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Research report

Inhibition of mammalian target of rapamycin activation in the rostral anterior cingulate cortex attenuates pain-related aversion in rats

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HIGHLIGHTS

- A nociceptive stimulus induced an upregulation of p-mTOR and p-p70S6K in the rACC.
- Intra-rACC injection of rapamycin attenuated pain-related aversion.
- Rapamycin infusion into the rAAC did not affect nociceptive behaviors.

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ABSTRACT

Pain is a complex experience that comprises both sensory and affective dimensions. Mammalian target of rapamycin (mTOR) plays an important role in the modulation of neuronal plasticity associated with the pathogenesis of pain sensation. However, the role of mTOR in pain affect is unclear. Using a formalin-induced conditioned place avoidance (F-CPA) test, the current study investigated the effects of the mTOR specific inhibitor rapamycin on noxious stimulation induced aversion in the rostral anterior cingulate cortex (rACC). Intraplantar injection of 5% formalin was associated with significant activation of mTOR, as well as p70 ribosomal S6 protein (p70S6K), its downstream effector, in the rACC. The inhibition of mTOR activation with rapamycin disrupted pain-related aversion; however, this inhibition did not affect formalin-induced spontaneous nociceptive behaviors in rats. These findings demonstrated for the first time that mTOR and its downstream pathway in the rACC contribute to the induction of pain-related negative emotion.

1. Introduction

Pain is a multidimensional experience composed of both sensory and affective dimensions. The sensory dimensions include the location, intensity, and quality of the stimuli, whereas the affective dimensions include anxiety, aversion and depression, which results in the desire to terminate or escape from the noxious stimuli [1,2]. Clinical observations in chronic pain patients have indicated that the emotional component of pain is a greater metric of quality of life than the pain itself [1].

Although the neuronal pathways and brain regions involved in the sensory component of pain have been extensively investigated, the neuronal systems that underlie the affective component of pain remain elusive. Recent accumulating evidence has implicated the anterior cingulate cortex (ACC) in functions related to the emotional processing of pain [3–9]. In clinical reports, patients with surgical ablation of the ACC continued to feel pain, but experienced substantial decreases in pain-related depression and unpleasantness [10,11]. Animal behavioral studies have demonstrated that the destruction of neurons that originate from the rostral ACC (rACC) blocks formalin-induced conditioned place aversion (F-CPA), which comprises a pain-related aversive learning that reflects aspects of the negative affective components of pain, without reducing pain sensation [3,12,13]. Moreover, microinjection of excitatory amino acids into the rACC of uninjured rats produces conditioned place

Abbreviations: mTOR, mammalian target of rapamycin; p70S6k, p70 ribosomal S6 protein kinase; ACC, anterior cingulate cortex; rACC, rostral anterior cingulate cortex; F-CPA, formalin-induced conditioned place avoidance; CPP, conditioned place preference; AMPA, a-amino-3-hydroxy-5-methyl-isoxozole propionic acid; KA, kainite.

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aversion without sensory threshold alterations [6]. However, the molecular mechanisms that underlie pain affect in the ACC circuitry are poorly understood.

Mammalian target of rapamycin (mTOR) is a serine-threonine protein kinase that regulates cell proliferation and growth [14]. The activation of mTOR, especially in a complex sensitive to rapamycin (mTOR complex 1), promotes the phosphorylation of downstream molecules, such as p70 ribosomal S6 protein kinase (p70S6K), which subsequently activates S6 ribosomal proteins to initiate new protein synthesis [15,16]. Accumulating evidence indicates that mTOR plays an important role in the modulation of neuronal plasticity and memory processes [17–21]. For example, mice with deletions of mTOR downstream effectors exhibit deficits in synaptic plasticity and various forms of long-term memory [19,22,23]. Parallels between the molecular mechanisms of long-term memory and pain plasticity have implicated mTOR as a potential target for the induction of pathological pain states [24,25]. Recent studies have demonstrated that peripheral noxious insults caused by intraplantar carrageenan or bone cancer lead to increases in the phosphorylation of mTOR (p-mTOR) in the rat spinal dorsal horn [26,27]. Furthermore, intrathecal administration of rapamycin, a specific inhibitor of mTOR, produces anti-nociception in models of neuropathic, inflammatory, and bone cancer pain [26–30]. However, whether mTOR is involved in pain affect pathways remains elusive. Therefore, the current study aimed to determine whether mTOR is activated in the ACC following peripheral noxious stimulation, as well as to investigate the effects of mTOR inhibition on persistent pain-induced negative emotion in rats.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats that weighed 250–300 g were used in all experiments. All animals were obtained from the Experimental Animal Center of Zhejiang Academy of Medical Sciences, China. The animals were housed in a climate-controlled room under a 12 h light/dark cycle (lights on at 6:00 a.m.); they were provided food and water ad libitum. The animals underwent a 5 day period of acclimation to the new surroundings prior to the start of experimental manipulations. All experimental protocols were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of Ningbo University (Ningbo, China).

2.2. Inflammatory pain model

To induce inflammatory pain, a 5% formalin solution (50 μl, Sigma-Aldrich, St. Louis, MO, USA) was subcutaneously injected into the plantar surface of the left hind paw.

2.3. rACC cannulation and microinjections

Rats were anesthetized with isoflurane and placed in a stereotaxic apparatus (RWD Life Science, Shenzhen, China). A stainless steel guide cannula (24-gauge, RWD Life Science, Shenzhen, China) was implanted into the bilateral rACC [anteroposterior (AP) +2.6 from bregma, mediolateral (ML) ±0.6, dorsoventral (DV) −2.5] [31]. The cannula was attached to the bone with stainless steel screws and acrylic cement (RWD Life Science, Shenzhen, China). A dummy cannula (28-gauge stainless steel wire) was inserted into the guide cannula to prevent clogging. The rats were allowed to recover for at least 5 days prior to experimentation. Drugs were administered through a 28-gauge injection cannula that protruded 2 mm beyond the guide cannula tip. Rapamycin (7 ng/0.5 μl/side, Cell Signaling Technology, Beverly, MA, USA) [32] or vehicle [0.5 μl 1% dimethylsulfoxide (DMSO)] was administered at a rate of 0.1 μl/30 s. Following the completion of the drug infusion, the injection cannula was maintained in place for an additional 5 min to minimize the backflow of the drug up the cannula track. All behavioral tests were performed blind with respect to the drug injections.

2.4. CPA

A shuttle-box that consisted of three white acrylic compartments was used in this behavioral experiment [3]. Two larger conditioning compartments (A and B: 30 cm × 30 cm × 30 cm) were placed parallel, and one smaller neutral compartment (C: 15 cm × 20 cm × 30 cm, length × width × height) was positioned in front of them. To ensure the two conditioning compartments had distinct visual, tactile, and olfactory cues, one compartment had horizontal stripes on the walls and an odor of 1.0% acetic acid, whereas the other compartment had vertical stripes and a standardized cinnamon scent. Furthermore, the floors of the two compartments were also different (one floor was smooth, whereas the other floor was textured). The neutral compartment was characterized by a uniform wall color in the absence of a distinctive odor. There were removable doors (10 cm × 10 cm) between the three compartments to enable room isolation, when necessary.

The CPA was performed without light during the day (6:00 a.m.–6:00 p.m.). The procedure consisted of 3 distinct sessions: a pre-conditioning session (days 1 and 2), a conditioning session (days 3 and 4), and a post-conditioning session (day 5) [3,33]. On day 1, the rat was placed in the neutral compartment. Following 5 min of acclimation, the doors that lead to each conditioning compartment were opened. When the rat entered a conditioning compartment, the 2 doors that connected the neutral and conditioning compartments were closed. The rat was allowed to freely explore between the 2 conditioning compartments for 15 min. A timer automatically recorded the time spent in each compartment. The same trial was performed on day 2. The rats that spent >80% (>720 s) in one side on day 2 or that spent >600 s in one side on day 1 and >600 s on the other side on day 2 were eliminated from the subsequent experiment. Days 3 and 4 comprised the place conditioning periods, during which all doors were closed. The rat received no treatment and was randomly restrained in one conditioning compartment for 45 min in the morning. After at least 4 h, in the afternoon, the rat was conditioned with a formalin injection or a foot-shock. For the formalin-induced CPA (F-CPA), the rat was administered a left hind paw intraplantar (i.pl.) injection of 5% formalin (50 μl) and was subsequently confined to the other conditioning compartment for 45 min. For the electric foot-shock-induced CPA (S-CPA), the rat received an electric shock (0.5 mA for 2 s) every 8–10 min throughout the 45-min training session. On day 5, the same trial as day 1 or 2 was repeated, and the time spent in each compartment was measured. The CPA scores were calculated by subtracting the time spent in the pain-paired compartment during the post-conditioning session from the time spent in the same compartment during the pre-conditioning session.

In the experiments that investigated rapamycin-induced conditioned place preference (CPP) or CPA, training procedures were performed as previously described for F-CPA, with the exception that the injection of formalin/normal saline (i.pl.) on days 3 and 4 was replaced with an intra-rACC microinjection of rapamycin/vehicle. The time that the rats spent in the rapamycin/vehicle-paired compartment was recorded on the pre-conditioning and post-conditioning days.

2.5. Formalin test

Based on methods described by Coderre et al. [34], the animals were anesthetized with isoflurane (1.5%, inhalation), and 50 μl of a