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European Journal of Pharmacology 405 (2000) 161–166

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Stress-induced expression of co-localized neuropeptides in hypothalamic and amygdaloid neurons

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Accepted 28 June 2000

Abstract

This short review summarizes the effect of various stressful stimuli on the expression of neuropeptides which co-localize in corticotrophin releasing hormone (CRH)-synthesizing neurons in the hypothalamic paraventricular nucleus, as well as in oxytocin and vasopressin neurons in the supraoptic nucleus. Stress-induced changes failed to act on CRH neurons in the central amygdaloid nucleus but formalin-evoked pain enhanced galanin mRNA expression in the medial subdivision of this nucleus. Changes in the expression of enkephalin, galanin, dynorphin and cholecystokinin mRNA in response to restraint and formalin-induced pain are documented in hypothalamic and amygdaloid nuclei by in situ hybridization histochemical technique.

Keywords: Stressful stimuli; Neuropeptide; c-*fos*; Paraventricular nucleus; Supraoptic nucleus; Amygdaloid nucleus, central

1. Introduction

Different stressors elicit marked heterogeneity of neuronal, neuroendocrine and behavioral (Gaillet et al., 1991; Pacak et al., 1998) responses. Each stressor may activate brain structures and differs in their evoked responses in the expression of neuropeptides in hypothalamic, limbic and autonomic neurons. Consequently, they differ also in their neuronal circuits.

Immunohistochemical studies demonstrated that a variety of neuropeptides are present in neurons of parvicellular subdivisions of the paraventricular nucleus in co-localization with corticotrophin releasing hormone (CRH). Enkephalin, galanin, vasoactive intestinal polypeptide (VIP), neurotensin, cholecystokinin have been localized in the medial parvicellular subdivision (Ceccatelli et al., 1989). The magnocellular neurons in the paraventricular nucleus and the supraoptic nucleus express dynorphins (co-localized with vasopressin), cholecystokinin and enkephalin (co-localized with oxytocin) genes (Weber et al., 1982; Vanderhaeghen et al., 1983; Watson et al., 1983; Sherman et al., 1986; Lightman and Young, 1987).

In previous studies, we analyzed six different stressors (immobilization, formalin-induced pain, insulin hypoglycemia, hemorrhage, cold and audiogenic stress) under acute conditions by recording such parameters as plasma adenocorticotropic hormone (ACTH), corticosterone, norepinephrine and epinephrine levels, investigated c-*fos* activation by Fos immunostaining and applied immunostaining and in situ hybridization histochemistry for various neuropeptides in hypothalamic, limbic and brainstem neurons (Pacak et al., 1995, 1998; Palkovits et al., 1995, 1996). Immobilization, audiogenic stress, and formalin-pain resulted in intense c-*fos* and CRH activations in the parvicellular subdivisions of the paraventricular nucleus, cold stress and insulin failed to have such effect. In contrast, hemorrhage induced c-*fos* expression in the magnocellular portion of the paraventricular nucleus (Palkovits et al., 1995).

2. Stress-induced expression of neuropeptides in the paraventricular nucleus

Afferent stressors elicited different c-*fos* activation in the paraventricular nucleus. Immobilization, a mixture of physical and psychological stressors including decreased body temperature and pain represents a strong stressful stimulus. One to three hours immobilization elicit signifi-

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00549-5

cantly increased CRH mRNA expression in the paraventricular nucleus (Darlington et al., 1992; Bartanusz et al., 1993; Ceccatelli and Orazzo, 1993, Harbuz et al., 1993; Kalin et al., 1994; Pacak et al., 1996). Exposure to acute immobilization stress also increased CRH receptor mRNA levels in the paraventricular nucleus (Luo et al., 1994).

It is well established that vasopressin mRNA is present not only in the magnocellular but in parvicellular paraventricular neurons. Immobilization stress has been shown to increase vasopressin mRNA expression and vasopressin potentiate CRH to release ACTH from the anterior pituitary (Bartanusz et al., 1993; Herman, 1995).

Formalin-induced pain and audiogenic stress are strong stimuli which induce immediate c-*fos* expression in the parvicellular subdivisions of the paraventricular nucleus

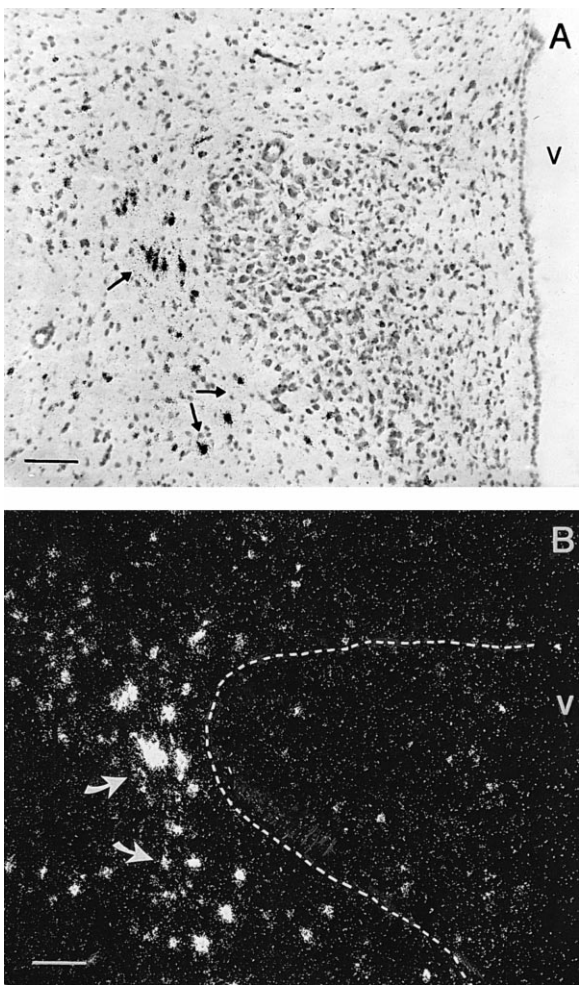

Fig. 1. Expression of enkephalin mRNA in neurons in and around the paraventricular nucleus. Coronal sections through the midportion of the nucleus, bright (A, C) and dark field (B, D) microphotographs. (A and B) intact control rats, (C and D) rats subjected to formalin stress. Highly labeled cells lateral and ventral to the paraventricular nucleus (arrows) were present in the intact rat and remained unaltered after stressful stimuli. Formalin elicited enkephalin mRNA expression in the parvicellular paraventricular nucleus neurons. (The paraventricular nucleus is outlined by dotted lines on Fig. B.) V = third ventricle. Bar scales: 100 μm.

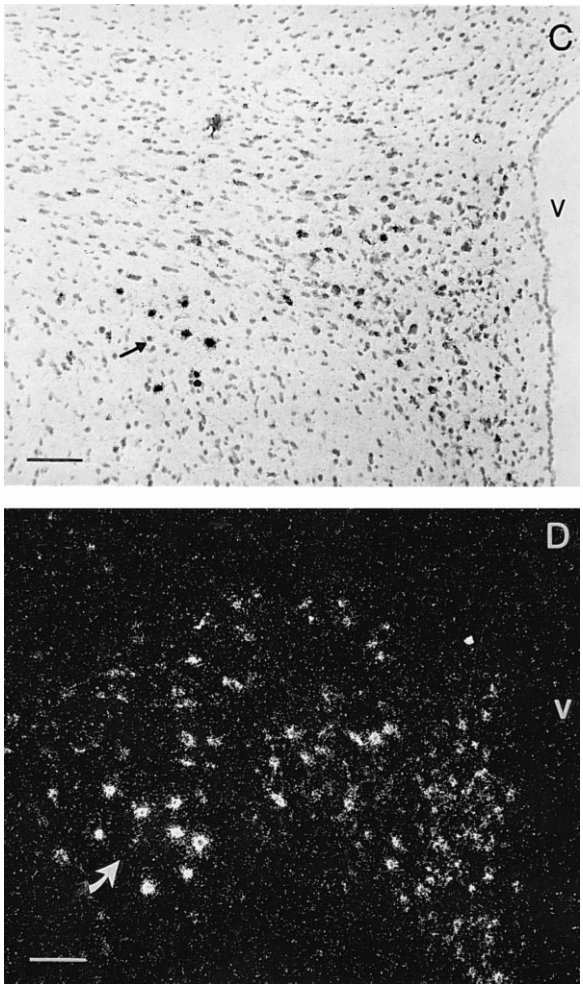

Fig. 1 (*continued*).

(Palkovits et al., 1995). Enhanced expression of CRH mRNA was also observed in these subdivisions 30–90 min after formalin injection indicating that paraventricular nucleus neurons were influenced by painful stimuli (Palkovits et al., 1999).

Enkephalin is expressed in neurons of several hypothalamic nuclei. In the parvicellular paraventricular nucleus, enkephalin is co-localized with CRH (Lightman and Young, 1987; Ceccatelli et al., 1989; Pretel and Piekut, 1990).

Some stressors, but not all, known to induce CRH gene expression, have been shown to activate also ENK gene expression in the parvicellular paraventricular nucleus (Lightman and Young, 1987; Harbuz and Lightman, 1989; Harbuz et al., 1994). Ceccatelli and Orazzo (1993) examined changes in expression of thyreotrop releasing hormone, enkephalin and neurotensin mRNA levels in the paraventricular nucleus, after immobilization but they found relatively slight changes.

In our experiments, injections of 4% formalin were associated with significant increases in enkephalin mRNA

levels in the parvicellular paraventricular nucleus. These enhanced expressions were observed only in the medial and dorsal parvicellular neurons (Fig. 1C and D) where enkephalin is co-localized with CRH (Ceccatelli et al., 1989). Enkephalin-synthesizing neurons in other hypothalamic nuclei remained also silent. Furthermore, enkephalin-positive neurons which are present immediately lateral and ventral to the paraventricular nucleus and show high enkephalin mRNA expression in intact, unstressed animals (Fig. 1A and B) did not show any changes after formalin injection (Fig. 1C and D). In contrast, parvicellular paraventricular nucleus neurons that were almost unlabeled for enkephalin mRNA in unstressed animals, established a significant expression 60 min after subcutaneous injection of 4% formalin (Fig. 2). Enkephalin-immunoreactive neurons in the parvicellular subdivisions of the paraventricular nucleus project to the external zone of the median eminence (Merchenthaler, 1992). Enkephalin-synthesizing neurons around the paraventricular nucleus fail to have such projections (no labels of retrograde tracers from the median eminence). Thus, they may not participate in the hypothalamo–pituitary–adrenal (HPA) regulatory axis.

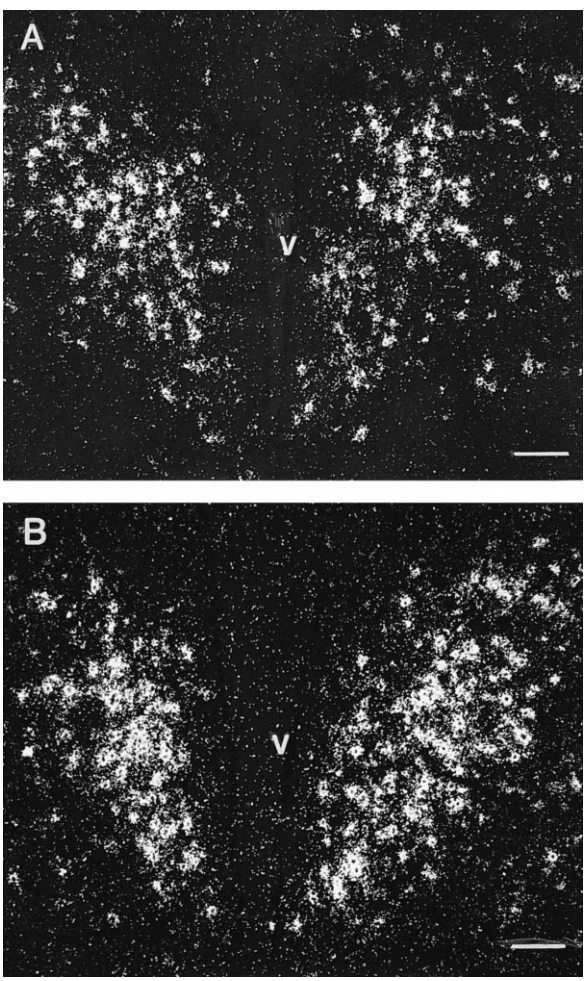

Fig. 2. Expression of galanin mRNA in the parvicellular paraventricular neurons following formalin (A) and immobilization (B) stress. V = third ventricle. Bar scales: 100 μm.

No significant differences in hypothalamic ENK mRNA expression were detected in response to cold or restraint stress (Lightman and Young, 1989). In our experiments, immobilization (Rusnak et al., 2000) and formalin-induced pain elicited high ENK mRNA levels in the parvicellular paraventricular nucleus.

Repeated immobilization (daily 2 h immobilization for 7 weeks) increased both CRH and enkephalin mRNA levels in the paraventricular nucleus but they did not exceed the levels that was elicited by a single immobilization. An additional stress induced further increase in the expression of ENK mRNA in the parvicellular paraventricular nucleus, but it failed to cause any significant changes in CRH mRNA levels in the paraventricular nucleus (Rusnak et al., 2000). Thus, synthesis of the co-localized enkephalin seems to be rather independent of CRH synthesis, at least in such an extreme condition.

By giving rats daily intraperitoneal injections of hypertonic saline for up to 12 days, Young and Lightman (1992) induced a marked increase in enkephalin expression in magnocellular neurons in the paraventricular nucleus and the supraoptic nucleus.

In agreement with previous findings, galanin mRNA signal was evident in the paraventricular nucleus, supraoptic, dorsomedial, arcuate nuclei, the lateral hypothalamus and the medial subdivision of the central amygdaloid nucleus. It has been proposed that hypothalamic galanin may be involved in stress response (Koenig et al., 1991; Akabayashi et al., 1994). In our experiments, formalin injection (Fig. 2A) and 3 h immobilization (Fig. 2B) elicited marked increases in galanin mRNA levels in the parvicellular paraventricular nucleus. Enhanced galanin gene expression was also seen in other hypothalamic (dorsomedial, arcuate, lateral hypothalamic) neurons in response to formalin-induced pain stimuli.

Reportedly, dynorphin mRNA levels are increased in the paraventricular nucleus in response to stress (Lightman and Young, 1987). In our studies, dynorphin gene expression was induced by immobilization, formalin injections and hemorrhage. Cold and insulin hypoglycemia failed to elicit measurable changes in dynorphin mRNA levels in the parvicellular paraventricular nucleus.

Insulin-induced hypoglycemia activates vasopressin in CRF neurons in the parvocellular subdivision of the paraventricular nucleus (Whitnall, 1989). In these rats, other co-localized neuropeptides (galanin, enkephalin, dynorphin) fail to show any changes in response to insulin-induced hypoglycemia.

3. Stress-induced expression of neuropeptides in the supraoptic nucleus

Immobilization, formalin, hemorrhage and audiogenic stress elicit c-*fos* expression in the supraoptic nucleus

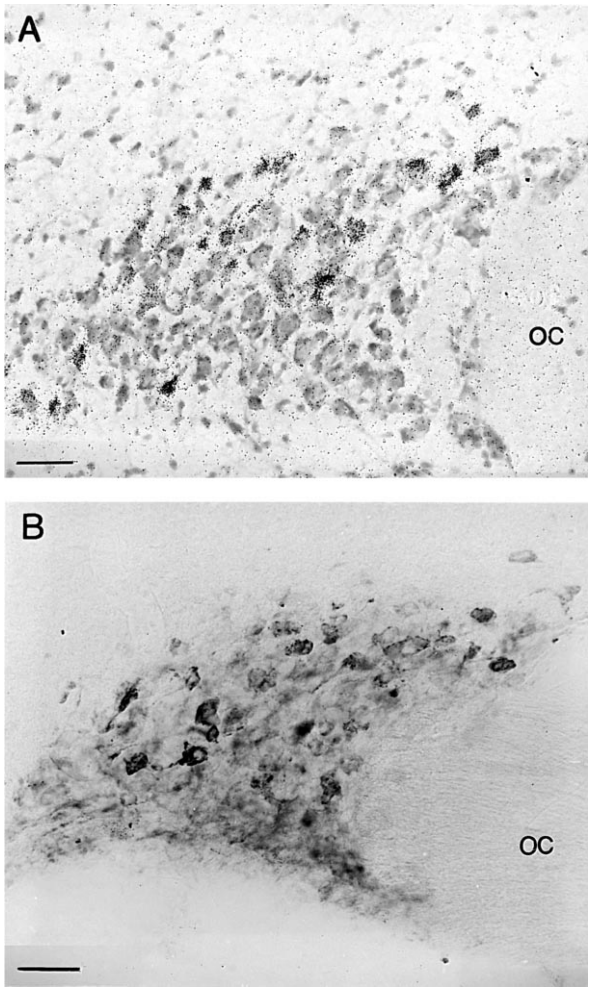

Fig. 3. Supraoptic nucleus, coronal sections. (A) Expression of cholecystokinin mRNA (A) after formalin-induced pain stress. (B) Dynorphin B immunopositive neurons after repeated immobilization. (OC) Optic chiasm. Bar scales: 100 μm.

(Miyata et al., 1995; Palkovits et al., 1996). While hemorrhage was mainly effective on vasopressin-synthesizing neurons in the ventral portion of the nucleus, the other stressors increased Fos immunopositivity mainly in the dorsal, oxytocin-synthesizing cells.

Despite stress-conduced c-*fos* activation in the supraoptic nucleus immobilization did not associate with significant increases in vasopressin mRNA in the supraoptic nucleus (Bartanusz et al., 1993; Herman, 1995). In our studies, only hemorrhage has been shown to increase vasopressin mRNA expression, while the other stressors failed to elicit it.

The gene expression of some other co-localized neuropeptides, however, altered significantly in the supraoptic nucleus after certain stressful stimuli. A number of supraoptic neurons showed dynorphin immunopositivity after repeated immobilization (Fig. 3B), and increased cholecystokinin (Fig. 3A) and galanin (not shown) mRNA expressions were observed 60–120 min after subcutaneous injection of formalin into the hind paw of the rat. Dynorphins are co-expressed with vasopressin in the supraoptic nucleus (Watson et al., 1982; Weber et al., 1982; Sherman et al., 1986). Combined surgical and neurochemical experiments showed that dynorphin and α-neoendorphin in the posterior pituitary are in processes of supraoptic neurons. These neurons also project to the median eminence (Palkovits et al., 1983). Four days after unilateral transection of the supraoptico-hypophyseal tract, dynorphin-immunoreactive material and preprodynorphin mRNA appeared in supraoptic neurons ipsilateral to the knife cut (Palkovits, 1995).

4. Stress-induced expression of neuropeptides in the central nucleus of the amygdala

The central nucleus of the amygdala contain CRH neurons which occupy the intermediate subdivision of the nucleus. It has been assumed that CRH neurons in the amygdala are involved in fear-related behavior in response to stressful stimuli (Davis, 1992; Menzaghi et al., 1993; Makino et al., 1995). Recent studies indicate that psychological stress represents a highly significant impact on the CRH mRNA levels in the central amygdala (Makino et al., 1999).

While neurons in the medial amygdaloid nucleus responded to stressful stimuli with increased expression of c-*fos* in our studies, neurons in the central nucleus reacted only moderately. After 3 h immobilization, c-*fos* immunoreactivity was found in the lateral subdivision of the nucleus. These cells, however, as it is indicated by double immunostaining, did not correspond to CRH-synthesizing neurons (Fig. 4). Immobilization did not elicit any changes in c-*fos* activation in the central amygdaloid nucleus (Arnold et al., 1992; Chen and Herbert, 1995; Pacak et al.,

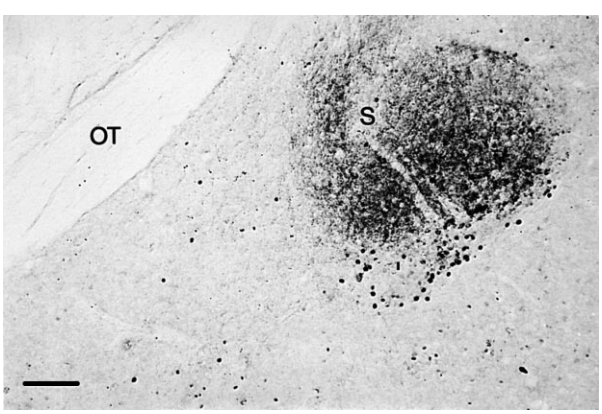

Fig. 4. Amygdala. Coronal section, double immunostaining for c-*fos* and CRH. Immobilization stress-induced c-*fos* expression in the ventral marginal and lateral subdivisions of the central amygdaloid nucleus. Area occupied by activated neurons does not correspond to the CRH-positive area in the nucleus. Abbr.: OT — optic tract, S — stria terminalis. Bar scale: 100 μm.

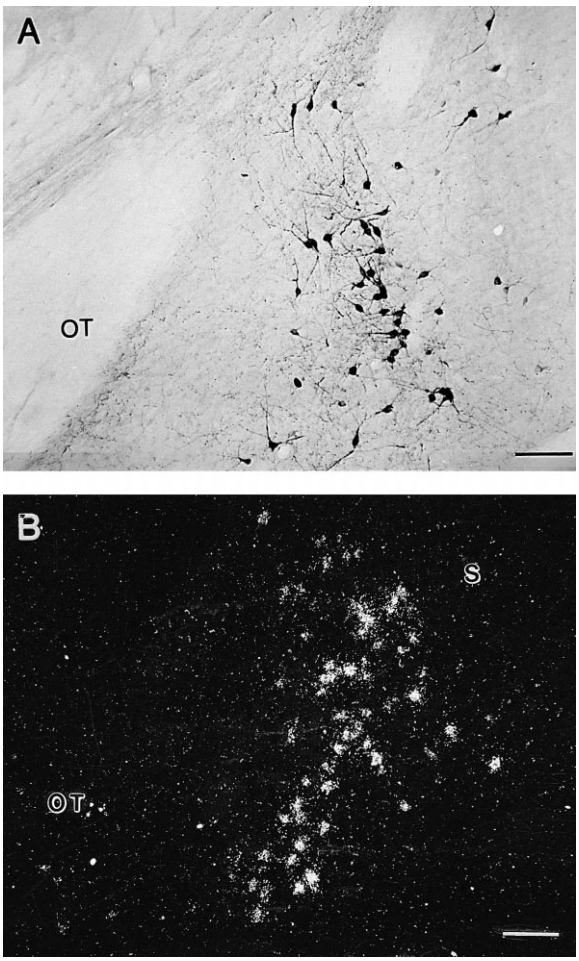

Fig. 5. (A) Galanin immunopositive neurons in the medial subdivision of the central amygdaloid nucleus. (B) Expression of galanin mRNA in the central amygdala after formalin stress. Abbr.: OT — optic tract, S — stria terminalis. Bar scales: 100 μm.

1996; Senba and Ueyama, 1997). Immobilization stress failed to affect CRH receptor mRNA expression in the amygdala (Makino et al., 1995). In contrast, others demonstrated increased CRH mRNA expression in the central nucleus of the amygdala immediately after 1 or 2 h of immobilization stress (Kalin et al., 1994; Mamalaki et al., 1992).

The amygdala has been considered to play a role in stress-related changes in pain sensitivity. A dense accumulation of c-*fos* positive cells were found in the lateral but not the intermediate subdivision of the central amygdaloid nucleus after formalin stress (Palkovits et al., 1996).

CRH mRNA expressions in the central nucleus of the amygdala were upregulated in rats with bilateral lesions of the paraventricular nucleus (Palkovits et al., 1998). This observation suggests that paraventricular nucleus-central amygdala connections are likely to be inhibitory. The bilateral neuronal connections between these two neuronal cell groups have been demonstrated by tract-tracing techniques (Gray et al., 1989; Palkovits et al., 1998).

Several other neuropeptides are synthesized by central amygdaloid neurons. Specific neurotransmitters that respond to stressful stimuli are unknown. Galanin-synthesizing cells occupy the medial subdivision of the nucleus (Fig. 5A). These cells slightly reacted to stressful stimuli: immobilization stress, formalin injection (Fig. 5B) and cold stress increased galanin mRNA levels in these neurons. We did not find any changes in the expression of somatostatin and neurotensin mRNA of the central amygdaloid nucleus in response to any acute stressful stimuli applied in our experiments. It is worth to note that insulin activated a small group of the central amygdaloid neurons as it is indicated by an increased c-*fos* expression in the most ventral neurons of the nucleus (Palkovits et al., 1996).

5. Conclusion

Observations summarized here support the view that so-called co-localized neuropeptides in hypothalamic neurons may participate in the organization of responses to stressful stimuli. In response to certain stressors, these neuropeptides may act in accordance to CRH, but there are evidences for their individual responses to some specific stressors like formalin-evoked painful stimuli.

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