

Novel Hippocampal Interaction between Spexin and Corticotropin Releasing Factor

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Nowadays, people pay more and more attention to homeostatic regulation, which is the detrimental effect of stress on physiological and psychological well-being and cannot be ignored. The public perception of anxiety has been associated with the hypothalamic hormones, because of the pivotal role of the hypothalamic-pituitary-adrenal axis to promote the pituitary-adrenal functions and endocrine responses. Although modulation of learning and memory seems to be one of the major roles of corticotropin releasing factor (CRF) in rodent and human brain [1], increasing evidences suggest that CRF has an involvement in the development of anxiety-related and mood disorders. Especially, CRF treatment can directly induce anxiety at a high dose [2]. In the *in vivo* mouse hippocampal study, stress could increase CRF mRNA expression at least 5-fold in various brain areas including olfactory bulb, hippocampus, hypothalamus, hypophysis, striatal and prefrontal cortex in mice [3]. By contrast, Bale and colleagues have found that dysregulation of CRF or its family members in stress responsivity can lead to the onset of anxiety-like behaviors and depression [4], further indicating the importance of CRF in the regulation of anxiety and depression. Spexin (SPX) is a novel neuropeptide with multiple functions in central effects [5]. Meanwhile, the transcript and protein signals of SPX are found to be widely located in various brain areas and brain nuclei [6], where the up-regulation in the hippocampus and striatum but down-regulation in the hypothalamus has been reported in the rat with chronic treatment of escitalopram, a depression- and anxiety-related serotonin reuptake inhibitor [7]. Thus, this has raised the concern for a possible link of SPX with mood disorder/related psychiatric diseases. In hippocampus, the activation of CRF receptor (CRFR) enhances

learning indicated by fear conditioning [8]. In this mouse hippocampal model, it is clear that the negative correlation exists between the co-expressed hippocampal CRF and SPX in the stress state [3]. The authors are first to provide evidence that CRF shows an inhibitory role in the regulation of SPX expression in hippocampus [3].

The organization of hippocampus as small neurons distributed all over this tissue may hinder the viability of the *in vitro* study based on the hippocampus model. Cell lines obtained from central nervous system have limitations because these neurons are not ideal for the restoration and manifestation of characteristics from intact central neurons, including the regenerative ability to form well-defined axons, dendrites and synapses. Therefore, primary mouse hippocampal cell culture techniques have been adopted to study these neurons *in vitro*. In the article by Beaudoin III and colleagues [9], the method for mouse hippocampal neuron isolation was described with great details and designed to permit functional testing. This is supported by the *in vitro* mouse hippocampal study with constantly stable CRF-reduced SPX responses.

To demonstrate the effects of CRF treatment on SPX mRNA expression, mouse hippocampal cells were challenged with CRF in the dose- and time-dependent manner. The mediation of CRF receptors 1 and 2 (CRFR1 and CRFR2) contribute to the stress responses during adult life, their persistent sensitization is related to the sustained stress exposure in childhood [10]. As shown in other studies, transgenic mice with global CRF overexpression is manifested with evoked anxiety-like defensive behavior, which also leads to the Cushing's syndrome-like phenotype [11]. Bale et al. found that while

the mutation of different CRFR isoforms exert opposite behavioral effects that the stress response in CRFR1-mutant mice is depleted with an anxiolytic-like behavior while the stress response in CRFR2-mutant mice is reinforced with anxiogenic-like behavior; the basal feeding and weight gain remain unchanged in both CRFR-mutants, yet CRFR2-mutant mice exhibit decreased food intake following a stress of food deprivation [12]. Accumulative studies have also indicated that CRF could initiate anxiety-like defensive responses via both CRFR1 and CRFR2 signaling in an associative pattern [1]. Thus, the pharmacological approach was to determine the involvement of the CRFR(s) in this inhibitory effect induced by CRF treatment on SPX mRNA expression, and the inhibitory effect of CRF treatment on mouse hippocampal SPX expression was found to be mediated specifically by CRFR2 but not CRFR1 by using CRFR1 and CRFR2 specific blockers. In previous studies, the functional role of CRFR2 in stress responsiveness has been controversial. Nevertheless, the convincing evidence has shown that CRFR2 play a role in mediating stress behaviors. The approach-avoidance conflict paradigms in the elevated plus maze and open field tests in mice has shown that the constitutive gene deletion of CRFR2 result in either increased or normal anxiety-like defensive behaviors basally or during stress [4,11,13,14], which further supported the pivotal mediatory role of CRFR2 in hippocampal SPX regulation.

Since the transmembrane and intracellular domains of both CRF1 and CRF2 are highly homologous, studies summarized by Hauger et al. have shown the two CRFR subtypes coupling to the same G_{α} proteins activate signaling via similar second messengers [15]. There is a consensus among researchers that the dominant mode of CRFR signaling is activated via the adenylyl cyclase (AC)-protein kinase A (PKA) pathway [16,17]. AC/cAMP/PKA pathway has been reported to couple with the activated CRFR, while the involved mitogen-activated protein kinase (MAPK) cascades may be cAMP-independent in the CRFR signaling [18]. With the over-expression of CRFR2 in HEK293 cells, AC/cAMP/PKA pathway was found to be coupled with CRFR2 in the CRF-reduced SPX promoter activity by using pharmacological approach [3]. Although CRFR can be mediated through MAPK cascades including extracellular signal-regulated kinase (ERK), p38 and c-JUN N-terminal kinase (JNK) components, ERK MAPK is the most active signaling component for CRFR activated by mitogen-activated kinase (MEK) [18,19], which deserves to be focused as the target pathway to be tested in the subsequent studies. This ERK_{1/2} signaling was tested in the primary mouse hippocampal cell culture as well as the HEK293 cell line with CRFR2 over-expression. While a consensus on the basic features of cAMP signaling has been reached,

there is a necessity to detect which downstream signaling components contribute to the activation of the MAPK cascades. Exchange protein directly activated by cAMP (Epac) is a guanine nucleotide exchange factor for the small GTPases Rap1 and Rap2. In locus coeruleus neurons, CRFR-mediated cAMP production can further activate Epac instead of PKA [20]. Since Epac is also known to mediate cAMP crosstalk with the MAPK cascades and potentiate BDNF-stimulated TrkB signaling via CRFR actions [20], it is tempting to speculate that Epac may be involved in CRFR-coupled signal transduction targeting on the regulation of SPX transcription. In this way, the idea is supported by the findings based on cAMP analogs with differential selectivity for PKA and Epac. By using the pharmacological approach, the cross-talk between the cAMP/Epac and ERK_{1/2} signaling pathways were also found in the CRF-inhibited SPX promoter activity, as Epac was also shown to worked as the upstream signal component of MEK_{1/2}. Although this CRF-reduced SPX transcription study has provided great evidence for differential crosstalk of PKA and Epac with the MARK cascades, the possibility cannot be excluded that direct stimulation of MARK signaling may also be achieved by CRFR activation, e.g., by signal transduction via $G_{\beta\gamma}$ protein [21].

In previous reports in women as well as in lower vertebrates (e.g., goldfish), the studies on SPX regulation have focused on SPX secretion/transcript expression [22,23] and no information is available for CRF regulation of SPX gene transcription; thus, this study on SPX transcription can be considered as a milestone to date. Of note, the demonstration that the luciferase luminescence correlates with the reduced SPX gene expression levels determines the more detailed mechanisms for CRF effects on hippocampal cell function. The precise mechanisms of SPX promoter activity, however, are based on the lipofectamine-based transfection of luciferase reporter in HEK293 cells. The limitations for the transcriptional study in mouse hippocampal model are those which generally apply to the non-neuronal HEK293 cell line. The variance of the regulation of SPX transcription by CRF treatment may exist in the original mouse hippocampal cell and the HEK293 cell line, though the condition was optimized for CRF-inhibited SPX promoter activity in HEK293 cell. In addition, the measurement of cAMP production induced by CRF treatment in the primary hippocampal cell culture can directly highlight the role of cAMP signaling in the regulation of SPX gene expression, which is worthy of consideration for the future studies. Nevertheless, for basic research there is still the case for the use of non-neuronal cell line, in particular when used in luciferase studies; hence, this dual-reporter transfected cell line is still of great value for transcription studies [24].

In conclusion, the study presented by Zhuang and colleagues is particularly novel to determine the potential interaction between CRF and SPX in mouse hippocampus by *in vivo* physiological and *in vitro* pharmacological approaches, which should be of considerable interest for the therapeutic considerations to solve the problem for anxiety or translational research in general. The role of CRFR2 in the stress responsiveness, anxiety, and depressive pathophysiology are still being investigated and, to date, no small molecules targeting the CRF2 receptor have been developed. Conceivably, it is imminent to develop small-molecule medicine targeting on CRFR2 in a pathway-specific way to provide new considerations on the therapies of stress-related disorders and depression.

Disclosure Summary

The authors declare no conflict of interest.

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