The involvement of hippocampal CA3 TRP channels in anxiety and avoidance memory consolidation in rats tested in elevated plus maze

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ABSTRACT

In the current study, we assessed the role of transient receptor potential (TRP) channels on avoidance memory and anxiety states in CA3 area of the hippocampus. We explored the anxiety and avoidance memory states using test-retest protocol in the elevated plus maze to understand whether TRP channels can affect the above mentioned states in CA3 area. To investigate the consolidation phase of memory, the drugs were injected into the CA3 region before the test. Our data showed that the application of SKF-96365 did not alter anxiety-like behaviors but induced avoidance memory impairment. It was revealed that CA3 TRP channels could affect the avoidance memory consolidation and their role must be considered in future research.

Keywords: TRP Channels; SKF-96365; Memory; Anxiety; Elevated Plus Maze; Rats

INTRODUCTION

Transient receptor potential (TRP) channels, have emerged as important players in seizure and excitotoxicity. They are non-selective cation channels permeable to sodium and Ca^{2+} ions. TRP channels are categorized as: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPP (polycystin), TRPN (nompC), and TRPML (mucolipin). TRPC subfamily, the first mammalian cloned, are sub-grouped into TRPC1/4/5, TRPC3/6/7, and TRPC2 [1-3]. In many previous reports, 1-[2-(4-methoxyphenyl)-2- [3- (4-methoxyphenyl) propoxy]ethyl-1H-imidazole hydrochloride (SKF-96365) has been used as the inhibitor of the TRP channels [4-7].

The superfamily of TRP channels share a common architecture with voltage-gated potassium channels and calcium channels [8]. The expression of TRPC1 in most limbic areas has been reported from moderate to high [9, 10] and TRPC5 is highly expressed in the CA1-CA3 region of the hippocampus and the amygdala [11, 12]. It has been shown that the gating of these channels can be done either by G-protein coupled receptors [13] or directly by a rise in free intracellular Ca^{2+} [14, 15].

All this clearly shows the important role of TRP channels in calcium signaling.

Calcium entrance can regulate a number of important functions of the cell from muscular contraction to action potential propagation in neurons [16]. Calcium is also a key player in cognitive functions such as learning and memory [17]. TRP channels as alternative pathways for Ca^{2+} entrance have been considered in a number of studies on cognitive and non-cognitive behaviors [18-20]. For example, it has been reported that decreasing the reuptake of serotonin in synapses, the activation of TRPC6 can be used as a treatment for depression [21]. The TRP channels contribution has also been investigated in growth cone guidance [22] excitotoxicity [2], cancer [18], and memory consolidation [23] and they seem to be promising molecular targets for

the treatment of head trauma, stoke and epilepsy [2].

Hippocampal formation has been widely recognized for its crucial role in spatial and contextual learning and memory [24, 25] and anxiety [26, 27]. Considering the structure and connections of hippocampus, the CA3 area receives connection from entorhinal cortex and dentate gyrus as well as a number of subcortical structures like amygdala which gives it a special position in processing emotional and memory related data [28].

Based on existing evidence of the role of TRP channels in cognitive processes, the current study aimed at investigating the involvement of CA3 TRP channels in the anxiety and avoidance memory states in a test-retest protocol in elevated plus maze using SKF-96365.

MATERIALS AND METHODS

Animals

A sum of 28 male Wistar rats purchased from Pasteur Institute of Iran (weight: 220–270 g, age: 7-8 weeks at the time of surgery) were used and housed 6-7 per cage in a room with a 12:12 h light/dark cycle (lights on 07:00 hours). The temperature was controlled at 23±1°C. Animals had free access to food and water and were adapted to the laboratory conditions for one week prior to the surgery. The rats were handled about 3 minutes/day in advanced to behavioral testing. The experiments were conducted in accordance with the guide for care and use of laboratory animals established by the National Institute of Health of the United States of America (1996).

Stereotaxic surgery and drug microinjections

The animals were anesthetized using 2 ml/kg intraperitoneal injection of ketamine hydrochloride 10% (50 mg/kg) and xylazine 2% (4 mg/kg). Using the rat brain atlas by Paxinos and Watson [29] and to aim the ventral CA3 area (AP -4.5, ML ±5.2, DV -7.6; Figure 1), the rats were placed in Kopf stereotaxic frame and two stainless steel guide cannulas with a length 11.0 mm and an outer diameter of 0.6 mm were implanted bilaterally and fixed. Drug injections were made by a 2 µl Hamilton syringe connected by a polyethylene tube to an internal cannula (27-gauge, terminating 1.3 mm below the tip of the guides). Injections were made over 1 minute into the CA3 area and 5 minutes before testing [30].

Elevated Plus-Maze (EPM)

The device consisted of two opposite arms (50×10 cm) surrounded by a 1 cm high Plexiglas ledge and two enclosed-arms (50×10×40 cm) set up 50 cm above the floor and was made of wood. The junction area of four arms was an area of 10×10 cm [31]. The animals were individually placed in the center of the EPM, as described by Pellow and File [32], facing an open arm and allowed free exploration. The EPM model is a common test of animal anxiety [33] and can also be used to measure the effect of emotional states on the memory in a repeated measures protocol [34]. Based on the observed experience-dependent behavioral changes, the test-retest protocol gives a measure of acquisition and memory retention [35].

Drugs

Ketamine and xylazine - purchased from Alfasan Chemical Co, Woerden, Holland - were used for animal anesthesia. SKF-96365 was purchased from Tocris (Bristol, UK).

Histology

After the experiments, a lethal dose of pentobarbital (100 mg/kg, i.p.) was intraperitoneally injectioned. Then, 0.5 µl of a 50% Indian ink solution was injected to mark the implantation sites. The brains were removed and fixed in 10% neutral buffered formalin for at least 48 hours. Slices (50 µm thick) were obtained and mounted on glass microscope slides for localization according to the diagrams from Paxinos and Watson's rat brain atlas [29]. If the drug infusion was outside the CA3 region, the data of the rat was excluded from the analysis.

General conditions and data collection

The tests were undertaken in a low illuminate (40-lux) environment, during the diurnal phase. The apparatus was cleaned with a wet tissue paper (10% ethanol solution) after each test to avoid urine impregnation. The following behavioral measures were scored: the number of open-(OAE) and enclosed-arms entries (EAE) with the four paws, and the time spent in open-arms (OAT). The resulted data were used to calculate the percentage of time spent in open

arms [34]. The percentage of OAE and OAT as the standard anxiety indices were calculated as follows: (a) %OAT (the ratio of time spent in the open arms to total times spent in any arm×100); (b) %OAE (the ratio of entries into open arms to total entries×100). (c) EAE (total closed arm entries were measured as a relative pure index of locomotor activity)[36].

Experimental protocol

Seven rats were used in each experimental group. After a one-week recovery, the rats were subjected to the following experimental procedures. The microinjection cannulas were inserted into the guide cannulas and the rats were injected bilaterally (0.5 µl on each side) with either saline or 0.9% sterile saline dissolved SKF-96365 (0.01 µg/rat). Five minutes after the completion of the injection, the rats were placed at the center of the EPM apparatus. The response of each animal was recorded for 5 minutes (test) and the videotapes were scored later. After a 24-hour interval, the animal's response on the EPM was recorded again (retest).

Statistical analysis

Data displayed normality of distribution and homogeneity of variance, hence repeated measure and t-tests was used for data analysis. SPSS (Version 16; SPSS Inc., Chicago, IL, USA) was used for data processing. P<0.05 represented a significant result in all comparisons.

RESULTS

Effects of pretest intra-CA3 microinjection of SKF96365 on open-arms exploratory behaviors

Repeated measure and dependent and independent t-test between the test and retest days showed that the intra-CA3 injection of SKF-96365 increased %OAT (p<0.001; Fig. 1, panel 2A) and %OAE (p<0.01; Fig. 1, panel 2B) at a dose of 0.01 µg/rat on the retest day as compared to their respective control group, but did not alter %OAT or %OAE on the test day. The data suggest that SKF-96365 induced avoidance memory impairment at the mentioned dose while it did not alter anxiety-like behaviors. (Table 1)

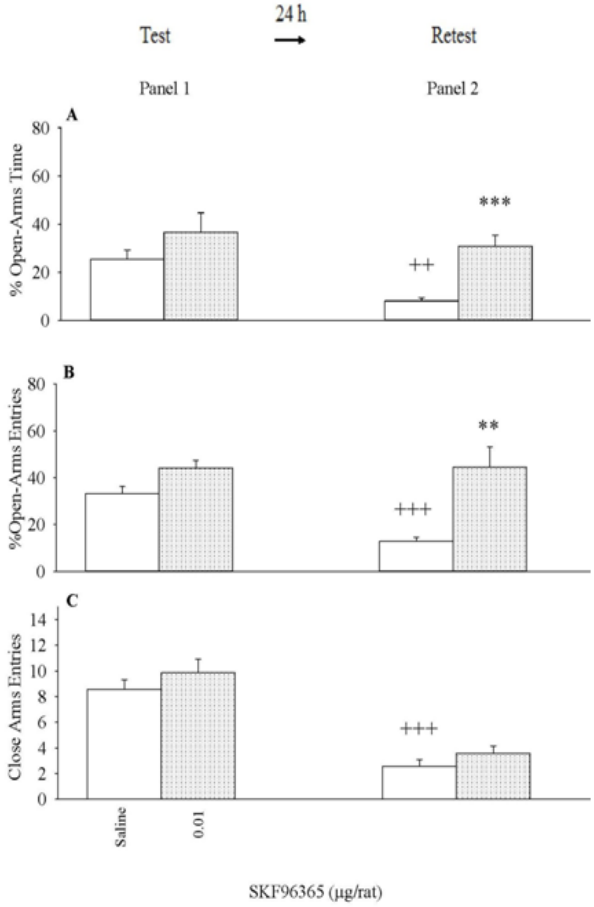

Figure 1. Panels 1 and 2: The effect of SKF-96365 on anxiety (Panel 1) and memory (Panel 2). Rats (n=7) were injected with saline (1 µl/rat) or SKF-96365 (0.01 µg/rat). The tests were performed 5 minutes after intra-CA3 injection of the drug. Each bar indicates mean ± SEM. (A) %OAT (percentage open arm time), (B) %OAE (percentage open arm entries) and (C) EAE (enclosed-arm entries). ++p<0.01 and +++p<0.001 as compared with the control group in panel 1. **p<0.01 and ***p<0.001 as compared with the control group in panel 2.

Table 1. Data analysis in the experimental groups

Experiments	Behaviors	Inter-group		Intra-group		Inter-Intra group	
		F(1, 18)	P	F(2, 18)	P	F(2, 18)	P
Repeated measure analysis for SKF-96365 between panels 1 and 2 Fig.1)	%OAT	7.981	0.011	5.221	0.016	0.64	0.539
	%OAE	5.64	0.029	10.523	0.001	2.173	0.143
	EAE	106.143	0.001	1.256	0.309	0.619	0.549

DISCUSSION

Our data showed that SKF-96365, the inhibitor of TRP channels, induced avoidance memory impairment at the applied dose, which is an indication of the involvement of these channels in memory consolidation. However, no significant change in anxiety-like behaviors was observed at the applied dose, which is in line with previous report that SKF-96365 did not appear to alter anxiety-like behaviors on the EPM task [37]. The SKF-96365 effect on avoidance memory impairment was significant, which is in contradiction with the effects shown for the prelimbic cortex [37] and intra-medial septum [23]. Differences in the involved areas might be regarded as contributing factors. Reports showed that Ca^{2+} dependent molecules are involved in hippocampal synaptic plasticity and related processes [38-41]. As Ca^{2+} entrance pathways, TRP channels have recently been considered as novel targets for the development of therapeutic drugs for head trauma, epilepsy, and stroke [2]. It is shown that TRPC channels have seven members (TRPC1-7) in their family and they may have a share in various human diseases [42].

Also, it has been shown that TRPC1/4/5 channels are "polymodal" and can be activated either by G-protein coupled receptors like mGluR1 [13], or directly by an increase in intracellular free Ca^{2+} [14, 15].

"The authors declare no conflict of interest"

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TRPC3 and 6 channels are receptor-operated or store-operated [1, 3]. TRP channels are activated by phospholipase C produced diacylglycerol or tyrosine kinases [3]. They also permit Ca^{2+} influx through IP3 receptor-dependent mechanisms [3, 43]. As TRP channels let the influx of cations down

their electrochemical gradients, they can cause depolarization of the cell and regulate spontaneous firing activities [44]. T-type Ca^{2+} channels blockade under physiological conditions by SKF-96365 has also been reported. T-type Ca^{2+} channels are involved in cellular mechanisms of maintaining LTP [45].

For example, 10 μM SKF-96365 has been used to identify the contribution of non-selective cation channels in maintaining intracellular Ca^{2+} levels and spontaneous firing in midbrain dopamine neurons [44]. However, Singh reported that SKF-96365 may not be the ideal choice to study TRP channels in some tissues (Singh et al., 2010). Modulation of the Na^{+}/Ca^{2+} exchanger by SKF-96365 is another effective method to disrupt $[Ca^{2+}]_i$ homeostasis [46].

CONCLUSION

The experiment data showed that the TRP channels in the CA3 area are involved in producing amnesia and the role these channels must not be ignored in future memory related research.

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