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Research report

NK_1 receptors antagonism of dorsal hippocampus counteract the anxiogenic-like effects induced by pilocarpine in non-convulsive *Wistar* rats

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HIGHLIGHTS

- NK_1 receptor antagonist's FK888 did not alter emotional responses in CA_1 or LSD.
- Infusion of FK888 into CA_1 inhibits long-term anxiogenic responses induced by PILO.
- PILO changes anxiogenic responses modulated by SP-NK_1 receptor signaling in CA_1.

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ABSTRACT

Recent evidence supports a role for the substance P (SP) in the control of anxiety and epilepsy disorders. Aversive stimuli alter SP levels and SP immunoreactivity in limbic regions, suggesting that changes in SP-NK_1 receptor signaling may modulate the neuronal excitability involved in seizures and anxiogenesis. The involvement of NK_1 receptors of the dorsal hippocampus and lateral septum in the anxiogenic-like effects induced by a single injection of pilocarpine (PILO) was examined in non-convulsive rats evaluated in the elevated plus-maze (EPM). Male Wistar rats were systemically injected with methyl-scopolamine (1 mg/kg) followed 30 min later by saline or PILO (350 mg/kg) and only rats that did not present status epilepticus were used. One month later, vehicle or FK888 (100 pmol) – an NK_1 receptor antagonist – were infused in the dorsal hippocampus or the lateral septum of the rats and then behaviorally evaluated in the EPM. Previous treatment with PILO decreased the time spent in and the frequency of entries in the open arms of the EPM, besides altering risk-assessment behaviors such as the number of unprotected head-dipping, protected stretch-attend postures and the frequency of open-arms end activity, showing thus a long-lasting anxiogenic-like profile. FK888 did not show any effect *per se* but inhibited the anxiogenic responses induced by PILO when injected into the dorsal hippocampus, but not into the lateral septum. Our data suggest that SP-NK_1 receptor signaling of the dorsal hippocampus is involved in the anxiogenic-like profile induced by PILO in rats evaluated in the EPM test.

1. Introduction

Depression and anxiety disorders are major comorbidities in epilepsy [1–6]. Several animal models demonstrated that the *status epilepticus* (*SE*) observed in epileptic animals induces aggressive, depressive and anxiety-like behaviors [7–14] supporting the existence of a shared causation of epilepsy and affective disorders.

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Substance P (SP) – a member of the neurokinin family – exhibits an excitatory outline in the brain and has been implicated in a variety of physiological processes including the regulation of stress, anxiety and depression responses [15–20]. The exposure of animals to aversive and stressful stimuli alter the SP levels or SP immunoreactivity (SP-ir) in several brain regions – such as lateral septum, dentate gyrus, periaqueductal gray, hypothalamic nuclei, nucleus accumbens and amygdala – involved in the regulation of mood and affective behaviors [21–26]. Blocking SP transmission by NK_1 antagonists or genetic disruption attenuates the effects of stress and induces anxiolytic-like effects in animal models [20,27–29] – effects that can be associated with an attenuation of neuroendocrine stress responses [26]. SP also exhibits a critical role in the control of neuronal excitability and maintenance of the *SE* [30–33]. *In vivo* studies have shown that there is a strong correlation between SP levels and the propensity, severity and duration of seizures [31,32]. An increase in preprotachykinin-A (PPT-A) mRNA and SP-ir in the CA_1, CA_3, and dentate gyrus of the hippocampus was observed after *SE* induced by the injection of lithium-pilocarpine or the stimulation of the perforant path. The intrahippocampal injection of SP triggers *SE* and induces hippocampal damage whereas the SP receptor antagonists block both the development and maintenance of the self-sustaining *SE* [32].

Over the last years, our research group showed that a single systemic administration of subconvulsant doses of pilocarpine (PILO, 75–350 mg/kg) – a non-selective cholinergic agonist – triggers anxious-like behaviors in rats evaluated in the EPM test 24 h up to three months after the treatment, suggesting that the anxiogenesis is not totally dependent of convulsive episodes [34–36]. In addition, we showed that the septo-hippocampal pathway is critically involved in these anxiogenic responses [36]. In the current work, we assume the hypothesis that the SP-NK_1 receptor pathway may modulate neuronal hyper-excitability involved in anxiogenesis in non-convulsive rats. Thus, changes in the SP signaling in the dorsal hippocampus and lateral septum – areas richly innervated by SP-NK_1 receptor-containing neurons and widely affected by stress conditions and seizures – would be expected to interfere with the anxiety-related behavioral alterations in these animals. We here investigated the potential participation of the NK_1 receptors of the CA_1 region of the dorsal hippocampus and lateral septum in the anxiogenesis induced by PILO treatment in non-convulsive rats.

2. Material and methods

2.1. Animals

Male Wistar rats, 2.5 months old, were housed in groups of four animals *per* cage and kept in a room with controlled temperature ($22 \pm 2\,^{\circ}C$) and a 12-h light/dark cycle (lights on at 07:00 a.m.) with food and water *ad libitum*, except during the experiments. Rats were allowed to adapt to the laboratory conditions for one week before the experiments. All experiments were conducted in accordance with international standards of animal welfare recommended by the Brazilian Society of Neuroscience and Behavior (Act, 1992) and the experimental protocols were approved by the Animal Care and Use Committee of the Federal University of Santa Catarina (#23080.001156/2001-50/UFSC). The minimum number of animals (7–10 animals *per* group) and duration of observation required to obtain consistent data were used.

2.2. Drugs and treatment schedule

Methyl-scopolamine bromide (a muscarinic receptor antagonist; RBI, USA) was given subcutaneously and used to prevent the peripheral cholinomimetic effects elicited by PILO. Pilocarpine hydrochloride (a non-selective muscarinic receptor agonist; Merck SA, Brazil) was injected intraperitoneally afterward. FK888 (a selective NK_1 receptor antagonist; Fujisawa Pharmaceutical Co., Osaka, Japan) was previously prepared as a stock solution (10^{-3} M) in ethanol and diluted in phosphate-buffered saline (PBS, pH = 7.4; Sigma, USA) immediately before the experiments. Methyl-scopolamine and PILO were dissolved in saline (NaCl 0.9%). All doses were taken from previous studies [27,28,34,36–39].

Rats received methyl-scopolamine (1 mg/kg) followed 30 min later by a single systemic injection of saline or PILO (350 mg/kg).

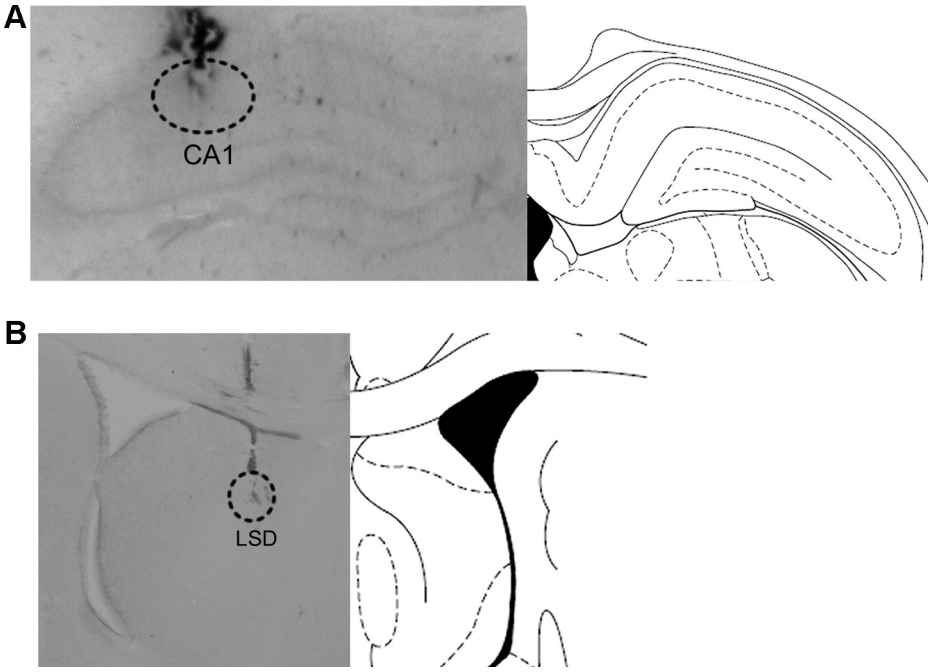

Fig. 1. Photomicrographs (left) and schematic drawings (right), based on the atlas of Paxinos and Watson (1986), of coronal sections from 4.2 mm and 0.2 mm posterior to bregma of the rat brain showing the microinjection sites (black dotted circles) of PBS or FK888 (100 pmol) into the CA_1 region of the dorsal hippocampus (A) or lateral septum (B) of male Wistar rats.