulation of the hypothalamic-pituitary-adrenocortical axis (Pacák et al. 1995a,b). This is further supported by the presence of noradrenergic synapses on CRH cells in the paraventricular nucleus (Liposits et al. 1986). Thus, surgical transection of the ascending ventral noradrenergic bundle markedly decreases CRH mRNA and CRH immunostaining in the ipsilateral paraventricular nucleus and the concentration of CRH in hypophyseal portal blood (Alonso et al. 1986, Sawchenko 1988, Szafarczyk et al. 1988). To understand better the central noradrenergic regulation of CRH in the paraventricular nucleus and the central nucleus of the amygdala (the region involved in the regulation of the hypothalamic-pituitary-adrenocortical axis), we have recently assessed the effects of ipsilateral surgical hemisections of the brainstem on expression of CRH mRNA in the paraventricular nucleus and the central nucleus of the amygdala under baseline and stress conditions (Pacák et al. 1996). Rats with brainstem hemisections had lower paraventricular CRH mRNA expression ipsilateral to the lesion and markedly blunted responses after 3 h immobilization. In contrast, neither hemisection nor immobilization affected CRH mRNA expression in the central nucleus of the amygdala. These results suggest a different role of norepinephrine in the regulation of CRH neurons in these two brain regions. In contrast to the paraventricular nucleus, norepinephrine in the central nucleus of the amygdala does not participate in CRH regulation and CRH neurons in this brain region do not participate in immobilization-induced activation of the hypothalamic-pituitary-adrenocortical axis.

Existence of stressor-specific neuroendocrine responses

Differences in the activation of the adrenomedullary and sympathoneuronal systems in response to different stressors provide some of the most convincing evidence for distinctive neuroendocrine stress response patterns. From the differential activation of these effector systems during exposure to different stressors, one would predict differential activation of central neural pathways. It has been unknown until recently whether there is stressor-specific activation of central noradrenergic systems underlying differential adrenocortical and sympathoneuronal responses to various stressors (Pacák et al. 1995b, 1998). This was partially due to the fact that the available literature has involved only few stressors to test the notion of stressor-specific activation of central neural pathways.

Using a microdialysis technique and peripheral plasma sampling, we simultaneously measured central (the paraventricular nucleus) and peripheral catecholamine release and activation of the hypothalamic-pituitary-adrenocortical axis in response to different stressors. Conscious rats were assigned randomly and exposed to one of several experimental stressors: 2 h immobilization; insulin injected i.v. at one of three doses (0.1, 1.0, and 3.0 IU/kg); exposure to cold at one of two temperatures (4 °C and –3 °C) ; hemorrhage of 10 % or 25 % of estimated blood volume (the latter producing hemorrhagic hypotension); formalin given s.c. at one of two doses (1 % and 4 %) to produce pain and tissue damage; physiological saline injected s.c. after handling in preparation for the injection. A painless i.v. injection of saline (without handling) was used as a control. An indwelling femoral arterial catheter served for plasma ACTH and catecholamine collection.

The microdialysis probe was perfused continuously at 1.0 µl/ min with artificial cerebrospinal fluid as described previously (Pacák et al. 1992). The acute experiment began with 30-min microdialysate baseline collection intervals, with a blood sample obtained at the middle of each interval. After baseline sample collection, each animal was exposed to a single stressor and several microdialysate collection periods followed, with arterial blood sampled in the middle of each interval. Animals that were exposed to cold were carefully transferred (less than 30 s) in their home cages (next to the cold chamber) into the cold chamber, with chamber temperature maintained at either 4 °C or –3 °C and they remained in the chamber for 3 h. Two hours of immobilization were performed according to the method of Kvetňanský and Mikulaj (1970), which consisted of taping each of the rat's limbs to a metal frame with hypoallergic tape.
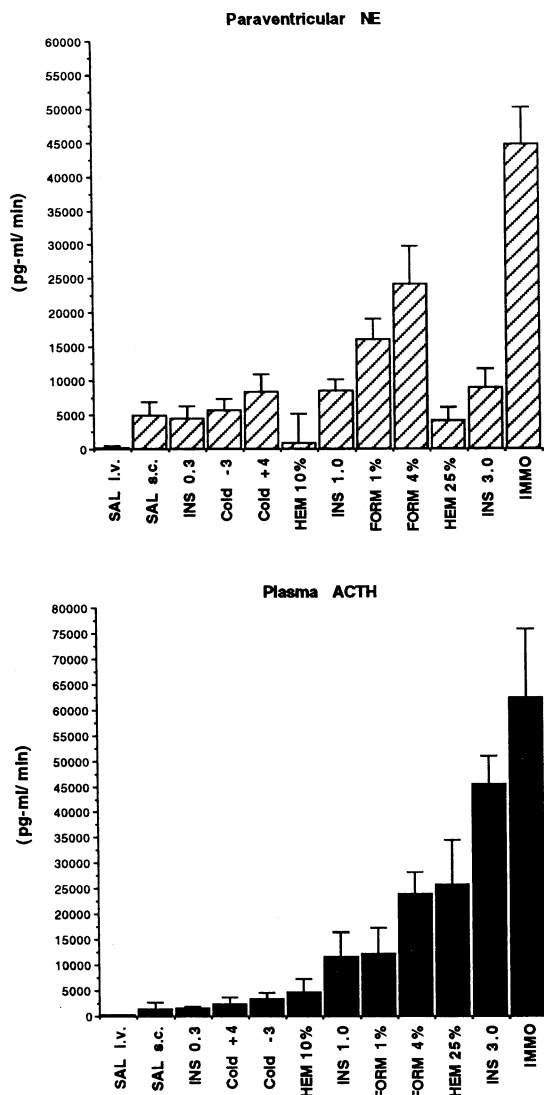
Fig. 1. *Responses of microdialysate norepinephrine (NE) in the paraventricular nucleus and plasma ACTH levels during exposure of conscious rats to various stressors. Each column represents the mean value for the net areas under the curve (AUCs) for particular stressor. In each animal the net AUC was calculated by subtraction of the baseline AUC from the total AUC. Vertical bars denote S.E.M.*

Responses of paraventricular microdialysate norepinephrine and plasma ACTH levels varied significantly across the stressors (Fig. 1). When compared to an i.v. saline injection, all of the stressors, at their highest intensity, significantly increased paraventricular microdialysate levels of norepinephrine and plasma levels of ACTH. Immobilization evoked large increases in paraventricular microdialysate norepinephrine and plasma ACTH, norepinephrine, and epinephrine levels. In

response to hemorrhage or hypoglycemia, relatively large increments in plasma ACTH levels were obtained compared to the modest increments in microdialysate norepinephrine levels. In response to pain (formalin injection), increments in ACTH levels were modest compared to the microdialysate norepinephrine levels. There were only small responses in paraventricular microdialysate norepinephrine and plasma ACTH levels during cold exposure. For all stressors except cold, mean plasma ACTH levels correlated positively with mean microdialysate NE levels. However, slopes of the regression lines differed substantially among stressors (Fig. 2). The correlation of plasma ACTH responses with stress-induced responses of the paraventricular noradrenergic system demonstrated a role of paraventricular norepinephrine in ACTH regulation and showed that norepinephrine participation in stress-induced HPA axis activation is stressor-specific.

These differences in stress responses lead to a new concept of stressor-specific activation of the hypothalamic-pituitary-adrenocortical axis and the existence of stressor-specific central neural pathways (Fig. 3). In response to hemorrhage and hypoglycemia, stressors with metabolic demands without involving marked discomfort or distress, ACTH release did not require a mechanism involving major activation of paraventricular norepinephrine release, suggesting that other (e.g. non-catecholaminergic) pathways exist to regulate ACTH release. In response to painful stimuli, however, a striking increase in paraventricular norepinephrine release, with relatively modest increments in plasma ACTH levels, indicated that paraventricular norepinephrine could be the most important neurotransmitter participating in the regulation of pain-induced ACTH release. The plasma ACTH response to immobilization, which involves both metabolic demands attending struggling and distress, was intermediate relative to paraventricular norepinephrine release, consistent with the view that immobilization-induced ACTH release is controlled by both paraventricular norepinephrine and other mechanisms.

Although individual differences could explain the weak or absent correlations between the effector responses, neither individual differences nor the existence of a unitary stress system would predict stressor-specific differences of effector system activation. Our studies tackled these difficult issues directly, by assessing ACTH as well as peripheral and central catecholaminergic responses to different intensities of various stressors, all
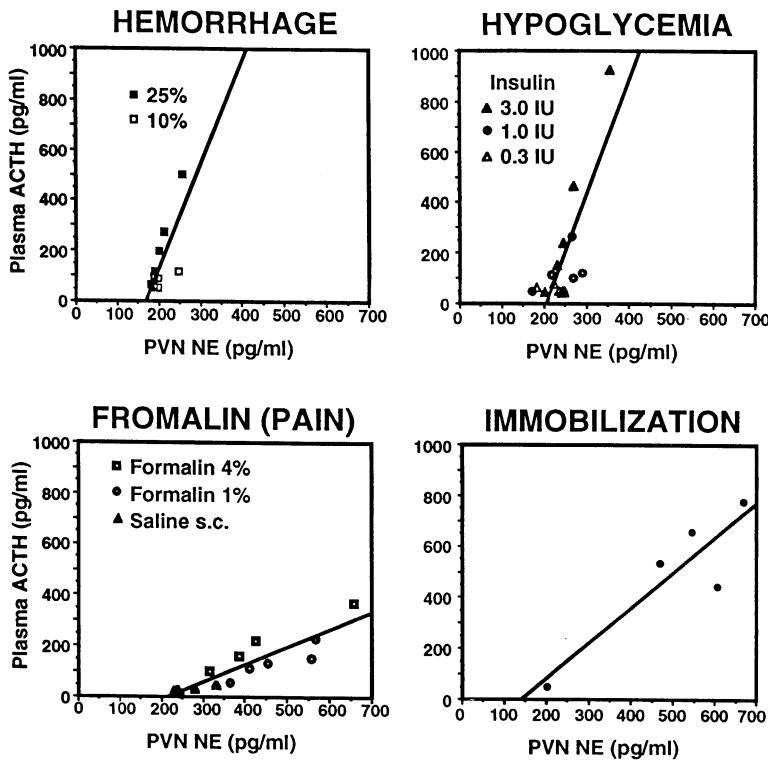
HEMORRHAGE

Plasma ACTH (pg/ml)

■ 25%
□ 10%

PVN NE (pg/ml)

HYPOGLYCEMIA

Insulin
▲ 3.0 IU
● 1.0 IU
△ 0.3 IU

PVN NE (pg/ml)

FROMALIN (PAIN)

Plasma ACTH (pg/ml)

□ Formalin 4%
● Formalin 1%
▲ Saline s.c.

PVN NE (pg/ml)

IMMOBILIZATION

PVN NE (pg/ml)

Fig. 2. *Scatter plots relating mean microdialysate NE levels in the paraventricular nucleus to mean plasma ACTH levels.*

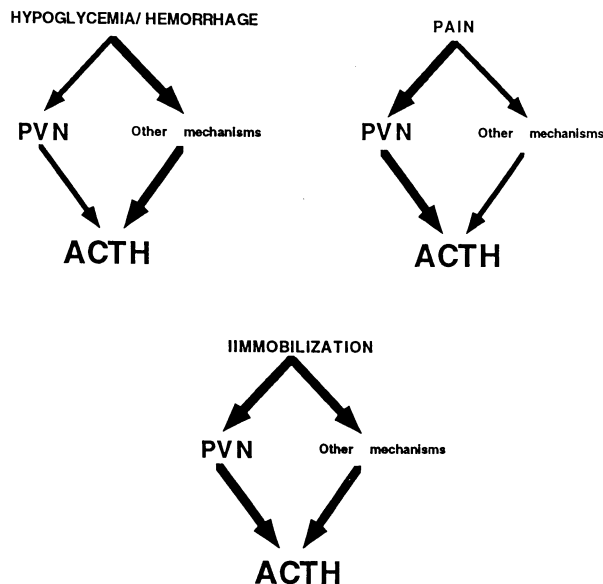HYPOTHESIZED ROLES OF HYPOTHALAMIC PARAVENTRICULAR NOREPINEPHRINE IN STRESS-INDUCED ACTH RELEASE

HYPOGLYCEMIA/ HEMORRHAGE

PVN   Other mechanisms

ACTH

PAIN

PVN   Other mechanisms

ACTH

IIMMOBILIZATION

PVN   Other mechanisms

ACTH

Fig. 3. *Proposed central mechanisms of ACTH regulation upon exposure to various stressors. PVN – hypothalamic paraventricular nucleus, HYP – hypoglycemia, HEM – hemorrhage, IMMO – immobilization.*

of which are known to evoke large increases in circulating ACTH levels and therefore evoke hypothalamic-pituitary-adrenocortical activation. We introduced a new alternative proposal that each stressor has a neurochemical "signature", with quantitatively, if not qualitatively, distinct central and peripheral mechanisms (Pacák et al. 1998). As originally described by Goldstein (1995), our alternative proposal views stress not as a noxious aspect of the environment, or a pathological response pattern, but as a condition where expectations, whether genetically programmed, established by prior learning, or deduced from circumstances, do not match current or anticipated perceptions of the internal or external environment. The resulting discrepancy between what is observed or sensed and what is expected or programmed elicits patterned, compensatory responses. From these considerations one would hypothesize the existence of distinctive patterns of activation of central neural pathways mediating both specific stress responses and nonspecific distress responses.

Therefore, our future studies will focus on delineating stressor-specific patterns of central neurotransmitter release and regional neuronal activation. This can be achieved, e.g. by the gene-targeting (knockout) method to replace the gene of interest with one that is altered or inactive. At the present time there is no knockout mouse model that could be characterized by increased susceptibility to stress-related disorders.

Nevertheless, CRH knockout mice often used in many stress laboratories are a first attempt at addressing this hypothesis (Majzoub et al. 1996). Another approach for mapping stressor-specific pathways is to use novel imaging methods such as positron emission tomography. Simultaneous recording of brain function and images obtained by positron emission tomography or observations of responses of stress effector systems after selective stimulation of various brain regions are undoubtedly further steps ahead in the description of stressor specificity and its application for future treatment of stress-related disorders.

Acknowledgements

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