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Research report

Acute systemic rapamycin induces neurobehavioral alterations in rats

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HIGHLIGHTS

- We analyzed in rats the neurobehavioral impact of a single low dose rapamycin.
- Rapamycin increased amygdaloid neuronal activity (EEG, FOS).
- Rapamycin induced anxiety-like behaviors (elevated plus-maze, open-field).
- Rapamycin lead to over-expression of anxiety-related proteins (KLK8, FKBP51).

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ABSTRACT

Rapamycin is a drug with antiproliferative and immunosuppressive properties, widely used for prevention of acute graft rejection and cancer therapy. It specifically inhibits the activity of the mammalian target of rapamycin (mTOR), a protein kinase known to play an important role in cell growth, proliferation and antibody production. Clinical observations show that patients undergoing therapy with immunosuppressive drugs frequently suffer from affective disorders such as anxiety or depression. However, whether these symptoms are attributed to the action of the distinct compounds remains rather elusive. The present study investigated in rats neurobehavioral consequences of acute rapamycin treatment. Systemic administration of a single low dose rapamycin (3 mg/kg) led to enhanced neuronal activity in the amygdala analyzed by intracerebral electroencephalography and FOS protein expression 90 min after drug injection. Moreover, behavioral investigations revealed a rapamycin-induced increase in anxiety-related behaviors in the elevated plus-maze and in the open-field. The behavioral alterations correlated to enhanced amygdaloid expression of KLK8 and FKBP51, proteins that have been implicated in the development of anxiety and depression. Together, these results demonstrate that acute blockade of mTOR signaling by acute rapamycin administration not only causes changes in neuronal activity, but also leads to elevated protein expression in protein kinase pathways others than mTOR, contributing to the development of anxiety-like behavior. Given the pivotal role of the amygdala in mood regulation, associative learning, and modulation of cognitive functions, our findings raise the question whether therapy with rapamycin may induce alterations in patients neuropsychological functioning.

1. Introduction

Immunosuppressive or anti-proliferative drugs are widely used for treatment of inflammatory autoimmune diseases, cancer, or solid-organ transplantation. But, the knowledge of the effects in the central nervous system (CNS) is limited. For example, clinical evidence indicates that in a number of patients treatment with immunosuppressive drugs such as cyclosporin A (CsA) or tacrolimus is accompanied by increased incidence rates of developing affective or anxiety symptoms such as depression, aggression,

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paranoia, and apathy [1–5]. Likewise, it was demonstrated in rodents that chronic administration of CsA decreases calcineurin activity in the brain resulting in neurobehavioral activation [6], or elevated levels of anxiety-behavior [7].

Rapamycin (also known as sirolimus), a product of the bacterium *Streptomyces hygroscopicus*, has first been identified as a macrolid in the 1970s [8,9]. It inhibits the activity of the mammalian target of rapamycin (mTOR), a serine/threonine protein kinase and member of the phosphatidyl inositol 3′ kinase (PI3K) family that is known to play an important role in cell growth and proliferation [10–13]. Dysregulation of mTOR signaling occurs in various human tumors, and has been associated with cancer pathogenesis, disease progression, and treatment resistance [14]. Since treatment with mTOR inhibitors such as rapamycin has been shown to exert broad anti-tumor activity in experimental animal models as well as in patients [15–17] this drug reached importance in clinical practice. Due to its properties to inhibit antigen-induced proliferation of T and B cells, as well as antibody production [8], rapamycin is furthermore widely used as immunosuppressant in organ transplantation [18,19].

Data revealed that peripheral administration of rapamycin results in brain drug concentrations and in CNS effects [20]. More important, beneficial impact of rapamycin on behavioral disturbances accompanying neurological diseases such as epilepsy, tuberous sclerosis complex, or traumatic brain injury have been documented [11,21–24]. Furthermore, rapamycin-induced mTOR inhibition was able to attenuate traumatic fear memory reconsolidation and also inhibited contextual fear memory [25,26]. These findings suggest that inhibition of mTOR signaling by rapamycin may be used as a potential therapeutic strategy for treating affective symptoms accompanying neurological diseases [11,20].

However, despite the known beneficial effects of rapamycin and its widespread application in clinical conditions such as transplantation medicine and oncology, the knowledge of neurobehavioral effects of acute treatment in healthy subjects is surprisingly sparse. In order to extend our understanding of the involvement of compound in neurobehavioral outcomes, the present study investigated in rats, possible neurobehavioral consequences of a single systemic low dose of rapamycin.

2. Materials and methods

2.1. Animals

Male Dark Agouti rats (DA/HanRj, 200–230 g; Janvier, France) were single housed with ad libitum access to food and tap water. The vivarium was temperature (20 °C) and humidity (55 ± 5%) controlled and maintained on a reversed 12-h dark–12-h light cycle (7:00 a.m. to 7:00 p.m.) to allow the experiments to be conducted during the active phase of the rats' awake/sleep cycle. Animals were allowed to acclimate to the new surroundings for 2 weeks before initiation of the experiments. The animal facilities, surgical and experimental procedures were in accordance with National Institutes of Health and Association for the Assessment and Accreditation of Laboratory Animal Care guidelines and were approved by the Institutional Animal Care and Use Committee (LANUV Düsseldorf, North Rhine-Westphalia and LDS Leipzig, Saxony, Germany).

2.2. Drugs

Based on previous studies [27,28] employing low therapeutic doses of rapamycin (LC Laboratories, Woburn, MA, USA), the mTOR inhibitor was dissolved in a vehicle solution, containing cremophor (62%), ethanol (33%), and aqua dest. (5%) at a dose of 3 mg/kg. The vehicle solution was diluted with sterile saline according to animal's individual weight to gain a final injection volume of 0.5 ml and was administered intraperitoneally (i.p.).

2.3. Experimental design

The present study used three separate groups of rats. In *group 1* ($n = 16$) the neuronal activity in the amygdala was recorded as baseline electroencephalogram (EEG) for 200 min post-injection of rapamycin. Subsequently, two different groups were used to determine the acute behavioral and molecular effects of single systemic rapamycin treatment. In *group 2* rats performance in the elevated plus-maze ($n = 26$), and the open-field was assessed ($n = 16$). Finally, *group 3* ($n = 16$) was used for analyzing protein expressions in the amygdala.

Data indicate that following single intravenous administration, rapamycin plasma concentration peaks at 1 h post injection, and maintains for a long period of time with a half-life of over 60 h [29]. According to these findings and because the present study employed i.p. administration, behavioral as well as molecular analyses were conducted 90 min following systemic injection of the drug.

2.4. Intracerebral EEG recordings

In *group 1* electrode implantation and EEG recordings were performed as described previously with modifications [30]. Animals were deeply anesthetized with ketamine hydrochloride (90 mg/kg, Ketanest®, Parke–Davis, Karlsruhe, Germany) and xylazine hydrochloride (15 mg/kg; Rompun®, Bayer Health Care, Leverkusen, Germany). A monopolar stainless steel EEG electrode (outer diameter: 0.25 mm, insulated with polyurethane except for the tip) was stereotaxically implanted into the left or right central amygdala (distribution was equally over both sites). The coordinates used for EEG recording were as follows: antero-posterior −2.5 mm; lateral ±4.3 mm; dorso-ventral −8.0 mm relative to bregma [31]. As indifferent electrode, a stainless steel screw was positioned at the surface of the cerebellum. The electrodes were soldered to a socket (TSE Systems, Bad Homburg, Germany) and fixed to the skull with dental cement (Technovit 3040, Heraeus Kulzer, Wehrheim, Germany). For postoperative care rats were treated with antibiotic (Retacillin compositum, 200,000 IE, i.m.; mibe GmbH Arzneimittel, Brehna, Germany) and analgesic (Rimadyl®, 5 mg/kg, s.c.; Pfizer, USA), and were allowed to recover from surgery for 14 days. On testing days, animals were transferred to the experimental room and habituated to the new environment for at least 1 h. EEG transmitters (TSE Systems) were plugged to the implanted electrode socket and baseline EEG activity (three 5 min blocks separated by 10 min intervals) was recorded from the freely moving rats. Subsequently, animals were gently lifted and injected with rapamycin or vehicle and EEGs were recorded for 200 min. EEG signals were telemetrically received via pulse position modulation, transmitted to a decoder, and digitized with a sample rate of 128/s. After removing artifacts from the recordings, power density spectra were computed for periods of 4 s by fast Fourier transformation and averaged for each 5 min block [32]. Absolute EEG power (μV) of the recorded frequency range (0.6–30 Hz) was summarized and mean total EEG power was calculated [6,33]. Data are expressed as percentage of baseline EEG activity. At the end of the experiment, animals were euthanized, the brains were removed and the correct placement of the electrode tips was histologically verified and mapped onto standardized coronal sections (Fig. 1C) of a rat brain stereotaxic atlas [54].

2.5. Elevated plus-maze and open-field

Behavioral testing was performed in *group 2* during the dark phase (activity period) of the animals under red-light illumination.