The contextual fear conditioning deficit presented by spontaneously hypertensive rats (SHR) is not improved by mood stabilizers

Mariana Bendlin Calzavara a,b,*, Wladimir Agostini Medrano a, Raquel Levin a,b, Tânia Cristina Libânio a,b, Rosana de Alencar Ribeiro a, Vanessa Costhek Abílio a,b

a Department of Pharmacology, Universidade Federal de São Paulo, São Paulo, SP, Brazil
b Laboratório Interdisciplinar de Neurociência Clínica (LiNC), Department of Psychiatry, Universidade Federal de São Paulo, São Paulo, SP, Brazil

1. Introduction

Psychiatric disorders, including schizophrenia, bipolar disorder and attention deficit/hyperactivity disorder (ADHD), are associated with impairments in emotional processing (Addington and Addington, 1998; Marsh and Williams, 2006; Phillips et al., 2003). In rodents, fear conditioning is extensively used to investigate the biological basis of emotion (LeDoux, 2000; Pezze and Feldon, 2004). In this paradigm, an emotionally neutral conditioned stimulus (e.g., contextual stimulus) is paired with an aversive unconditioned stimulus, e.g., a foot shock, during the acquisition phase. As a result, the contextual stimulus comes to elicit conditioned fear response during expression phase, without foot shock presentation, because it acquires aversive reinforcing properties (LeDoux, 2000; Pezze and Feldon, 2004). While fear is an adaptive component of response to aversive stimuli, inappropriate fear responses may be present in many common psychiatric problems. For instance, diminished or exaggerated emotional response to aversive stimuli may be observed in schizophrenia, ADHD, and bipolar disorder (Addington and Addington, 1998; Marsh and Williams, 2006; Phillips et al., 2003) and may trigger nonadaptive responses like suicide (Kohler and Martin, 2006).

We have recently reported that spontaneously hypertensive rats (SHR) present a deficit in contextual fear conditioning (CFC) that is reverted by antipsychotics but not by acute treatment with mood stabilizers or psychostimulants (used to treat bipolar disorder and ADHD, respectively). In addition, this deficit is potentiated by pharmacological (ketamine and amphetamine) and environmental manipulations (sleep deprivation) that enhances psychosis in patients (Becker et al., 2003; Herz and Melville, 1980; Janowsky and Risch, 1979). Based on these findings, we suggested that the deficit in CFC presented by SHR could be a useful animal model to study abnormalities in emotional context processing related to schizophrenia (Calzavara et al., 2009).

However schizophrenia and bipolar disorder share some therapeutic management: both are treated with atypical antipsychotics. In our previous study (Calzavara et al., 2009), we evaluated the acute effects of some mood stabilizers—carbamazepine, lamotrigine and valproic acid—but we did not test lithium. In this respect, lithium has been in use in medicine for decades and has been tried and tested across the full range

**Keywords:** Bipolar disorder; Contextual fear conditioning; Lamotrigine; Lithium; Schizophrenia; SHR
of mood disorders. It is unquestionable that lithium is the best mood stabilizer of all and, because of its unique properties, it may even constitute a class of its own (Malhi et al., 2009). Moreover, although acute treatment with mood stabilizers is effective in a series of animal models (Arban et al., 2005; Corrêa et al., 2007; Lamberty et al., 2001), clinical trials suggest that the therapeutic effect is better observed after long-term treatment (reviewed by Amann et al., 2007; Fountoulakis and Vieta, 2008). Specifically related to lithium and lamotrigine, some placebo-controlled studies have shown that there is no acute effect of lamotrigine (reviewed by Bowden, 2002), and lithium seems to be moderately efficacious for acute bipolar depression (Van Lieshout and MacQueen, 2010).

Based on the above, the aim of the present study was to extend the pharmacological characterization of the CFC deficit presented by SHR by evaluating a possible beneficial effect of acute treatment with lithium and/or of long-term treatment with lithium or lamotrigine. Based on the described antinociceptive action of lamotrigine (Gilron et al., 2006; Munro et al., 2007) we also verified the effects of lamotrigine on sensitivity to the shock. In addition, we evaluated the effects of this drug on locomotion.

2. Material and methods

2.1. Subjects

Male adult (five-month-old) Wistar Rats (WR) and SHR of our own colony were housed under conditions of controlled temperature (22–23 °C) and lighting (12/12 h light/dark cycle, lights on at 07:00 am). Groups of 5–6 animals were kept in Plexiglass cages (41×34×16.5 cm), with free access to food and water. The animals were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA. This study was approved by the Ethical Committee of Federal University of Sao Paulo.

2.2. Drugs

Lithium (Li2CO3, Eurofarma – Sao Paulo, Brasil) and lamotrigine (Torrent – Sao Paulo, Brazil) were diluted in 0.9% saline. Saline was used as control solution. All the solutions were injected intraperitoneally in a volume of 1 ml/Kg of body weight. The doses and schedules used in these experiments were chosen based on previous studies showing the mood stabilizer effect of the drugs in animal models (Arban et al., 2005; Calzavara et al., 2009; Corrêa et al., 2007; Eren et al., 2007; Frey et al., 2006).

2.3. Behavioral tasks

2.3.1. CFC task

On the first day (training session), the rats were individually placed in a dark chamber with a grid floor (22×22×22 cm). After 150 s, 0.4 mA foot shocks lasting 5 s were applied every 30 s for the subsequent 150 s. Thirty seconds after the last foot shock, the animal was removed from the apparatus. The contextual conditioning test (test session) was performed 24 h after the training. Each animal was placed in the same dark chamber, without receiving foot shocks. The freezing duration (defined as complete immobility of the animal with the absence of vibrissae movements and sniffing) was scored during 5 min.

2.3.2. Sensitivity to the shock

Rats were individually placed in a dark chamber with a grid floor (22×22×22 cm). After 150 s, 0.4 mA foot shocks lasting 5 s were applied every 30 s for the subsequent 150 s. The freezing duration (defined as complete immobility of the animal, with the absence of vibrissae movements and sniffing) was quantified during 5 min.

2.3.3. Locomotor activity

Rats were individually placed in an open-field (97 cm in diameter and 32.5 cm high, with an open top and a floor divided into 19 similar parts) and the number of floor squares entered were quantified during 10 min (locomotion frequency).

Each animal was used in only one experiment. All the experiments were performed between 2:00 and 6:00 pm. All the behavioral parameters were hand-scored by blinded observers.

2.4. Experimental design

2.4.1. Experiment 1 – Effects of acute treatment with lithium on the CFC of WR and SHR

WR and SHR (n = 6) were treated with 47.5 mg/kg lithium or saline, twice a day. Sixty minutes later, the animals were submitted to the training session of the CFC task.

2.4.2. Experiment 2 – Effects of repeated treatment with lithium on the CFC of WR and SHR

WR and SHR (n = 6) were treated with 47.5 mg/kg lithium or saline, twice a day. This treatment was performed during 7 days. Sixty minutes later after the second injection on the 7th day, the animals were submitted to the training session of the CFC task. The duration of this treatment was determined based on previous works showing that a seven-day treatment with lithium is able to attenuate the behavioral alterations and the increase in oxidative stress and mitochondrial dysfunction presented in an animal model of mania (Corrêa et al., 2007; Frey et al., 2006).

2.4.3. Experiment 3 – Effects of repeated treatment with lamotrigine on the CFC of WR and SHR

WR and SHR (n = 9–10) were treated with saline or 20 mg/kg lamotrigine during 20 days. Thirty minutes after the last injection, rats were submitted to the training session of the CFC task. The duration of this treatment was determined based on a previous work showing that a similar treatment (28 days) with lamotrigine is able to attenuate the increase in oxidative stress observed in an animal model of depression (Eren et al., 2007).

2.4.4. Experiment 4 – Effects of lamotrigine on the foot shock sensitivity

Reactivity to the shock was evaluated in the same apparatus used for fear-conditioning training. WR and SHR (n = 10) were treated with saline or 20 mg/kg lamotrigine. After thirty minutes, rats were submitted to the training session of the CFC task. After each shock presentation, freezing time, jump (withdrawal of three or four paws from the grid floor) and flinch (withdrawal of one paw from the grid floor) were quantified.

2.4.5. Experiment 5 – Effects of lamotrigine on locomotion

WR and SHR (n = 10) were treated with saline or 20 mg/kg lamotrigine. Thirty minutes later, rats were submitted to the open-field for quantification of locomotion.

2.5. Statistical analysis

Data were analyzed by two-way ANOVA followed by Duncan’s test. The p < 0.05 was used as a criterion for statistical significance.

3. Results

3.1. Experiment 1 – Effects of acute treatment with lithium on the CFC of WR and SHR

The freezing time observed after an acute treatment with lithium is depicted in Fig. 1. Two-way ANOVA revealed a significant strain effect [F(1,20) = 20.02; p < 0.05]. Post hoc analysis revealed that the
freezing response was significantly reduced in SHR group when compared to WR group treated with saline or lithium. Lithium did not modify the freezing response of both strains when compared to the respective saline groups.

3.2. Experiment 2 – Effects of repeated treatment with lithium on the CFC of WR and SHR

The freezing time observed after a repeated treatment with lithium is depicted in Fig. 2. Two-way ANOVA revealed a significant strain effect [F(1,20) = 16.78; p < 0.05] and treatment effects [F(1,35) = 10.17; p < 0.05]. Post hoc analysis revealed that the freezing response was significantly reduced in SHR group when compared to WR group treated with saline or lithium. Lithium did not modify the freezing response of both strains when compared to the respective saline groups.

3.3. Experiment 3 – Effects of repeated treatment with lamotrigine on the CFC of WR and SHR

The freezing time observed after a repeated treatment with lamotrigine is depicted in Fig. 3. Two-way ANOVA revealed significant strain [F(1,36) = 5.979; p < 0.05] and treatment effects [F(1,35) = 12.945; p < 0.05] and an interaction between strain and treatment factors [F(1,36) = 6.204; p < 0.05]. Post hoc analysis revealed that the freezing response was significantly reduced in SHR group when compared to WR group treated with saline or lamotrigine. Repeated lamotrigine treatment decreased the freezing response in both strains when compared to the respective saline groups.

3.4. Experiment 4 – Effects of lamotrigine on the foot shock sensitivity

The shock sensitivity parameters observed after a treatment with lamotrigine is displayed in Table 1. For freezing time, two-way ANOVA revealed a significant strain effect [F(1,36) = 163.20; p < 0.05] and treatment effects [F(1,35) = 10.17; p < 0.05]. Post hoc analysis revealed that the freezing response was significantly reduced in SHR group when compared to WR group treated with saline. For jump, two-way ANOVA did not reveal any significant effect. For flinch, two-way ANOVA revealed significant strain effect [F(1,36) = 12.945; p < 0.05] and an interaction between strain and treatment factors [F(1,36) = 6.204; p < 0.05]. Post hoc analysis revealed that the flinch response was significantly reduced in SHR group when compared to WR group treated with saline.

3.5. Experiment 5 – Effects of lamotrigine on locomotion

The locomotion frequency is depicted in Fig. 4. Two-way ANOVA revealed a significant strain [F(1,36) = 7.05; p < 0.05] and treatment effects [F(1,36) = 4.18; p < 0.05] and an interaction between these factors [F(1,36) = 5.06; p < 0.05]. Post hoc analysis revealed that the locomotion frequency was significantly increased in SHR treated with saline when compared to WR with the same treatment. Lamotrigine decreased the locomotion frequency of SHR when compared to SHR treated with saline and WR treated with lamotrigine.

4. Discussion

The decrease in the performance of SHR in the CFC task observed in this study corroborates our previous work (Calzavara et al., 2009). Interestingly, although others have described memory deficits in some tasks using Wistar-Kyoto as controls (Hernandez et al., 2003; Meneses et al., 2011), we have described that SHR did not present deficits in learning/memory tasks other than the CFC—as the discriminative avoidance task (Calzavara et al., 2004) or locomotor habituation in an open-field (Calzavara et al., 2011)—when compared to Wistar Rats (as in this and in our previous works). In this sense, the deficit in CFC seems to be related to disturbances in the processing of emotional context and not to a general impairment in learning/memory. This inability to process emotional information (which would lead to a deficit in the acquisition of CFC) could be associated with some psychiatric disorders, such as schizophrenia or bipolar disorder. Based on a previous extensive pharmacological characterization, we suggested that the deficit presented by SHR could model the impairment in the emotional context processing related to schizophrenia. Indeed, this deficit was specifically reverted by antipsychotics, potentiated by proschizophrenia manipulations (amphetamines, ketamine and sleep deprivation—Becker et al., 2003; Herz and Melville, 1980; Janowsky and Risch, 1979), and not altered by acute treatment with mood stabilizers (Calzavara et al., 2009). Nevertheless, in this previous work we did not evaluate the effects of lithium, the prototype mood stabilizer for the treatment of bipolar disorder, as well as the effects of a repeated treatment with the mood stabilizers. In this respect,
clinical trials suggest that the therapeutic effect of these drugs is better observed after long-term treatment (reviewed by Amann et al., 2007; Fountoulakis and Vieta, 2008).

In relation to lithium, the clinical literature suggests that this drug is useful during the acute manic and the maintenance phase (Fountoulakis and Vieta, 2008). Our data showed that neither an acute nor a repeated treatment with lithium were able to ameliorate the deficit in CFC presented by SHR. In addition, they did not modify the acquisition of CFC presented by WR, corroborating and extending previous studies showing the absence of effect of a repeated lithium treatment on the acquisition or expression of this conditioning (Muraki et al., 1999; Tsaltas et al., 2007).

As reviewed elsewhere (Bowden, 2002), some studies show that there is no acute antimanic effect of lamotrigine. Our previous work (Calzavara et al., 2009) demonstrated that acute treatment with lamotrigine did not change the deficit in CFC presented by SHR. The present data show that a repeated treatment with this drug even potentiated this deficit, reinforcing the absence of a beneficial effect of mood stabilizer. Considering WR, the acute impairment in CFC produced by lamotrigine observed in our previous work (Calzavara et al., 2009) is not tolerated after a repeated treatment (present data).

As far as we know, this is the first study addressing the effects of lamotrigine on fear conditioning.

The decrease in fear conditioning after repeated treatment with lamotrigine could be considered from different points of view. Firstly, an antinoceptive action of lamotrigine (Gilron et al., 2006; Munro et al., 2007) could interfere with the perception of the shock and, therefore, impair the acquisition of the fear conditioning. Contrary to this possibility, lamotrigine did not alter freezing time after the presentation of the shock (experiment 4) in both Wistar and SHR.

Secondly, it could be suggested that this drug impairs the acquisition of an emotional memory and this effect should be taken into account when considering long-term treatment with this mood stabilizer. On the other hand, clinical data indicate that lamotrigine produces fewer untoward cognitive effects in healthy individuals and bipolar patients when compared to other mood stabilizers (Daban et al., 2006; Meador et al., 2005; Shannon and Love, 2007). Indeed, the effects of a lamotrigine monotherapy in emotional processing was tested in bipolar disorder patients and the authors suggest that lamotrigine treatment “may result in more efficient processing of facial affect for anger with reduction in ambiguity and “normalization” of the pattern of activation” (Haldane et al., 2008). Conversely, based on our results, we could speculate that in the case of the emotional processing deficits associated with schizophrenia, lamotrigine could instead be harmful. In schizophrenia, lamotrigine has been used as an adjuvant treatment. Nevertheless, a systematic review and meta-analysis suggests that lamotrigine may be effective only for a substantial proportion of patients with clozapine-resistant schizophrenia (Tiihonen et al., 2009).

From another standpoint, this result could be related to the supposed antidepressive effect of lamotrigine. Interestingly, differential analysis of depressive and manic symptoms revealed that lamotrigine was significantly superior to placebo in prevention and time to intervention of new depressive episodes, and reversely, lithium was better than lamotrigine and both were better than placebo in prevention of manic episodes (reviewed by Amann et al., 2007). In this way, long-term antidepressive treatment promotes impairment in the acquisition of CFC (Burghardt et al., 2004; Inoue et al., 1996, 2004; Santos et al., 2006). Hence, in this study, the decreased acquisition of CFC in both WR and SHR observed after long-term treatment with lamotrigine could be related to the antidepressive profile of this drug. In this respect, the effects of lamotrigine could be interpreted as beneficial. However, a recent clinical paper pointed out that the antidepressive effect of lamotrigine is not established yet (Calabrese et al., 2008).

Alternatively, mood stabilizers, such as carbamazepine, valproic acid and lamotrigine, have been evaluated in clinical trials for a variety of anxiety-related disorders, including posttraumatic stress disorder, social phobia, obsessive–compulsive disorder and generalized anxiety disorder (Berlin, 2007; Mula et al., 2007). In addition, an anxiolytic effect of lamotrigine has been reported in animal models of anxiety (Foreman et al., 2009). In this respect, it is well-established that anxiolytic drugs impair the acquisition of fear conditioning (Brignell and Curran, 2006; Calzavara et al., 2009; Fanselow and Helmstetter, 1988; Resstel et al., 2006). In this way, the decreased fear conditioning induced by lamotrigine treatment could be related to its anxiolytic effect.

From a methodological point of view, one might think that the described hyperlocomotion presented by SHR (Calzavara et al., 2011; Knardahl and Sagvolden, 1981; Van den Buuse and de Jong, 1989) could interfere with the ability to freeze in this strain. Nonetheless, lamotrigine decreased hyperlocomotion in SHR (experiment 5) without changing freezing response to the shock (experiment 4). In addition, the improvement in the CFC deficit of SHR induced by antipsychotics (Calzavara et al., 2009) is not accompanied by changes in their hyperlocomotion (data not shown). It is also interesting to note that SHR present a decreased freezing time in response to shock (as previously observed for a loud noise—Hard et al., 1985) but their flinch response is enhanced (experiment 4). This seems to indicate that, although they are able to perceive and react to the shock, this strain might display different behaviors when submitted to aversive stimuli. Importantly, although SHR exhibit a decrease in conditioned freezing response, they are able to display this behavior (increase in freezing response induced by previous exposure to the shock compared with animals that were not exposed to this aversive stimulus—Calzavara et al., 2009).

5. Conclusion

In conclusion, our data reinforce the absence of beneficial effects of mood stabilizers on the deficit of CFC presented by SHR. From a clinical point of view, both schizophrenia and bipolar disorder are associated with emotional processing deficits (Addington and Addington, 1998;
Phillips et al., 2003). Considering the pharmacological therapeutic treatment for these disorders, although antipsychotics are the first-choice treatment for schizophrenia and mood stabilizers are the most common prescribed drugs for bipolar disorder, these drugs have been employed to treat at least some aspects of both disorders (Chen et al., 2007; Gao et al., 2005). Our data indicate that the impairment in emotional context processing modeled by SHR seems to be sensitive only to antipsychotics.

Acknowledgments

This research was supported by fellowships from FAPESP, CNPq and CAPES. The authors would like to thank Mrs. Teotila R.R. Amaral and Mr. Cleomar S. Ferreira for capable assistance.

References


