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Angiotensin-(1-7)/Mas axis modulates fear memory and extinction in mice

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ABSTRACT

Inappropriate defense-alerting reaction to fear is a common feature of neuropsychiatric diseases. Therefore, impairments in brain circuits, as well as in molecular pathways underlying the neurovegetative adjustments to fear may play an essential role on developing neuropsychiatric disorders. Here we tested the hypothesis that interfering with angiotensin-(1-7) [Ang-(1-7)]/Mas receptor axis homeostasis, which appears to be essential to arterial pressure control, would affect fear memory and extinction. Mas knockout (MasKO) mice, in FVB/N background, showed normal cued fear memory and extinction, but increased freezing in response to context. Next, as FVB/N has poor performance in contextual fear memory, we tested MasKO in mixed 129xC57BL/6 background. MasKO mice behaved similarly to wild-type (WT), but memory extinction was slower in contextual fear conditioning to a weak protocol (1CS/US). In addition, delayed extinction in MasKO mice was even more pronounced after a stronger protocol (3CS/US). We showed previously that Angiotensin II receptor AT1 antagonist, losantan, rescued object recognition memory deficit in MasKO mice. Here, losartan was also effective. Memory extinction was accelerated in MasKO mice after treatment with losartan. In conclusion, we showed for the first time that Ang-(1-7)/Mas axis may modulate fear memory extinction. Furthermore, we suggest MasKO mice as an animal model to study post-traumatic stress disorder (PTSD).

1. Introduction

One of the most important physiological functions in animals is learning how to properly react to danger. Suitably, learning how to fear a threat is evolutionarily conserved and rapidly acquired (Martinez, Carvalho-Netto, Ribeiro-Barbosa, Baldo, & Canteras, 2011; Schmitz & Grillon, 2012). Furthermore, defense-alerting reaction to fear comprises species distinctive behaviors and neurovegetative adjustments (Diamant, Croiset, De Zwart, & De Wied, 1991; Stiedl & Spiess, 1997; Stiedl, Tovote, Ogren, & Meyer, 2004). For example, in Pavlovian fear conditioning, mice learn to associate an innocuous stimulus (CS, conditioned stimulus: tone or light) with an aversive stimulus (US, unconditioned stimulus: electric foot shock). After conditioning, freezing behavior and altered cardiovascular parameters are indicatives of learning (Hsu et al., 2012; LeDoux, Sakaguchi, & Reis, 1982).

Both elevation of blood pressure and freezing response reflect fear conditioning (Iwata, LeDoux, & Reis, 1986; LeDoux et al., 1982). Additionally, blood pressure rapidly increases in response to CS, whereas sympathetic tone decreases in response to US during fear-potentiated startle conditioning (Hsu et al., 2012). Same authors also showed that as better was these progressive changing during learning, better was the performance 24 h later. Together, these results indicate that blood pressure variations may predict fear learning and memory. Therefore, it is reasonable to suggest that fear learning and cardiovascular function shares at least some of the same molecular mechanisms.

Angiotensin-(1-7) [Ang-(1-7)] and its receptor Mas, combined with other components of renin–angiotensin system, are important mediators of cardiovascular function (Dias-Peixoto et al., 2008; Trask & Ferrario, 2007). Ang-(1-7) acting directly into the brain attenuates the development of DOCA-salt hypertension (Guimaraes et al., 2012) and lowers blood pressure in transgenic hypertensive rats (Garcia-Espinosa, Shaltout, Gallagher, Chappell, & Diz, 2012). In contrast, knockout of Mas receptor (MasKO), in

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FVB/N genetic background, induces mild-hypertension and endothelium dysfunction (Xu et al., 2008). Furthermore, MasKO mice, in mixed background 129xC57BL/6, are normotensive and have no alteration in baseline heart rate, though have increasing blood pressure variability (Walther, Wessel, et al., 2000).

Mas receptor expression, as well as Ang-(1-7) production are not restricted to brain areas involved in controlling cardiovascular functions. Mas receptor is highly expressed in hippocampus and amygdala (Freund, Walther, & von Bohlen und Halbach, 2012), as well as ACE2, the main enzyme responsible to synthesize Ang-(1-7) (Doobay et al., 2007; Elased, Cunha, Marcondes, & Morris, 2008). Ang-(1-7)/Mas axis functionality in areas like hippocampus seems to be important to maintain hippocampal plasticity. Ang-(1-7) increases hippocampal LTP magnitude and this effect was abolished in MasKO mice (Hellner, Walther, Schubert, & Albrecht, 2005; Walther, Voigt, Fink, & Bader, 2000). MasKO mice presents object recognition memory deficit that was rescued by blocking Angiotensin II AT1 receptor. Furthermore, Mas deletion increased hippocampal Ang-(1-7) production (Lazaroni et al., 2012).

Considering Ang-(1-7)/Mas axis role on controlling blood pressure and the evident intimacy between fear memory and cardiovascular parameters we hypothesized that fear memory, as well as fear memory extinction, are affected by changes in Ang-(1-7)/Mas axis homeostasis. Using genetic and pharmacological approach, our results showed for the first time that Ang-(1-7)/Mas axis modulates fear memory and extinction.

2. Material and methods

2.1. Animals

Adult male mice (8–12 weeks of age) were group-housed in acrylic cages, under 12 h light/dark cycle and controlled temperature (22 ± 1 °C), with water and food *ad libitum*. All behavioral protocols were conducted in light phase of cycle. We used two mouse strains: MasKO on FVB/N background (showed as MasKO and its control, FVB/N) and MasKO on mixed 129xC57BL/6 background (showed as MasKO and its control, WT).

All protocols were approved by Institutional Animal Care and Ethics Committee of Universidade Federal de Minas Gerais (136/2011).

2.2. Auditory fear conditioning

Conditioning chamber (Insight Equipamentos, Ribeirão Preto, Brazil) was a 23 × 23 × 30 cm Plexiglas box with black walls. Floor was made with stainless-steel grid rods (0.4 cm in diameter, spaced 0.6 cm apart) and at sealing there was a video camera system. Chamber was located inside a soundproof box. At the time of conditioning, mice were habituated to chamber during 120 s. After, a tone (CS: conditioned stimulus; 85 dB, 1KHz) was delivered during 30 s, being the last 2 s paired with foot-shock (US: unconditioned stimulus; 0.7 mA). After 30 s, animal was removed from conditioning chamber and returned to their home cage. Twenty-four hours later, animal was exposed to the same context where it was conditioned and during 5 min freezing behavior was measured. Freezing behavior (defined as complete lack of movement, except for respiration) was scored for 2 s in every 5 s (Radwanska et al., 2011). Three hours after, animal was put in a different context, with same size, but black and white walls and distinct smell, during 180 s and right after, same tone used in conditioning was delivered during additional 180 s. Mice were exposed to tone presentation exactly as explained before for two consecutive days, once a day. Freezing was quantified during the entire period.

2.3. Contextual fear conditioning

Contextual fear-conditioning chamber was the same used to cued fear conditioning experiments. 1CS/US protocol consisted in after 120 s of context (CS) habituation, animals received a 2 s foot-shock (0.7 mA) and 90 s after were removed from chamber. In 3CS/US protocol, animals received two additional shocks, with 90 s interval between them. Contextual fear memory was evaluated 24 h after training by measuring freezing during 600 s of context re-exposure. Memory extinction was evaluated by exposing animals to the same context during 600 s, once a day, along 3 or 5 consecutive days. Freezing behavior (defined as complete lack of movement, except for respiration) was scored for 2 s in every 5 s (Radwanska et al., 2011).

2.4. Locomotor activity

Spontaneous locomotor activity was evaluated automatically (Acrylic Cage – 11.5″L × 7.375″W × 5″H, Accuscan Instruments Inc. Columbus, OH) and analyzed with the VersaMax® software. Horizontal, vertical and total activity was measured during 120 min, in blocks of 5 min (Sotnikova et al., 2004).

2.5. Shock threshold test

Mice were introduced in an acrylic chamber (23 × 23 × 30 cm) with metallic grid floor. After, 1 s duration foot shocks with intensities varying from 0.1 mA to 1.2 mA were delivered at random intervals (30–60 s). Each animal received only five consecutive foot shocks. Minimal shock intensity to produce flitching, movement, jumping, freezing and vocalization was recorded (Klemenhagen, Gordon, David, Hen, & Gross, 2006).

2.6. Drugs

Losartan (Sigma–Aldrich), antagonist of Angiotensin II type I receptors, was administered intraperitoneally, at 10 mg/kg, immediately after each extinction session in 3CS/US contextual fear conditioning protocol.

2.7. Data analysis

Gaussian distribution of data was analyzed using KS normality test. Simple comparisons between two groups were analyzed by unpaired *t*-test (Figs. 1A, 2A and B, 4A and B). All other data were analyzed with repeated measures two-way ANOVA and Bonferroni's post-hoc. Factors were genotype and trial in Figs. 1B, 3A and B. In Figs. 2C, D, 4C and D factors were genotype and time, while in Fig. 5 were treatment and trial.

3. Results

3.1. Auditory fear-conditioning

MasKO mice were trained in the auditory fear-conditioning paradigm and twenty-four hours after tested for contextual and cued-fear memory. As observed, 1CS/US protocol elicits a weak behavioral response for contextual fear memory in both groups (between 10% and 20% of freezing), but knockout mice spent more time freezing than FVB/N mice ($t(14) = 2.8$, $p = 0.01$) (Fig. 1A). In cued fear memory there was no interaction between factors ($F(3,42) = 1.51$, $p = 0.22$), though we observed a main effect of genotype ($F(1,42) = 5.06$, $p = 0.04$), which failed to reach statistical significance in the post-hoc test. We also detected a main effect of