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Research Report

Increased susceptibility to transcriptional changes with novel stressor in adrenal medulla of rats exposed to prolonged cold stress

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Abstract

The response to stress is influenced by prior experience with the same or different stressor. For example, exposure of cold pre-stressed rats to heterotypic (novel) stressors, such as immobilization (IMO), triggers an exaggerated release of catecholamines and increase in gene expression for adrenomedullary tyrosine hydroxylase (TH), the rate limiting catecholamine biosynthetic enzyme. To study the mechanism, we examined induction or phosphorylation of several transcription factors, which are implicated in IMO-triggered regulation of TH transcription, in rats exposed to cold (4 °C) for up to 28 days and then subjected to IMO. Levels of c-*fos* increased transiently after 2–6 h and returned to basal levels after 1–28 days cold stress. Fra-2, was unaffected by short term cold, but was induced about 2-fold by 28 days continual cold. In contrast, there were no significant changes in CREB phosphorylation or Egr1 induction. Rats, with and without pre-exposure to 28 days cold, were subjected to single IMO for up to 2 h. Phosphorylation of CREB after 30 min IMO was greater in cold pre-exposed rats. Induction of Egr1 was three times higher in cold pre-exposed rats and remained significantly elevated even 3 h after cessation of IMO. Exposure to IMO triggered a 10–20-fold elevation in Fra-2 in both groups, which was even higher 3 h after the IMO. However, Fra-2 was more heavily phosphorylated following IMO stress in cold pre-exposed animals. The results reveal that sensitization to novel stress in cold pre-exposed animals is manifested by exaggerated response of several transcription factors.

Theme: Neural basis of behavior
Topic: Stress

Keywords: CREB; Egr1; Fra-2; c-*fos*; Adrenal medulla; Stress; Tyrosine hydroxylase

1. Introduction

Stress elicits important adaptive changes which enable the organism to cope with environmental challenges [12,43]. The adrenergic/noradrenergic systems in the periphery and the brain together with the hypothalamic pituitary adrenocortical (HPA) axis play key roles in responding to stress. Prolonged or repeated stress is well recognized as a major risk factor for many disorders including cardiovascular diseases and psychiatric illnesses, such as hypertension, myocardial infarction and depression. In addition, stress alters the progression of other serious illnesses, such as diabetes and cancer (reviewed in [6,26]).

One of the important and complex issues of stress research is that the response to the same physiological stressor is not always identical. It is influenced by many factors, including perception of the stress, personal behavior (diet, drugs, smoking), individual differences, including genetic factors, and prior experience with the same or other

Abbreviations: AP1, activating protein1; CRE, cAMP response element; CREB, cAMP response element binding protein; DBH, dopamine β-hydroxylase; Egr1, early growth response gene1; Fra, fos-related antigen; GAPDH, glyseraldehyde-3-phosphate dehydrogenase; HPA, hypothalamic pituitary adrenocortical axis; IMO, immobilization stress; P-CREB, phosphorylated CREB; PTSD, post-traumatic stress disorder; TH, tyrosine hydroxylase; WKY, Wistar Kyoto rat

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stressors (reviewed in [25]). For example, when exposed to a heterotypic (novel) stressor, an exaggerated activation of sympathetic-adrenomedullary responses, as indicated by a greater rise in plasma norepinephrine and epinephrine, is observed in animals pre-exposed and adapted to a different stressor [16]. Sensitization of the stress response is thought to be one of the mechanisms underlying the onset and/or recurrence of some stress-related disorders [4,13,14,39].

Enhanced adrenergic/noradrenergic activity and/or HPA axis activity and reactivity have been reported in some psychopathological states, particularly in depressive and anxiety disorders [4,14,39]. Patients with post-traumatic stress disorder (PTSD), while displaying low plasma cortisol, have increased urinary and plasma concentrations of norepinephrine and/or epinephrine compared to controls with non-trauma-related anxiety [44,48]. These individuals are also more sensitive to stress and display increased startle responses. Catecholamines, together with glucocorticoids, are involved in mediating the "flash bulb" memories associated with strong emotions. The encoding and retention of memories are strengthened by peripheral administration of epinephrine [11,45]. It is speculated that stress-triggered elevations in norepinephrine may be related to the subsequent symptoms of re-experiences, intrusive memories and nightmares in PTSD (reviewed by [44]).

The chromaffin cells of the adrenal medulla synthesize the catecholamines, epinephrine and norepinephrine, and are the major source of epinephrine in the periphery. A number of earlier studies revealed that activity and gene expression of catecholamine biosynthetic enzymes are increased in adrenal medulla in response to numerous types of stress, including physical (restraint, immobilization), metabolic (hypoglycemia) and environmental (cold) (reviewed in [18]).

Transcriptional regulation of adrenomedullary catecholamine biosynthetic enzymes, such as tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH), was shown to be a prominent mechanism mediating the stress response. TH and DBH transcription, as measured by run-on assays, are significantly elevated by immobilization stress (IMO) [32]. In addition, acute and repeated IMO or three days cold stress led to substantial elevations of reporter activity under control of the TH promoter in the adrenals of transgenic mice [35]. Acute IMO elicits rapid activation or induction of several transcription factors which can regulate TH transcription. Phosphorylation of cAMP response element (CRE) binding protein (CREB) on Ser-133, which is needed for transactivation, is observed within 5 min and can stimulate TH transcription at the perfect consensus CRE [40]. Subsequently, a single IMO or cold stress triggers induction of expression of AP1 factors, especially c-*fos* [28,46]. With single IMO, Fos-related antigen 2 (Fra-2) and Egr1 are also induced [31,33,36].

With repeated daily IMO, the transcriptional activation is more sustained and associated with induction of different combination of transcription factors. There is no longer induction of c-*fos*, while Fra-2 becomes a predominant fos-family member induced by stress and has been proposed to mediate the more sustained elevation in TH and DBH mRNAs, proteins and enzymatic activities in adrenal medulla with repeated IMO [33]. Egr1 is also induced with long term repeated IMO [36,47]. Interestingly, there was no attenuation of the adrenomedullary response to IMO. Even a 42nd daily consecutive IMO still significantly elevated TH mRNA and triggered a robust induction of Fra-2 [22,23].

In contrast to the response to IMO, the effect of prolonged cold stress on these transcription factors has not been previously studied. TH mRNA levels and activity in adrenal medulla after a month or more of prolonged cold stress are no longer different from values in untreated animals [20,21]. Nevertheless, when these cold pre-exposed rats were exposed to a heterotypic stress, there was an exaggerated response. When rats exposed to 4 weeks cold were subjected to IMO, the elevation of plasma norepinephrine was more than double that in naïve animals. The initial elevation of plasma epinephrine was the same in both groups of rats; however, by 1 h, it was declining in the naïve animals and remained high for the entire 2 h IMO in the cold pre-exposed rats [8]. Higher plasma levels of norepinephrine and epinephrine were also observed in response to restraint stress in rats pre-exposed to cold swim stress daily for 26 days [16]. Several heterotypic stressors such as IMO, insulin-induced hypoglycemia or glucopenia after 2-deoxyglucose administration, also lead to an exaggerated elevation in TH mRNA in the cold pre-exposed animals [21].

The mechanism for the exaggerated response is unclear. Long term cold stress may alter the pathways or factors which, while not sufficient by themselves to trigger the changes in gene expression, facilitate the response to the novel stressor. The present study investigates the effect of acute and prolonged cold stress on alterations in transcription factor phosphorylation/expression, which were shown to regulate TH transcription. In addition, we investigated the mechanism of sensitization to a heterotypic novel stressor (IMO) in cold pre-exposed rats. The results reveal that the exaggerated response to the heterotypic stressor is manifested in terms of transcription factor induction or activation, and thus is likely to have broad physiological consequences.

2. Material and methods

2.1. Animal procedures

All animal experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee. Male, murine pathogen-free, Sprague–Dawley rats (280–320 g) were obtained from Taconic Farms (Germantown, NY, USA) or Charles River (Suzfild, Germany). The animals were maintained under controlled conditions of a 12 h light–dark cycle (lights on from 7 am