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Research report

Time-dependent effects of rapamycin on consolidation of predator stress-induced hyperarousal

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HIGHLIGHTS

- Inhibition of mTOR via rapamycin blocks consolidation of predator stress-induced fear memories.
- Rapamycin blocks predator stress-induced hyperarousal.
- A second, late phase mTOR-dependent process following predator stress exacerbates startle.
- Rapamycin, depending on time of administration, may be a viable treatment for PTSD.

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ABSTRACT

Previous studies have indicated that rapamycin, a potent inhibitor of the mammalian target of rapamycin (mTOR) pathway, blocks consolidation of shock-induced associative fear memories. Moreover, rapamycin's block of associative fear memories is time-dependent. It is unknown, however, if rapamycin blocks consolidation of predator stress-induced non-associative fear memories. Furthermore, the temporal pattern of mTOR activation following predator stress is unknown. Thus, the goal of the current studies was to determine if rapamycin blocks consolidation of predator stress-induced fear memories and if so, whether rapamycin's effect is time-dependent. Male rats were injected systemically with rapamycin at various time points following predator stress. Predator stress involves an acute, unprotected exposure of a rat to a cat, which causes long-lasting non-associative fear memories manifested as generalized hyperarousal and increased anxiety-like behaviour. We show that rapamycin injected immediately after predator stress blocked consolidation of stress-induced startle. However, rapamycin injected 9, 24 or 48 h post predator stress potentiated stress-induced startle. Consistent with shock-induced associative fear memories, we show that mTOR signalling is essential for consolidation of predator stress-induced hyperarousal. However, unlike shock-induced fear memories, a second, persistent, late phase mTOR-dependent process following predator stress actually dampens startle. Consistent with previous findings, our data support the potential role for rapamycin in treatment of stress related disorders such as posttraumatic stress disorder. However, our data suggest timing of rapamycin administration is critical.

1. Introduction

Post-traumatic stress disorder (PTSD), now classified as a trauma- and stress-related disorder, is characterized by a cluster of symptoms that manifest following exposure to a severely traumatic stressor, in which victims experience feelings of intense fear, vulnerability, or helplessness. Symptoms include re-experiencing memories of the traumatic event, avoiding trauma-associated stimuli, negative cognition or mood, and hyperarousal (5th ed; DSM-V, American Psychiatric Association, 2013). Ultimately, the symptoms can disrupt activities and impair one's ability to function, thus impeding overall quality of life. In the United States and Canada, the lifetime prevalence of PTSD is currently 6.8% and 9.2%, respectively [1,2].

Stress related disorders such as PTSD can be characterized as disorders involving the disturbance of emotional learning and memory processes, resulting in enhanced consolidation of fear memories. Identification of the neural mechanisms underlying fear memory consolidation may therefore aid in understanding the development and/or treatment of stress related disorders such as PTSD.

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The mammalian target of rapamycin (mTOR), a serine/threonine protein kinase, controls the initiation and capacity of a subset of mRNA translation in neurons primarily through phosphorylation of two downstream targets, p70-kDa ribosomal s6 kinase (p70s6K) and eukaryotic initiation factor 4E-binding protein 1 (4EBP1) [3,4]. Previous research using the selective mTOR kinase inhibitor rapamycin (RAP) showed that mTOR signalling is a critical constituent of activity-dependent synaptic plasticity in a range of preparations [5–7]. In addition, the mTOR pathway appears to be engaged following a variety of learning paradigms, exhibiting increased phosphorylation of p70s6K in hippocampal and amygdala nuclei [8–11]. Furthermore, treatment with RAP around the time of training has implicated the mTOR pathway in consolidation of both shock-induced cue and contextual associative fear memory [8,10,12–14]. Interestingly, we and others have shown a recurrent, protracted, RAP-sensitive consolidation event that contributes to the consolidation of shock-induced fear memory [14,15].

While these data highlight the importance of mTOR in formation of cue/context-specific fear memories, the role of mTOR in consolidation of predator stress-induced, non-associative fear memories is less clear. Predator stress is an ecologically relevant animal model of PTSD in that it presents animals with a traumatic event (exposure to a predator or predator cues) that they may encounter in nature [16–18]. Predator stress paradigms reliably induce non-associative fear memories manifested as hyperarousal (enhanced acoustic startle response) and anxiety-like behaviour (ALB) [17,19–21]. Predator stress-induced behavioural changes in hyperarousal and anxiety are apparent in environments different from the cat exposure, suggesting these changes are not conditioned, but rather are measures of non-associative fear. Recent data from our lab suggests that mTOR signalling regulates acquisition of predator stress-induced non-associative fear memories [22]. However, to our knowledge, a precise role of mTOR in consolidation of predator stress-induced fear memories has not been identified. The first aim of this study was therefore to examine the effects of systemic RAP on consolidation of predator stress-induced non-associative fear memories.

As described above, prior studies have indicated that biphasic mTOR signalling is critical for the consolidation of shock-induced fear memories [14,15]. Thus, the second aim of this study was to examine mTOR-sensitive time windows following predator stress that regulate non-associative fear memories. Elucidating the molecular factors contributing to both associative and non-associative fear memories will provide valuable insight into the nature of stress related disorders such as PTSD.

2. Methods

2.1. Subjects

A total of 320 male Long Evans rats (Charles River, Canada) were used in Experiment 1, 2 and 3. Rats were individually housed in clear plastic cages with wire tops (42 cm × 25 cm × 20 cm). Food and water were available ad libitum and rats were habituated to the housing room for two weeks on a 12 h light/dark reverse light cycle (lights off at 7 am). Animals were handled for five consecutive days prior to the start of the study. Handling consisted of petting and lifting rats for approximately 30 s to 1 min under a red lamp in the colony room. The colony rooms for the rats were at the point farthest possible from the room where the cats were housed to ensure isolation from olfactory cues. After exposure to the cat, predator stressed rats were housed in a different room away from handled control rats. Residual olfactory cues from the cat exposure may have been present on predator stressed rats; therefore, housing these rats away from handled controls would eliminate the effect of any olfactory cues on unstressed rats. Procedures for Experiments 1-3 adhered to the guidelines of the Canadian Council on Animal care, and were approved by the Institutional Animal Care committee of Memorial University.

2.2. Groups and procedures

2.2.1. Experiment 1

Rats were randomly assigned to one of four groups ($n = 20$): handled controls (HC), predator stressed only (PS), predator stressed plus vehicle (PSV) and predator stressed plus rapamycin (PSR). Rats in the HC group were handled on predator exposure day and remained undisturbed in their home cage until behavioural testing. Predator stressed animals (rats in PSR, PS, and PSV groups) received a 10 min unprotected exposure to a cat. Full details of the cat exposure can be found in the section 2.4.1. Immediately following cat exposure, rats in the PSV and PSR groups received an i.p. injection of vehicle or rapamycin (40 mg/kg), respectively. Rats were returned to the housing room immediately after cat exposure and left undisturbed until behavioural testing.

Seven days after cat exposure or handling, all rats underwent several tests of anxiety-like behaviour (ALB) and hyperarousal including EPM, HB, LD box, and response to acoustic startle. Behavioural tests were run over three days with HB and EPM on the first testing day, LD box on the second day and acoustic startle response on the third. A detailed description of the behavioural tests can be found below in the section 2.4.

2.2.2. Experiment 2

Rats were randomly assigned to one of eight experimental groups ($n = 20$): handled control (HC), predator-stressed animals (PS), rapamycin (RAP) injection 3 h post-stress (PSR3), vehicle injection 3 h post-stress (PSV3), RAP injection 9 h post-stress (PSR9), vehicle injection 9 h post-stress (PSV9), RAP injection 24 h post-stress (PSR24), and vehicle injection 24 h post-stress (PSV24). On test day, rats were exposed to a cat (PS, PSR3, PSV3, PSR9, PSV9, PSR24, PSV24) or handled (HC). Following cat exposure, rats were given an intraperitoneal (i.p.) injection of RAP or vehicle at the time designated by their experimental group. HC and PS groups did not receive an injection. Immediately following cat exposure or handling, rats were left undisturbed (except for injection) until behavioural testing. behavioural testing was similar to Experiment 1.

2.2.3. Experiment 3

Rats were randomly assigned into four groups ($n = 20$/group): Handled controls (HC), predator stressed only animals (PS), predator stressed animals plus an injection of rapamycin (PSR) and predator stressed animals plus a vehicle injection (PSV). As in the previous experiments, HC rats were handled only on cat exposure day. Predator stressed rats (PS, PSR, PSV) were exposed to a cat for a 10 min period. Forty-eight hours later, PSR and PSV rats were given an i.p. injection of rapamycin or vehicle, respectively. Behavioural testing was similar to Experiments 1 and 2.

2.3. Drug administration

Similar to previous studies [14,22], rats received an i.p. injection of rapamycin (40 mg/kg dose, injection volumes of 10 ml/kg, volume dependent on rat weight) or vehicle (5% ethanol, 4% PEG400, and 4% Tween 80 in sterile water, volume dependent on rat weight).

2.4. Behavioural testing

Groups were counterbalanced for both time of day tested and time of day exposed to a predator. This was done to control for