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Enhanced excitability in the infralimbic cortex produces anxiety-like behaviors

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ABSTRACT

The medial prefrontal cortex (mPFC) has been implicated in modulating anxiety. However, it is unknown whether excitatory or inhibitory neurotransmission in the infralimbic (IL) subregion of the mPFC underlies the pathology of anxiety-related behavior. To address this issue, we infused the $GABA_A$ receptor ($GABA_AR$) antagonist bicuculline to temporarily activate the IL cortex. IL cortex activation decreased the time spent in the center area in the open field test, decreased exploration of the open-arms in the elevated plus maze test, and increased the latency to bite food in the novelty-suppressed feeding test. These findings substantiate the GABAergic system's role in anxiety-related behaviors. IL cortex inactivation with the AMPA receptor (AMPAR) antagonist CNQX produced opposite, anxiolytic effects. However, infusion of the NMDA receptor (NMDAR) antagonist AP5 into the IL cortex had no significant effect. Additionally, we did not observe motor activity deficits or appetite deficits following inhibition of GABAergic or glutamatergic neurotransmission. Interestingly, we found parallel and corresponding electrophysiological changes in anxious mice; compared to mice with relatively low anxiety, the relatively high anxiety mice exhibited smaller evoked inhibitory postsynaptic currents (eIPSCs) and larger AMPA-mediated evoked excitatory postsynaptic currents (eEPSCs) in pyramidal neurons in the IL cortex. The changes of eIPSCs and eEPSCs were due to presynaptic mechanisms. Our results suggest that imbalances of neurotransmission in the IL cortex may cause a net increase in excitatory inputs onto pyramidal neurons, which may underlie the pathogenic mechanism of anxiety disorders.

1. Introduction

Abnormal excitability in the CNS is thought to be associated with the origin of anxiety disorders (Gorman, 2003). However, the functions of brain areas in regulating rodent neuroendocrine and autonomic responses to stress and anxiety-related behaviors have not been fully clarified. Classical 1,4-benzodiazepines, which act on the $GABA_AR$/chloride channel, have marked anxiolytic activity and are the established standard treatment for anxiety disorders (Johnson and Rodgers, 1996). However, benzodiazepines' sedative–hypnotic, muscle relaxant, and memory impairing effects may limit their therapeutic application. Therefore, a pharmacological challenge is to investigate the neurobiological mechanisms and pathophysiology of anxiety and help to develop improved treatment for anxiety.

The mPFC is important in the processing of cognitively relevant events and in modulating stress responses (Burghy et al., 2012; Kantak et al., 2012; MacDonald et al., 2000; Radley et al., 2006). Recent research has demonstrated that exposure to even brief periods of intense stress is sufficient to cause significant structural remodeling of neurons within the rodent PFC (Holmes and Wellman, 2009). The mPFC is composed of the medial agranular, anterior cingulate (AC), prelimbic (PL), and infralimbic (IL) cortices. The mPFC appears to play a rather complex role in mediating rodent anxiety-like behavior that may be due to the different functions of its subregions (Holmes and Wellman, 2009; Radley et al., 2006; Stern et al., 2010). Broader inferences drawn from work

Abrreviations: mPFC, medial prefrontal cortex; IL, infralimbic; PL, prelimbic; $GABA_AR$, $GABA_A$ receptor; AMPAR, AMPA receptor; NMDAR, NMDA receptor; AP5, D(−)-2-amino-5-phosphonopentanoic-acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; BMI, bicuculline methiodide; eIPSCs, evoked inhibitory postsynaptic currents; eEPSCs, evoked excitatory postsynaptic currents; mIPSCs, miniature inhibitory postsynaptic currents; mEPSCs, miniature excitatory postsynaptic currents.

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exploring the modulation of stress-related behaviors suggest that the ventral (IL) mPFC influences the hypothalamo–pituitary–adrenal (HPA) response to emotional stress (Heidbreder and Groenewegen, 2003; Radley et al., 2006). Electrolytic lesions of the rodent mPFC including both the PL and IL decrease anxiety-like behavior in the elevated plus maze, Vogel conflict tests and shock-probe burying tests (Lacroix et al., 2000; Shah and Treit, 2003; Sullivan and Gratton, 2002). The tentative conclusion that the mPFC promotes anxiety-like behavior can be drawn, and further functional and molecular mechanisms of mPFC in anxiety should be investigated to support this conclusion.

Studies have consistently demonstrated that GABAergic and glutamatergic transmission in laboratory animals is extremely sensitive to a variety of physical, neurological, and psychological stressors. There appears to be a balance between glutamate receptor-mediated excitation and gamma aminobutyric acid receptor (GABAR)-mediated inhibition in the regulation of behavioral responses associated with anxiety (Sajdyk and Shekhar, 1997). Both increases in GABAergic transmission and decreases in glutamatergic transmission in areas such as the hippocampus, amygdala, hypothalamus, and the periaqueductal gray matter can produce anxiolytic effects (Davidson, 2002; Davis, 1997). The total mPFC cortex's role in anxiety has also been studied via local infusion of drugs such as the $GABA_AR$ agonist muscimol (Shah et al., 2004). The PL cortex's role has been studied by local infusion of drugs such as glutamatergic N-methyl-D-aspartic acid (NMDA) receptor antagonist, adrenergic beta-1 receptor antagonists, and cobalt (Stern et al., 2010). Conflicting results have been reported regarding the anxiolytic-like effects of AMPAR antagonists in animal models; some authors have reported anxiolytic effects of AMPAR antagonists (Alt et al., 2006; Benvenga et al., 1995; Kotlinska and Liljequist, 1998; Matheus and Guimaraes, 1997), whereas other authors have found anxiogenic effects (Karcz-Kubicha and Liljequist, 1995). This discrepancy may be due to differences in the antagonists used. However, there is a lack of data on the regulation of anxiety by neurotransmission in the IL cortex.

The aim of this study was to evaluate the function of $GABA_A$, NMDA, and AMPA receptors in the IL cortex in animal models of anxiety. To distinguish the anxiolytic-like activities of the antagonist compounds from their potential side effects, motor activity in the open field test was examined. Whole-cell currents in the IL including excitatory and inhibitory postsynaptic currents were recorded to investigate the electrophysiological mechanisms underlying the pathophysiology of anxiety.

2. Materials and methods

2.1. Subjects

Adult male C57 BL/6 mice weighing 20–25 g and aged 10–12 weeks at the time of testing were housed in standard laboratory cages (four to five per cage) on a 12 h light/dark cycle (lights on at 8:00 A.M.) in a temperature-controlled room (21–25 °C). Mice were housed with free access to food and water. Behavioral testing was performed during the light cycle between 10:00 A.M. and 5:00 P.M. Procedures were conducted in accordance with the Chinese Council on Animal Care Guidelines (Zhu et al., 2010). Efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

D(−)-2-Amino-5-phosphonopentanoic-acid (AP5; Sigma–Aldrich, USA) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Tocris Bioscience, UK) were dissolved in artificial cerebrospinal fluid (ACSF). Bicuculline methiodide (BMI; Tocris Bioscience, UK) was dissolved in dimethyl sulfoxide (DMSO), and the concentration of DMSO (Sigma) used for all solutions was less than 0.1%. All other chemicals were from Sigma–Aldrich. Dose selections for these drugs were based on both pilot and previously published studies (Corcoran et al., 2011; Rossato et al., 2009; Sanders and Shekhar, 1995).

2.3. Intracerebral infusions

As previously reported (Rodriguez Manzanares et al., 2005), mice were anesthetized with an intraperitoneal injection of chloral hydrate (400 mg/kg) and placed into a stereotaxic instrument (Stoelting, USA). The mouse scalp was removed, and small burr holes were drilled into the skull (1 mm diameter) with a dental drill. Stainless-steel guide cannulas (Plastics One, INC; C315G/SPC; length, 6 mm) were lowered unilaterally into the IL cortex according to the following coordinates: anterior −1.9 mm; lateral ±0.4 mm; ventral −2.75 mm; and into the PL cortex according to the following coordinates: anterior −2.7 mm; lateral ±0.4 mm; ventral −1.5 mm (Johnson et al., 2010). The guide cannulas were fixed in place using glass ionomer cements. Dummy cannulas (Plastics One, INC; C315DC/SPC, length matched to the guide cannulas) were placed inside the guide cannulas to prevent occlusion. Mice recovered on an electric blanket and were then placed back into their home cages. Seven days after surgery, the behavioral tests were performed.

To perform local infusions, the dummy cannulas were removed quickly from the guide cannulas and replaced by infusion cannulas (Plastics One, INC; C315I/SPC, lengths matched to the guide cannulas). The infusion cannulas were connected, via polyethylene tubing (Plastics One, INC; C313C), to 10 μl microsyringes (Hamilton, Reno, NV) mounted on a microinfusion pump (RWD200, China). The injection amount was 0.5 μl/side, and the rate was 0.25 μl/min as previously reported (Huff and Rudy, 2004; Rodriguez Manzanares et al., 2005; Rosenkranz and Grace, 2002). Next, the infusion cannulas were held in place for another two minutes to allow diffusion of the drug before being replaced with the dummy cannulas.

2.4. Open field test

The open-field test was performed in a rectangular chamber (60 × 60 × 40 cm) composed of gray polyvinyl chloride. The center area was illuminated by 25 W halogen bulbs (200 cm above the field). Mice were gently placed into the testing chamber for a 5 min recording period, which was monitored by an automated video tracking system. Images of the paths traveled in those 5 min were automatically calculated using the DigBehv animal behavior analysis program.

2.5. Elevated plus-maze test

The elevated plus-maze test was composed of plastic and consisted of open arms (10 × 50 cm) located opposite one another and two enclosed arms (10 × 50 × 40 cm) located opposite one another that were 40 cm above the floor. The junction of the four arms (the central platform) measured 10 × 10 cm (Stern et al., 2010). At the beginning of the 5 min test, each mouse was placed onto the center platform of the elevated plus maze facing the closed arm. Computational software autonomously quantified the time spent in the closed and open arms.

2.6. Novelty suppressed-feeding test

One single pellet of food was placed on a white piece of paper positioned at the center of the testing box (50 × 50 × 20 cm), and the box floor was covered with 2 cm thick padding. After 24 h of food (but not water) deprivation, mice were placed into the testing box. A stop watch was used to measure 5 min. Latency was scored as the time at which the mice began biting the food. Immediately after that, the mice were transferred to their home cage for another 5 min, and food intake amount over this time was measured (home-cage food intake).

2.7. Electrophysiological recordings

This study's entire protocol is based on previous studies from our laboratory (Chen et al., 2010; Woo et al., 2007). Infralimbic cortical slices (0.4 mm) were prepared from P28–P36 mice using a Vibroslice (Leica VT 1000S) in an ice-cold solution that contained 220 mM sucrose, 2.5 mM KCl, 1.3 mM $CaCl_2$, 2.5 mM $MgSO_4$, 1 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 10 mM glucose. Slices were allowed to recover for at least 1.5 h (0.5 h at 34 °C followed by 1 h at 25 ± 1 °C) in an ACSF solution containing 126 mM NaCl, 26 mM $NaHCO_3$, 3.0 mM KCl, 1.2 mM NaH_2PO_4, 2.0 mM $CaCl_2$, 1.0 mM $MgSO_4$, and 10 mM glucose. Slices were placed in the recording chamber and superfused (1.5 ml/min) with ACSF at 32–34 °C. All solutions were saturated with 95%O_2/5%CO_2. Neurons were visualized with an IR-sensitive CCD camera with a 403 water-immersion lens (Zeiss, Axioskop2 Fsplus) and recorded using whole-cell voltage-clamp techniques (MultiClamp 700B Amplifier, Digidata 1320A analog-to-digital converter) and pClamp 9.2 software (Axon Instruments). All the recording cells were pyramidal neurons and located in layers V–VI of the IL (Ninan et al., 2012). Pyramidal neurons were identified by the pyramid-shaped cell body with a single apical dendrite and multiple basal dendrites.

EPSCs were recorded in the presence of the $GABA_AR$ antagonist BMI (20 μM). To record eEPSCs, glass pipettes were filled with the following solution: 105 mMK-gluconate, 30 mM KCl, 10 mM HEPES, 10 mM phosphocreatine, 4 mM ATP-Mg, 0.3 mM GTP-Na, 0.3 mM EGTA, and 5 mM QX314 (pH 7.35, 285 mOsm). To pharmacologically isolate AMPAR- or NMDAR-mediated EPSCs, we blocked NMDAR with 100 μM AP5 and AMPAR with 20 μM CNQX. To record mEPSCs, 1 μM TTX was added to the bath solution. $GABA_AR$-mediated IPSCs were recorded in the presence of AP5