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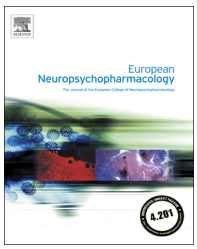

BDNF-TRKB signaling system of the dorsal periaqueductal gray matter is implicated in the panicolytic-like effect of antidepressant drugs

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Abstract
A wealth of evidence implicates the BDNF-TRKB system in the therapeutic effects of antidepressant drugs (ADs) on mood disorders. However, little is known about the involvement of this system in the panicolytic property also exerted by these compounds. In the present study we evaluated the participation of the BDNF-TRKB system of the dorsal periaqueductal gray matter (DPAG), a core structure involved in the pathophysiology of panic disorder, in AD-induced panicolytic-like effects in rats. The results showed that short- (3 days) or long-term (21 days) systemic treatment with the tricyclic ADs imipramine, clomipramine or desipramine increased BDNF levels in the DPAG. Only longterm treatment with the selective serotonin reuptake inhibitor fluoxetine was able to increase BDNF levels in this structure. After 21-day treatment, fluoxetine and the three tricyclic ADs used also increased BDNF concentration in the hippocampus, a key area implicated in their mood-related actions. Neither in the DPAG nor hippocampus did long-term treatment with the standard anxiolytics diazepam, clonazepam or buspirone affect BDNF levels. Imipramine, both after short and long-term administration, and fluoxetine under the latter regimen, raised the levels of phosphorylated TRKB in the DPAG.

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Short-term treatment with imipramine or BDNF microinjection inhibited escape expression in rats exposed to the elevated T maze, considered as a panicolytic-like effect. This anti-escape effect was attenuated by the intra-DPAG administration of the TRK receptor antagonist k252a. Altogether, our data suggests that facilitation of the BDNF-TRKB system in the DPAG is implicated in the panicolytic effect of ADs.

1. Introduction

Antidepressant drugs (ADs) are the first choice in pharmacotherapy for several neuropsychiatric conditions, including depression, generalized anxiety and panic disorders (APA [DSM V], 2013). The primary action of most of the existing ADs involves an increase in monoamine synaptic availability, mainly serotonin and/or noradrenaline, which may be achieved through different mechanisms, such as inhibition of reuptake transport proteins or degrading enzymes (Stahl et al., 2013). Besides this, all known antidepressants require 2–4 weeks of treatment to achieve clinical efficacy (Liebowitz et al., 1988; Nierenberg et al., 2000).

Several hypotheses have been formulated in an attempt to explain the late onset of ADs' beneficial effects. One of the most prominent was proposed by Blier et al. (1987). Broadly speaking, these authors suggest that a net increase in 5-HT neurotransmission in key areas, such as the hippocampus and frontal cortex, is required for ADs mood-relieving effects. However, at the beginning of the treatment, the full effect of ADs in increasing 5-HT synaptic availability is not achieved, since these drugs also raise 5-HT concentration in raphe nuclei (e.g. dorsal and median raphe nuclei), which are the main source of 5-HT innervation to these forebrain areas. In the raphe, 5-HT binds to inhibitory somatodendritic 5HT1A receptors, causing a decrease in the firing rate of 5-HT neurons and consequently inhibiting 5-HT release in terminal areas. Following repeated treatment, these autosomic 5HT1A receptors are desensitized and the 5-HT neurons regain their normal firing rate, allowing a higher accumulation of 5-HT in projection areas such as the hippocampus and frontal cortex (for a more detailed account of this proposal see Blier and El Mansari, 2013).

Nevertheless, over the past decade, molecular, cellular and behavioral studies have indicated that the mechanism of action of ADs goes beyond the monoamine hypothesis. Duman et al. (1997) postulated the involvement of neurotrophic factors, especially brain-derived neurotrophic factor (BDNF), in the pathogenesis of depression and in the mechanism of action of ADs. BDNF, acting through its receptor, tropomyosin-related kinase B (TRKB), has been implicated in neuronal survival and differentiation, in the regulation of synaptic changes and in the modulation of neurotransmitter release. The neurotrophic hypothesis of depression suggests that BDNF levels or its effects would be reduced in the hippocampus and/or frontal cortex in a depressive state and that AD treatment would restore such levels (Nestler et al., 2002).

Despite, a growing body of evidence implicating BDNF in the mechanisms of action of ADs in mood disorder treatment, less is known about the role of this neurotrophin in anxiety-related pathologies. Recently, we showed that BDNF microinjection into the dorsal periaqueductal gray matter (DPAG), a core structure involved in the modulation of fear and panic attacks (Mobbs et al., 2007; Schenberg, 2010), inhibits escape responses and autonomic changes induced by electrical stimulation of this midbrain area (Casarotto et al., 2010), suggestive of a panicolytic-like effect.

Although an AD-induced increase in BDNF and/or activated TRKB levels in forebrain structures, such as the hippocampus and prefrontal cortex, is consistently associated with their positive effects on mood disorders, it is still unknown whether chronic treatment with these drugs also increases BDNF levels in the DPAG and whether this effect is related to their panic-relieving effects. Therefore, the aim of the present study was to investigate the hypothesis that the panicolytic-like effect of ADs involves modifications in BDNF/TRKB signaling in the DPAG. Initially, we evaluated the effect of the tricyclic ADs imipramine, clomipramine and desipramine as well as the selective serotonin reuptake inhibitor (SSRI) fluoxetine on BDNF levels in the DPAG after acute-, short-or long-term systemic (i.p.) treatments. The effects of these drugs were compared to those caused by the standard anxiolytic drugs diazepam, clonazepam and buspirone, which are devoid of clinically relevant antidepressant properties. Also for comparative reasons, we determined BDNF levels in the hippocampus, an important structure for BDNF-mediated antidepressant effects (for review see Duman and Monteggia, 2006). Based on these results we determined the levels of activated (i.e. phosphorylated) TRKB in the DPAG after imipramine and fluoxetine administration. Finally, we also investigated whether the changes in DPAG BDNF/TRKB levels observed with imipramine were implicated in the panicolytic-like effect of this compound in rats submitted to the elevated T maze.

2. Experimental procedures

2.1. Animals

Male Wistar rats weighing 250–320 g on the day of the test were housed in groups of 4–6/cage under a 12-h each dark-light cycle (lights on at 07:00 h) at 22 ± 1 °C, with free access to food and water. All experiments were carried out in accordance with the Ethical Principles in Animal Research adopted by the Brazilian National Council for the Control of Animal Experimentation (CONCEA) and were approved by the local Animal Ethical Committee of the Ribeirão Preto Medical School, University of São Paulo (protocol number: 114/2007). All efforts were made to minimize animal suffering.

2.2. Drugs

The following drugs were used: imipramine hydrochloride (Sigma-Aldrich, USA), desipramine hydrochloride (Sigma-Aldrich, USA),