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DIFFERENCES IN PREFRONTAL CORTEX GABA/GLUTAMATE RATIO AFTER ACUTE RESTRAINT STRESS IN RATS ARE ASSOCIATED WITH SPECIFIC BEHAVIORAL AND NEUROBIOLOGICAL PATTERNS

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Abstract—In patients suffering from stress-related pathologies and depression, frontal cortex GABA and glutamate contents are reported to decrease and increase, respectively. This suggests that the GABA and/or glutamate content may participate in pathological phenotype expression. Whether differences in frontal cortex GABA and glutamate contents would be associated with specific behavioral and neurobiological patterns remains unclear, especially in the event of exposure to moderate stress. We hypothesized that an increase in prefrontal cortex GABA/glutamate ratio would be associated with a blunted prefrontal cortex activation, an enhanced hypothalamo-pituitary–adrenocortical (HPA) axis activation and changes in behavior. Rats being restrained for 1-h were then tested in an open-field test in order to assess their behavior while under stress, and were sacrificed immediately afterward. The GABA/glutamate ratio was assessed by ^{1}H high-resolution magic angle spinning magnetic resonance spectroscopy (^{1}H-HRMAS-MRS). The neurobiological response was evaluated through prefrontal cortex mRNA expression and plasma corticosterone levels. The stressed rats were distributed into two subgroups according to their high (H-G/g) or low (L-G/g) GABA/glutamate ratio. Compared to the L-G/g rats, the H-G/g rats exhibited a decrease in c-fos, Arc, Npas4, Nr4a2 mRNA expression suggesting blunted prefrontal cortex activation. They also showed a more pronounced stress with an enhanced rise in corticosterone, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), creatine kinase (CK) and lactate dehydrogenase (LDH) levels, as well as behavioral disturbances with decreased locomotion speed. These changes were independent from prefrontal cortex energetic status as mammalian target of rapamycin (mTOR) and adenosine monophosphate-activated protein kinase (AMPK) pathway activities were similar in both subpopulations. The differences in GABA/glutamate ratio in the frontal cortex observed in the stressed animals may participate in shaping individual differences in psychophysiological reactions.

Key words: GABA, glutamate, prefrontal cortex, locomotion, glucocorticoids.

INTRODUCTION

GABA (γ-aminobutyric acid) and glutamate are tightly linked intermediary products of energetic metabolism because they are involved in the same metabolic pathway, the glutamate/GABA-glutamine cycle (Bak et al., 2006). In neurons, glutamate is produced from the tricarboxylic acid (TCA) cycle and from the glutamine released by astrocytes, whereas GABA originates exclusively from glutamate from two glutamate decarboxylases (GAD), GAD-65 and GAD-67 (Soghomonian and Martin, 1998). Cytosolic GABA is catabolized into glutamate by GABA transaminase (GABA-T). Parts of the glutamate and GABA cell pools are released by neurons as neurotransmitters, representing respectively the major excitatory and inhibitory neurotransmitters. Both compounds are taken up by the astrocytes and catabolized through the TCA cycle (Roth and Draguhn, 2012). The GABA/glutamate tissue ratio may therefore reflect the dynamic equilibrium between GABA and glutamate in the context of cell metabolism. This balance is physiologically important since variations in GABA content via enzyme inhibitions are followed by changes in synaptic function (Golan et al., 1996; Engel et al., 2001).

GABA neurotransmission has been suggested to intervene in mood disorders (Petty, 1995; Brambilla

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Abbreviations: ALAT, alanine aminotransferase; AMPK, adenosine monophosphate-activated protein kinase; ASAT, aspartate aminotransferase; CK, creatine kinase; CPMG, Carr–Purcell–Meiboom–Gill; ECL, enhanced chemiluminescence; GABA-T, GABA transaminase; GAD, glutamate decarboxylases; ^{1}H-HRMAS-MRS, ^{1}H high-resolution magic angle spinning magnetic resonance spectroscopy; HPA, hypothalamo-pituitary–adrenocortical; LDH, lactate dehydrogenase; mTOR, mammalian target of rapamycin; NAA, N-acetylaspartate.

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et al., 2003), especially depression (Luscher et al., 2011). Changes in GABA-glutamate balance may also be involved in these particular disorders. Depressed patients exhibit increased glutamate (Sanacora et al., 2004) and decreased GABA (Sanacora et al., 1999; Hasler et al., 2007) cortical content, although the latter might differ according to depression subtypes (Sanacora et al., 2004). The decrease in brain GABA content may (Honig et al., 1988) or may not (Sanacora et al., 1999; Sanacora et al., 2004) correlate with depression scores. Also, remitted patients have similar cortex GABA content than healthy controls (Hasler et al., 2005). Consistent with these observations, depressed patients present a deficit in cortical inhibition (Levinson et al., 2010) and low plasma GABA levels (Petty et al., 1992; Petty et al., 1995), reflecting brain GABA content (Petty et al., 1987). However, low plasma GABA concentrations are not specific to depression and are also found in mania (Petty, 1995). Lastly, a decrease in hypothalamic GAD (Gao et al., 2013) and in prefrontal cortex GAD-67, but not GAD-65, levels (Karolewicz et al., 2010), were observed post-mortem in depressed patients, suggesting a deficit in GABA production.

Alterations in the GABA-glutamate equilibrium have also been described in stress and stress-induced pathologies. In humans, exposure to a mild stressor such as threat-of-shock decreases cortical GABA content (Hasler et al., 2010), such a reaction thought as being potentially predictive of a pejorative outcome. In accordance with this, peritraumatic plasma GABA levels are reduced in subjects who will develop acute stress disorder (Vaiva et al., 2004) and post-traumatic stress disorder (Vaiva et al., 2006), when compared to healthy volunteers and trauma-exposed controls. Alterations in the cortical GABA-glutamate equilibrium may be also present in post-traumatic stress disorder patients as they present a decrease in GABAergic functioning (Centonze et al., 2005; Rossi et al., 2009) and an increase in glutamatergic-driven functions (Rossi et al., 2009). In rats, frontal cortex GABA content remains unchanged after one acute stress (Otero Losada, 1988, 1989), but decreases after repeated exposure to stress despite an increased turnover (Otero Losada, 1988). In mice, chronic mild stress triggers depressive symptoms, while repeated social defeat leads to anxiety-like symptoms, both reactions being associated to a reduction in prefrontal cortex GABA content and GABA/glutamate ratio, but not in the hippocampus (Venzala et al., 2013). This is suggestive of specific brain area stress-induced GABA-glutamate equilibrium disturbances. However, rats exposed to a single intense prolonged stress and investigated 7 days later exhibit a decrease in glutamate and glutamine content in the medial prefrontal cortex without any change in GABA content (Knox et al., 2010). Altogether, intense and/or repeated stress exposures are associated with changes in frontal cortex GABA-glutamate equilibrium.

However, it is not known whether the differences in frontal cortex GABA-glutamate equilibrium shape the individual reactions after exposure to moderate stress. Although knowing that tissue GABA and glutamate contents do not equal GABA and glutamate neurotransmission, we hypothesized that a high GABA/glutamate ratio in the prefrontal cortex would be associated with a blunted prefrontal cortex activation and consequently, a decreased top-down hypothalamus inhibition resulting in greater hypothalamo-pituitary–adrenocortical (HPA) axis activation (Figueiredo et al., 2003).

To evaluate this hypothesis, rats were submitted to restraint, a stressor that is able to activate the frontal cortex (Drouet et al., 2010), then exposed to a 10-min open-field test to assess their behavior while developing stress. They were sacrificed a few minutes later. The prefrontal cortex metabolite content was assessed using ^{1}H high-resolution magic angle spinning magnetic resonance spectroscopy (^{1}H-HRMAS-MRS). Subsequently, the rats were distributed into two subgroups according to the value of their GABA/glutamate (G/g) ratio (*i.e.*, High vs. Low G/g ratio). The observed differences in the subgroups show imprints on their neurobehavioral phenotype due to the spontaneous differences in GABA/glutamate ratio when under stress. This procedure was completed by analyzing the correlations between the G/g ratio and the pertinent variables. Prefrontal cortex activation was evaluated by measuring the activity-dependent genes expression, such as c-fos (Kovacs, 1998), Arc (Bramham et al., 2008) and Egr1 (Thiel et al., 2010), using real-time quantitative RT-PCR. Plasticity-related genes, such as Nr4a2 (Barneda-Zahonero et al., 2012), Npas4 (Lin et al., 2008) and Bdnf (Lu, 2003), were also analyzed since their expression is related to the brain activation level. The HPA axis activation was evaluated using the plasma corticosterone concentration dosage. Since the GABA-glutamate equilibrium is related to metabolism, any possible prefrontal cortex metabolic imbalances among groups were evaluated using the phosphorylation level of proteins belonging to the adenosine monophosphate-activated protein kinase (AMPK, (Hardie et al., 2012)) and mammalian target of rapamycin (mTOR) pathways (Dennis et al., 2001).

EXPERIMENTAL PROCEDURES

Animals

The investigation was conducted with 30 male OFA Sprague–Dawley rats (Charles River Laboratories, L'arbresle, France) weighing 175–200 g upon arrival at the laboratory. Animals were housed at a constant temperature (22 ± 1 °C) and relative humidity (50 ± 10%), and kept on a 12-h-light–12-h-dark cycle (light on at 08h00). The animals had free access to water and food, including during the night before the day of experiments. During the 12 days preceding the investigation, the rats were accustomed to laboratory conditions and weighed daily to reduce handling stress (Briese and de Quijada, 1970).

All experimental procedures were approved by the institutional ethics committee for animal care and performed in accordance with animal care principles (NIH publication n° 86-23, revised 1985) and the European Community Council Directive (86/609 EEC).