STUDY OF DIURNAL RHYTHMS OF DEPRESSIVE STATE IN KAINATE MODEL OF EPILEPSY IN NORMOTENSIVE AND SPONTANEOUS HYPERTENSION RATS

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Abstract

Depression is one of the most frequently manifested mental complications in patients with epilepsy and the risk of development of clinical pattern is five fold higher compared to this in persons with normal status. Previously we have demonstrated that normotensive Wistar (WIS) and spontaneously hypertensive rats (SHRs) displayed different diurnal patterns of locomotor and exploratory activity and lower level of anxiety and exploratory activity in kainic acid (KA) model of temporal lobe epilepsy (TLE). The main focus of this study was to reveal strain-specific diurnal variations in depressive-like behaviour in WIS and SHRs during the chronic stage of KA model. The development of chronic epileptic stage was confirmed by the presence of spontaneous seizures detected by video monitoring. Epileptic WIS rats showed loss of taste preference (anhedonia) in sucrose consumption test (SCT) and increase in immobility time (despair-like state) in forced swim test (FST) during the light phase. In contrast, KA-treated SHRs exhibited depressive-like behaviour in SCT mostly during the dark phase while despair-like behaviour in FST was evident without diurnal variations. The present study suggests that both strains develop depressive-like behaviour during the chronic stage of KA epileptic model with differences in diurnal rhythms of its expression.

Key words: Wistar (WIS) rats, spontaneous hypertensive rats (SHRs), kainic acid (KA) model of temporal lobe epilepsy, sucrose consumption test, forced swim test

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**Introduction.** Affective disorder, including depression, represents one of the most common co-morbidities in epileptic patients \[^1, 2\]. Moreover, depression is reported more frequently in patients with temporal lobe epilepsy (TLE) \[^1\]. Mood disturbances as feelings of despair and depressive mood are among the common psychiatric features both in patients with TLE \[^2\] and patients with major depression but they may have a more abrupt start in epileptics than in non-epileptics \[^3\]. Establishment and validation of suitable animal models of co-morbidity between depression and epilepsy is necessary for elucidating the mechanism underlying depression state in epilepsy as well as for establishing of effective pharmacological approaches for treatment. Models of epilepsy and depression have high predictive validity when they respond to the same pharmacological treatment used in humans, but not to other drugs. For these reasons, experimental animals are evaluated for depressive-like behaviour in forced swim test (FST) and/or consumption preference in sucrose consumption test (SCT) which tests are valuable in predicting potential antidepressant properties of new substances. On the other hand, some of the best-studied epileptic models use convulsants kainic acid (KA) and pilocarpine, which can be administered systematically to reproduce the pathophysiological events of epileptogenesis in human TLE \[^4\]. Studies focused on depression-like state developed in epileptic models are limited and controversial. While some studies revealed no changes in SCT of kindled rats \[^5, 6\], A. Mazarati et al. (2008) reported loss of taste preference to sweet solutions in pilocarpine-treated rats \[^7\]. Furthermore, concerning despair-like behaviour in FST, the results varied from no changes in amygdala kindling \[^4\], increased immobility in both KA- and pilocarpine-treated rats \[^7, 8\] and improved performance in pilocarpine epileptic mice \[^9\]. It has been demonstrated that spontaneously hypertensive rats (SHRs) used as a model of human hypertension and cardiovascular disease may also be utilized to model certain Central Nervous System (CNS) changes associated with brain disorders including epilepsy \[^10\]. Based on the above finding, we hypothesized that WIS and SHRs would demonstrate loss of taste preference and despair-like behaviour and/or changes in their diurnal variations during the chronic stage in KA model of TLE.

**Materials and methods.** The experiments were performed on male 60-day old WIS and SHRs (n = 15) in the beginning of experimental procedures. They were kept under standardized conditions: temperature 21±2°C, photoperiod 12/12 with lights on at 8 a.m., in individual cages and fed with a regular pellet diet ad libitum. Systolic arterial blood pressure (ABP) was measured non-invasively in conscious unrestrained rats by the tail cuff method (Ugo Basile blood pressure recorder 5800) \[^11\]. The ABP value for each rat was obtained as a mean from three measurements. Status epilepticus (SE) was induced according to the method described by J. L. Hellier et al. \[^12\]. In brief, KA dissolved in saline was injected intraperitoneally (i.p.) at a dose of 5–2.5 mg/kg once per hour, in a total amount of 25–30 mg/kg, until class IV-V motor seizures (according to the
Racine’s scale \[^{13}\]) occurred for $\geq 3$ h. KA-treated rats were placed in labelled kennels and video-monitored (24 h) with light-sensitive black-white cameras (S-2016, AVTECH, Taiwan, No AVC307R) for detection of spontaneous seizures. Controls were i.p. titrated with saline in the same way as experimental groups and accommodated in individual cages.

Taste preference behaviour was evaluated using SCT starting from 2nd to 5th month after SE. On the first day (habituation), each cage was supplied with two identical graduated water bottles in volume of 100 ml. On the 2nd (habituation) and 3rd (test) day regular water in one of the bottles was replaced with 1% sucrose. Test started from 8:00 a.m. and ran for 24 h. During the test, both bottles were removed after 12 h for weighing, and were replaced by a second pair of pre-weighed bottles. Taste preference was expressed as percent of the volume of sucrose solution to a total volume of fluid (sucrose plus tap water) consumed over 12 h (light phase – 8:00-20:00 h and dark phase – 20:00–8:00 h).

The despair-like behaviour was evaluated 5 months after KA-induced SE, i.e. during developed chronic epileptic state by using classic Porsolt test (1979) which was shown to be relevant for both examining depressive-like behaviour and for screening antidepressant agents \[^{14}\]. The immobility behaviour was assigned.

Data were analyzed by three-way ANOVA (SCT) and two-way ANOVA (FST) followed by post hoc Bonferroni \(t\)-test, if appropriate. For samples that failed normality test, Mann-Whitney or Wilcoxon tests were employed. \(P < 0.05\) was accepted as an index of statistically significant differences.

The experimental procedures were carried out fully in accordance with the European Communities Council Directives of 24 November 1986 (86/609/EEC) on the use of laboratory animals.

**Results and discussion.** The main focus of this study concerns diurnal variations in depressive-like behaviour of WIS and SHRs during the chronic stage of epilepsy. Control SHRs exhibited significantly higher ABP compared with WIS controls \((p = 1.55^{-17})\). Analysis of variance have shown significant effect of strain (WIS vs SHRs) and KA treatment (controls vs KA) on sucrose preference during the 1st \((p < 0.001)\), 2nd \((p < 0.01\) and \(p < 0.001\), respect.), 3rd \((p < 0.006\) and \(p < 0.002\)), 4th \((p < 0.002\) and \(p < 0.001)\) and 5th \((p < 0.001)\) month of measurement, and of the phase (light vs dark) during the 1st \((p < 0.001)\), 3rd \((p < 0.032)\) and 5th \((p < 0.001)\) month of measurement (Fig. 1). The analysis of sucrose preference data revealed a significant 3-way interaction for the 1st month \([F(1, 84) = 6.901, p < 0.01]\), 3rd month \([F(1, 76) = 3.703, p < 0.05]\) and 5th month \([F(1, 69) = 7.022, p < 0.01]\). Our results revealed that the characteristics for controls diurnal rhythm in sucrose preference consumption (higher preference during the dark phase) were abolished in WIS but not in SHRs after the 1st month of measurement. The lack of “learned” adaptive response in SHR controls might be a reason for their sustained behavioural pattern.

The present data represent the first report on diurnal variations of sucrose consumption in WIS and SHRs.
preference and despair behaviour in FST in intact and KA-treated epileptic WIS and SHRs. Unlike controls, in KA-treated WIS rats the “learned” adaptive abolishment of diurnal rhythms in sucrose preference disappeared three months after SE while epileptic SHRs exhibited diurnal fluctuations in sucrose consumption similar to their controls only during the last two months of measurements, i.e. on the fourth and fifth month after SE (Fig. 1). It is widely accepted that lack of preference to sweet solution represents a depressive phenotype in animal models of depression \[^{15}\]. A decrease in sucrose preference of epileptic WIS rats was detected mostly during the light phase (2nd, 4th and 5th month) which could explain partly the phase shift compared to controls. However, diminished responses to sucrose consumption in KA-treated SHRs were detected both during the light (4th and 5th month) and the dark phase (2nd, 3rd, 4th and 5th month) (Fig. 1).
Three-way ANOVA revealed an overall strain, treatment and phase effect for immobility time in FST without any interaction ($F(1, 75) = 11.93$, $p < 0.001$), [$F(1, 75) = 4.091$, $p < 0.047$] and [$F(1, 75) = 8.208$, $p < 0.006$], respectively (Fig. 2). KA-treated WIS rats showed increased immobility time compared to controls with significant difference during the light phase ($p < 0.04$). Moreover, diurnal rhythm in despair-like behaviour was shown only in epileptic WIS rats. Unlike WIS rats, KA-treated SHRs demonstrated increased immobility time compared to their controls during both the light and dark phase ($p < 0.032$ and $p < 0.015$, respectively). Opposite to WIS rats, diurnal variations in forced swim behaviour were detected only in control SHRs. Similarly, recently, we have demonstrated that while both WIS groups (intact and KA-treated) were characterized with absence of diurnal anxiety rhythms, the diurnal anxiety patterns were KA-treatment dependent in SHRs and were present only in SHR controls [16]. Depressive-like behaviour (a loss of taste preference and increased despair reaction in FST) observed in our study confirmed previous reports in a model of absence epilepsy [17], pentylenetetrazol kindling in rats [18], pilocarpine and KA model of TLE [7]. Moreover, similarly to locomotor activity and anxiety, we have found strain- and KA-treatment-dependent difference in depressive-like rhythms which are not homogeneously distributed during the chronic phase of epilepsy. Both KA-treated strains showed loss of affinity to sucrose solutions as early as two months after SE. Specifically, epileptic WIS rats exhibited decreased sucrose preference and increased immobility time during the light phase while KA-treated SHRs showed

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Fig. 2. Dynamics of immobility time in Wistar and spontaneously hypertensive rats measured in the light phase (left) and the dark phase (right) in the forced swim test five months after KA-induced status epilepticus. Values are immobility time in seconds (means ± S.E.M., $n = 10–15$).

*p $< 0.05$ versus corresponding controls (Bonferroni post hoc t-test)
depressive-like responses both during the light and the dark phase. Validation of animal model of co-morbidity should include extensive analysis of complex behaviours as well as their diurnal rhythms which are suggested to being changed in depressive state. Our results provide evidence for strain-dependent difference in diurnal rhythms of depressive-like behaviour in KA model of TLE.

REFERENCES


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