Chapter

Role of Glucocorticoid Receptor in the Relation between Stress and Opiate Addiction

Javier Navarro-Zaragoza, María Victoria Milanés and María Luisa Laorden

Abstract

Stressful situations can result in relapse in dependent or abstinent causing reinstatement of drug-seeking. In fact, it has been suggested that activation of the brain stress system results in glucocorticoid release that affects the dopaminergic pathways. Also, the noradrenergic system innervates the extrahypothalamic BSS from the nucleus of tractus solitarius (NTS), resulting in a feedforward loop between the corticotropin-releasing factor (CRF) and noradrenaline (NA) crucial in drug addiction and relapses. Glucocorticoids interact with two receptors: mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) which bind to a GRE site located in tyrosine hydroxylase (TH), resulting in the upregulation of TH synthesis and, finally, increasing dopamine (DA) release in the nucleus accumbens. TH upregulation depends on the phosphorylation of serine 31 and/or serine 40. Previous research has shown that protein kinase C (PKC) activates extracellular signal-regulated kinase (ERK) pathway and in turn phosphorylates serine 31 in the NTS. Besides, cAMP response element binding protein (CREB) is regulated by PKA and PKC. The results shown after pretreating morphine-withdrawn rats with mifepristone and spironolactone (GR and MR antagonists, respectively) suggest that glucocorticoids have a prominent role in addiction because GR would activate ERK and CREB in the NTS, phosphorylating serine 31 and activating TH and indeed noradrenergic release in the paraventricular nucleus (PVN).

Keywords: glucocorticoids, stress, addiction, brain stress system, noradrenergic system, TH, ERK, CREB

1. Introduction

Drug addiction is a chronic disease characterized by recurrence of its signs: drug-seeking and drug-taking behavior, loss of control and impulsivity in consumption, and emergence of a negative state when the access to the drug is not possible [1]. Besides, drug relapse is very often even months and years after withdrawal [2].

Drug addiction has been described as a three-phase disease: During phase 1, drug- seeking behavior is exacerbated and it courses with sensibilization of dopaminergic system, altogether with an associative learning from environment [3]. Phase 2 consists of positive reinforcement pathway downregulation [4]. Finally, phase 3

is characterized by a negative emotional state and by an enhanced craving, which facilitates relapse to drug addiction [5]. Summarizing, individuals experience positive reinforcement in early stages of addiction when they consume drugs of abuse, but after several intakes, they continue that consumption only to avoid the negative state that appears during withdrawal [2, 6].

Previous research has described the importance of different neurotransmitters and neuronal systems in the distinct phases of addiction, being dopaminergic system the main responsible of positive reinforcement [7–10]. Differently, noradrenergic system and brain stress system activities are increased during drug dependence [11].

It is well known that dopaminergic system innervates the prefrontal cortex (PFC) and the nucleus accumbens (NAc), where consumption of major drugs of abuse produces dopamine (DA) release, what is attributed to be behind the development of drug addiction due to its positive reinforcement properties. In contrast, noradrenergic system is mainly related with the negative state that emerges when there is drug withdrawal. It has been shown that noradrenergic innervation from nucleus of tractus solitarius (NTS) to the paraventricular nucleus (PVN) is involved in drug-seeking and in the negative reinforcement produced by morphine withdrawal [12, 13]. Moreover, the existence of a loop between noradrenaline (NA) and corticotropin-releasing factor (CRF) has been described where the enhancement of NA system would result in the enhancement of CRF release (feedforward) and vice versa [14].

On the other hand, many pathways are involved in drug addiction resulting in intracellular responses once extracellular stimuli are processed. One of the more critical is the extracellular signal-regulated kinases (ERK) pathway which plays a main role in neuronal changes, being implicated, i.e., in reward after cocaine consumption [15]. Also, cAMP response element binding protein (CREB) is crucial being its activation through phosphorylation (pCREB). Previous studies from our laboratory have suggested an enhancement of pCREB during morphine withdrawal in the NTS [16]. Besides, CREB regulates TH phosphorylation, limiting enzyme for DA synthesis.

2. Brain stress system and addiction

Brain stress system is composed of two different linked structures: hypothalamic-pituitary-adrenal (HPA) axis and the extended amygdala [17]. Both structures are activated during drug intake and during withdrawal, resulting in CRF and glucocorticoid release [18].

2.1 HPA axis

Also known as hypothalamic brain stress system, as its name suggests, it is divided in three components: the PVN, the pituitary, and the suprarenal glands [1, 12, 19]. In the PVN, CRF is released from the medial parvocellular subdivision to the median eminence reaching the pituitary (**Figure 1**) where it stimulates the synthesis and release of adrenocorticotropic hormone (ACTH) through CRF1R and CRF2R activation [20, 21]. Consequently, ACTH stimulates the synthesis and release of glucocorticoids from the adrenal glands. These glucocorticoids regulate the HPA axis through a negative feedback system once they interact with glucocorticoid (GR) and mineralocorticoid receptors (MR). Changes in this system are proposed to mediate transition from acute consumption to chronic consumption in

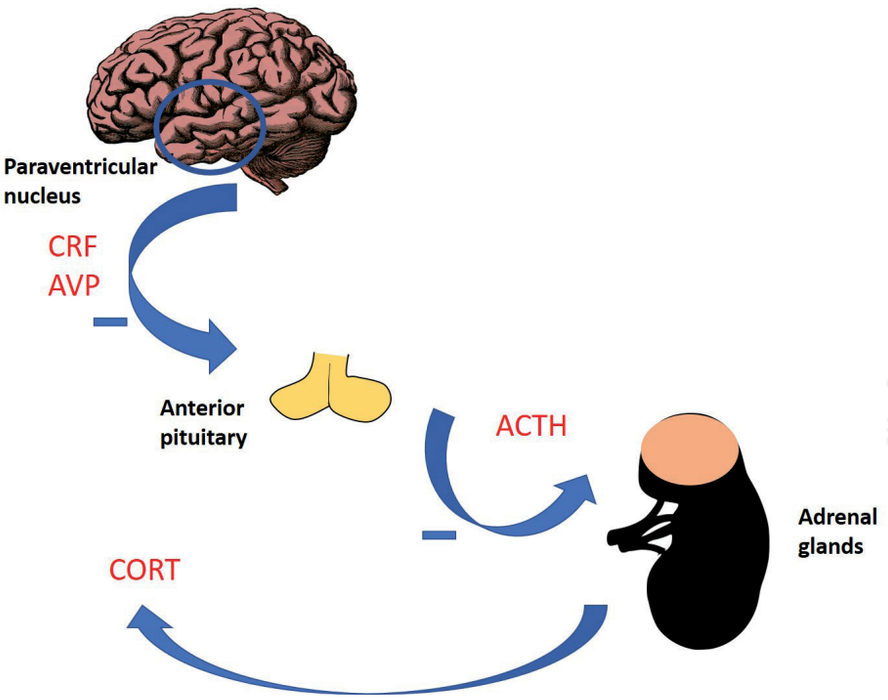

Figure 1.
Representation of the HPA axis. The hypothalamic brain stress system or HPA axis is composed by the PVN, the pituitary, and the suprarenal glands. CRF binds to CRF1R and CRF2R resulting in the activation of the pituitary which consequently, through ACTH, produces release of glucocorticoids (corticosterone, CORT) by the adrenal glands resulting in negative feedback over the previous steps.

addicted [12, 22]. Previous research has shown that different antagonists can block the negative state that come across during morphine withdrawal [23]. Besides, chronic exposure to opiates results in physic dependence and tolerance, and it is accompanied by enhanced ACTH and corticosterone release during morphine withdrawal [24]. Stressful situations can result in relapse in dependent or abstinent humans [25] and cause reinstatement of drug-seeking in different animal relapsing models [26].

2.2 Extended amygdala

The extrahypothalamic brain stress system or the extended amygdala (**Figure 2**) is composed of different nuclei as bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CeA), and the shell of the NAc [27, 28]. These nuclei have similar functions and are responsible of connecting the limbic structures as hippocampus, basolateral amygdala, or the midbrain [12, 29]. Also, limbic structures mediate responses and behavior guiding the individuals according to memories [30]. Here, CRF receptors and CRF neuron cell bodies have been seen in BNST and CeA innervating each other and others as the NAc [28, 31, 32]. Therefore, CRF has a prominent role in this structure. Moreover, the extended amygdala is a key component in the acquisition and development of different negative symptoms through the release of CRF together with other neurotransmitters or peptides like NA or dynorphin [17, 33, 34]. In addition, extended amygdala is linked to the NTS (a noradrenergic nucleus) through innervations from there to the BNST, CeA, or the NAc [35, 36]. Thereupon, the extended amygdala, a part of the brain stress system, connects with the noradrenergic system and the dopaminergic pathways [37]. In fact, it has been suggested that activation of the brain stress system would result in sensibilization of the dopaminergic pathways [38, 39].

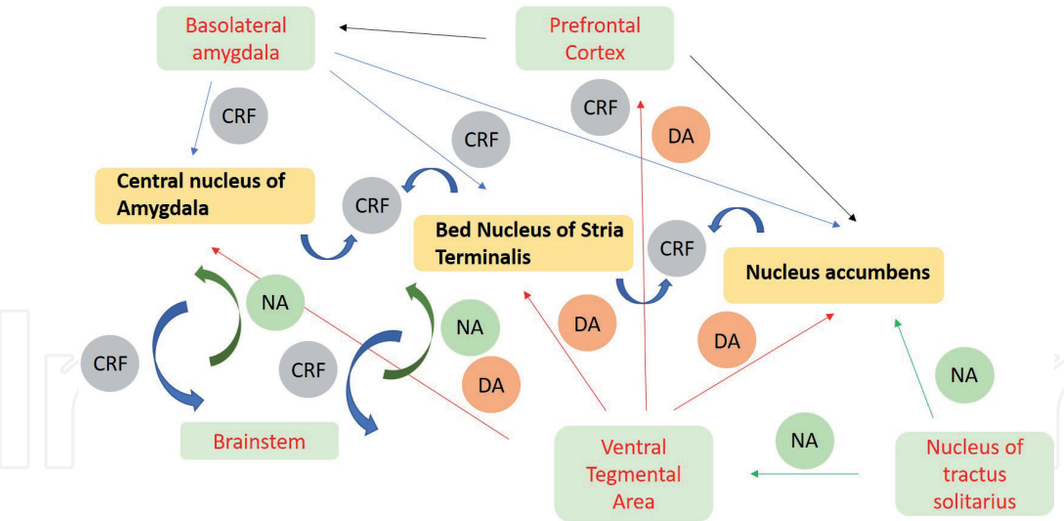

Figure 2.
Representation of the extended amygdala. The extrahypothalamic brain stress system or extended amygdala is shown here in a scheme with its main nuclei: BNST, CeA, and NAc. Noradrenergic innervations establish a feedforward loop between CRF and NA, which remains crucial for the development of drug addiction and relapses. Besides, there is dopaminergic innervation from ventral tegmental area to different nuclei establishing a relationship between NA system, DA system, and the brain stress system (hypothalamic and extrahypothalamic).

3. Role of glucocorticoids in addiction

Glucocorticoids are the final step of HPA axis, and their release takes place in response to stressful situations, becoming this activation one of the main mechanisms of adaption to stress [40]. Glucocorticoids make their function by interacting with two classes of receptors: MR or type I and GR or type II [41].

Whereas MR are located in limbic areas of the brain such as amygdala and also in the PVN or the locus coeruleus (LC) [42], GR have a more heterogeneous localization, with deep presence in the PVN, amygdala, or the hippocampus. MR have higher affinity for corticosterone than GR, but GR are activated when there are stressful facts differently to MR, which are important at basal levels. Both receptors have presence in the NTS, making this nucleus to be important in glucocorticoid effects [43]. Previous research has shown that MR blockade decreases self-administration of cocaine, suggesting a role for these receptors in addiction [44].

Moreover, stress affects GR, which are located through the dopaminergic pathways enhancing HPA axis and dopaminergic activity. In fact, glucocorticoids have been suggested to interact with a GRE site located in TH, resulting in the upregulation of TH synthesis and, finally, increasing DA release in the NAc [45]. Therefore, individuals with higher HPA axis activity would be more vulnerable to develop drug addiction [5].

4. Involvement of GR and MR in TH activity and phosphorylation in the NTS

The regulation in the biosynthesis of catecholamines by TH depends on its phosphorylation at serine 31 and serine 40. This has been proposed to be triggered by stressful situations considering that increased release of glucocorticoids results in uprising TH activity [46]. Moreover, morphine withdrawal induced by naloxone injection increased TH mRNA expression in the NTS and TH activity in the PVN [47]. Therefore, it was critical to elucidate if blocking GR and MR with mifepristone and

spironolactone would affect TH phosphorylation during morphine withdrawal in the NTS. Results from our laboratory showed that TH phosphorylation at serine 31 and serine 40 was increased during naloxone-induced morphine withdrawal in rats, a fact that, together with the existence of enhanced NA turnover in the NTS during morphine withdrawal, suggests that TH regulates noradrenergic activity [24, 31, 48–50]. Besides, the blockade of GR with mifepristone, selective antagonist of GR, significantly attenuated the phosphorylation at serine 31, but not at serine 40 in the NTS during morphine withdrawal [48, 50], different to the results after blockade of MR with spironolactone. Pretreatment with this antagonist decreased phosphorylation of serine 31 in the NTS but not significantly [49, 50] (**Figure 3**). These results would suggest that enhanced glucocorticoid release during morphine withdrawal results in TH phosphorylation at serine 31, consequently, also in enhanced TH activity, and finally in higher catecholamine levels in the PVN, innervated by noradrenergic system.

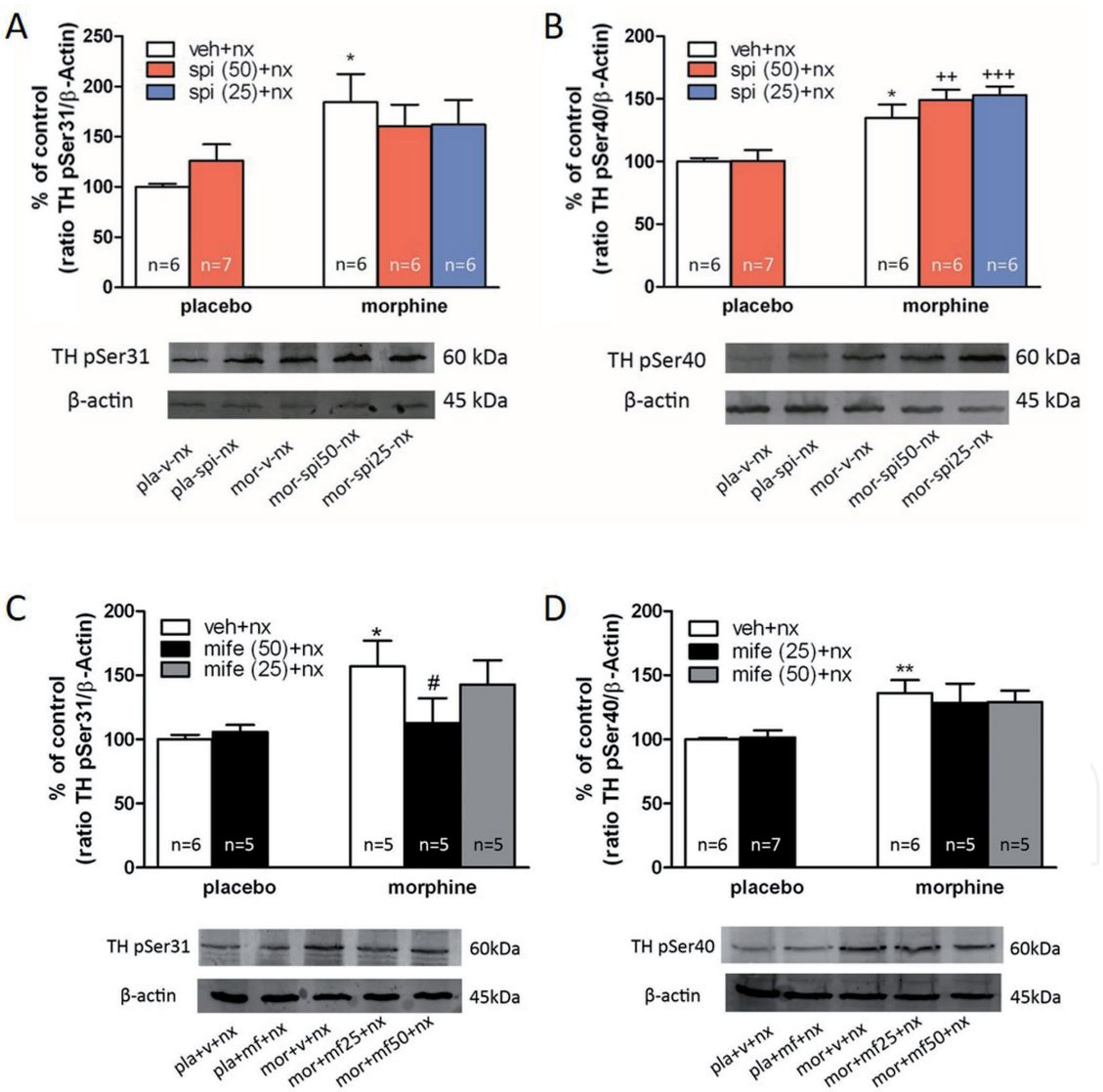

Figure 3.
Antagonization of TH phosphorylation at serine 31 by mifepristone (GR antagonist). Mifepristone (C) but not spironolactone (A) antagonized naloxone-induced morphine-withdrawal phosphorylation of TH at serine 31 in the NTS. Representative immunoblots of THpSer31 (A, C) and THpSer40 (B, D) in the NTS tissues isolated from placebo and morphine-dependent rats 60 min after administration of naloxone and the respective antagonist [mifepristone (C, D) or spironolactone (A, B)] or saline. Data represent the optical density of immunoreactive bands expressed as a percentage (%) of the mean ± SEM of placebo control band $^{*}P < 0.05$ versus placebo + vehicle + naloxone; $^{**}P < 0.01$ versus placebo + vehicle + naloxone; $^{\#}P < 0.05$ versus morphine + vehicle + naloxone; $^{++}P < 0.01$ versus placebo + spironolactone+ naloxone; $^{+++}P < 0.001$ versus placebo + spironolactone + naloxone.

5. Role of GR and MR in the activation of ERK pathway and CREB (via phosphorylation) in the NTS

Different studies have proposed the importance of ERK pathway in drug addiction, particularly, during morphine withdrawal [51, 52]. Protein Kinase C (PKC) regulates this pathway activated by the phosphorylation of ERKs [50, 52]. It is important to highlight that previous research has shown that ERK has a main role in the phosphorylation of TH at serine 31 in the NTS [53], supporting a synergic cooperation between the brain stress system, the noradrenergic system, and this pathway. GR but not MR blockade significantly decreased the enhanced activity (via phosphorylation) seen in pERK1 and pERK2 during morphine withdrawal in rats, supporting a role for glucocorticoids in activation of ERK pathway (**Figure 4**).

On the other hand, it is known that CREB has a main role in addiction to drugs of abuse as a transcription factor [54]. Nevertheless, CREB is the final step of protein kinase A (PKA) signaling pathway, although PKC pathway has been also proposed to be mediating its activation in the NTS [16]. As it happens with ERK,

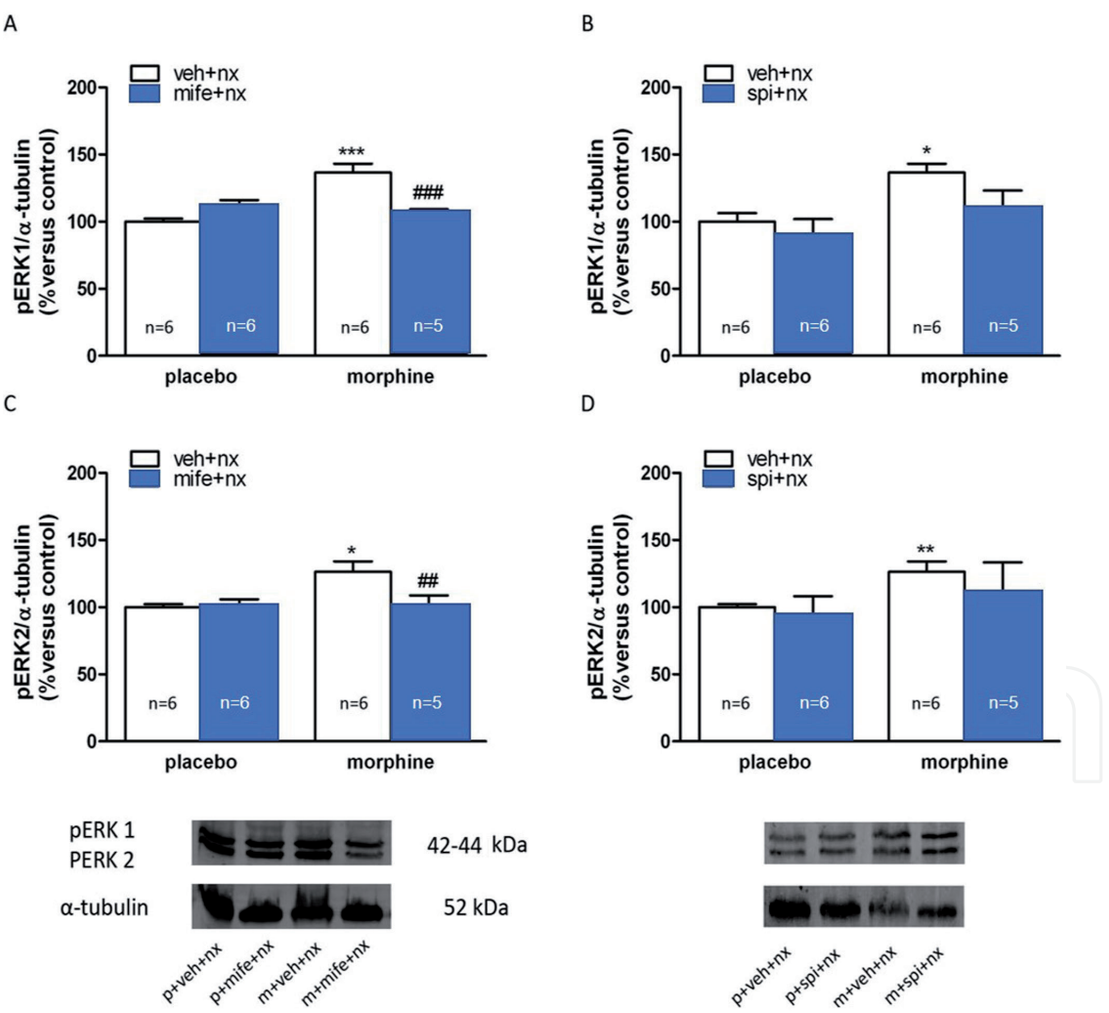

Figure 4.
Antagonization of ERK 1 and ERK 2 phosphorylation by mifepristone (GR antagonist). Mifepristone (A, C) but not spironolactone (B, D) antagonized naloxone-induced morphine-withdrawal phosphorylation of ERK 1 and ERK 2 in the NTS. Representative immunoblots of ERK 1 (A, B) and ERK 2 (C, D) in the NTS tissues isolated from placebo and morphine-dependent rats 60 min after administration of naloxone and the respective antagonist [mifepristone (A, C) or spironolactone (B, D)] or saline. Data represent the optical density of immunoreactive bands expressed as a percentage (%) of the mean ± SEM of placebo control band. $^{*}P < 0.05$ versus placebo + vehicle+ naloxone; $^{**}P < 0.01$ versus placebo + vehicle + naloxone; $^{*}P < 0.05$ versus placebo + vehicle + naloxone $^{\#\#}P < 0.01$ versus morphine + vehicle + naloxone; $^{\#\#\#}P < 0.001$ versus morphine + vehicle + naloxone.

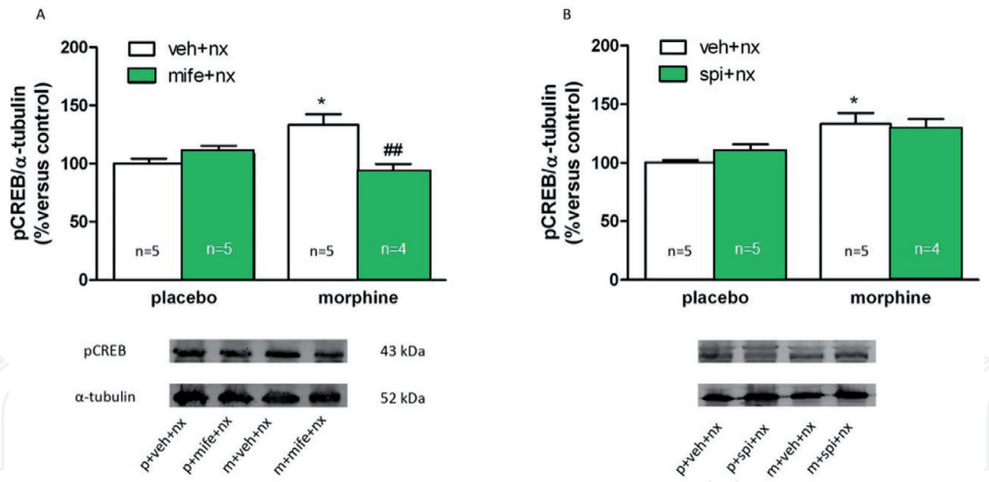

Figure 5.
Antagonization of CREB phosphorylation by mifepristone (GR antagonist). Mifepristone (A) but not spironolactone (B) antagonized naloxone-induced morphine-withdrawal phosphorylation of CREB in the NTS. Representative immunoblots of pCREB in the NTS tissues isolated from placebo and morphine-dependent rats 60 min after administration of naloxone and the respective antagonist mifepristone (A) or spironolactone (B) or saline. Data represent the optical density of immunoreactive bands expressed as a percentage (%) of the mean ± SEM of placebo control band. $^{*}P < 0.05$ versus placebo + vehicle + naloxone; $^{**}P < 0.01$ versus morphine + vehicle + naloxone.

CREB is activated via phosphorylation, and it has been shown to be enhanced in the NTS during morphine withdrawal [16, 50]. Once again, GR but not MR blockade significantly decreased the phosphorylation of CREB seen during morphine withdrawal [50] (**Figure 5**). Therefore, GR would be implicated in CREB activation during morphine withdrawal in the NTS.

6. Conclusion

Previous research has shown that CRE (binding site for CREB) and GRE (binding site for GR) are present in the gene promoters that regulate activity of TH [55], setting a relationship between NA system, the HPA axis and the extended amygdala, and finally, CREB. In contrast, little was known about the mechanisms underlying this regulation. This review suggests that stressful situations as naloxone-induced morphine withdrawal would result in glucocorticoid release which would activate GR. Immediately, GR would produce an activation of PKC signaling pathway that would regulate ERK pathway and CREB activation (via phosphorylation) in the NTS. Finally, TH activity would be enhanced in the NTS through the activation of different sites as CRE or GRE resulting in catecholamine release in the PVN, supporting a main role for glucocorticoids and the GR in drug addiction.

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Conflict of interest

The authors declare no conflict of interest.

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