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Effects of stress and etifoxine on pentobarbital-induced loss of righting reflex in Balb/cByJ and C57BL/6J mice

Marc Verleye[a,*], Isabelle Heulard[a], Philippe Nuss[b], Jean-Marie Gillardin[a]

[a]*Département de Pharmacologie, Laboratoires Biocodex, Zac de Mercières, Chemin d'Armancourt, 60200 Compiègne, France*
[b]*Département de Psychiatrie et de Psychologie Médicale, Hôpital Saint Antoine, 75571 Paris, France*

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Abstract

We hypothesized that functional changes in the GABAergic system induced by stress would differ between two inbred mouse strains BALB/cByJ and C57BL/6J. We compared the effects of restraint stress and of the anxiolytic drug etifoxine (EFX) on the duration of pentobarbital-induced loss of righting reflex (hypnotic effect) in the two strains. Naive BALB/cByJ mice were less sensitive than naive C57BL/6J mice to the hypnotic effect of pentobarbital. C57BL/6J mice exhibited a shortening in the duration of pentobarbital-induced hypnosis following stress whereas stress had no effect in BALB/cByJ mice. EFX reversed the shortening of pentobarbital-induced hypnosis elicited by stress in C57BL/6J and shortened the duration of pentobarbital-induced hypnosis after stress in BALB/cByJ mice. Alterations in the GABAergic function in BALB/cByJ mice could be corrected by EFX, an enhancer of GABAergic transmission.

Keywords: Inbred mice strains; BALB/cByJ mouse; C57BL/6J mouse; Pentobarbital-induced loss of righting reflex; GABAergic system; Etifoxine; Stress

Various animal models including inbred strains, selected lines of mice and rats, multiple marker strains and animals obtained from gene targeting technology, have been utilized to explain differences in sensitivity and susceptibility to anxiety disorders, and to correlate neurochemical parameters and behavioral response (for review see [8]). Among several strains, two inbred strains of mice, BALB/cByJ (BALB) and the C57BL/6J (C57) strains appeared to be of great interest because their emotional behavior has been reported as different or even opposite in the literature [6]. BALB mice are often described as 'anxious' mice while the contrary is claimed for C57 mice [2,20]. Abnormalities in various neurotransmitter systems and particularly in the GABAergic system could underlie the strain differences in behavioral responses following a stressful event. For example, it was reported that C57 and BALB mice differ in the density and/or affinity of the benzodiazepine (BZD) receptors [7,15]. Furthermore, using binding studies on brain membranes, it was shown that the amygdala of BALB mice exhibited a fivefold decrease in the density of BZD receptors compared to C57 mice [11]. However, the complex structure of the $GABA_A$ receptor, with its multiple binding sites, is the site of action of a number of drugs including benzodiazepines but also barbiturates, neurosteroids and other compounds like etifoxine [14,16]. Etifoxine (EFX), a non-benzodiazepine compound, has anxiolytic-like effects in animals [5] and is effective in the treatment of adjustment disorder with anxiety in humans [18]. This drug has been demonstrated to enhance GABAergic inhibition by allosterically interacting with the chloride channel of $GABA_A$ receptors [16,19] and was utilized in the present study as a probe compound.

The aim of this study was firstly to compare the $GABA_A$ receptor function in C57 and BALB mice confronted with an acute stress such as the restraint stress, and secondly to study the effects of EFX in the two inbred strains of mice with and without an acute stress. The changes in duration of pentobarbital (PTB)-induced loss of righting reflex (LORR) were chosen as a reliable index of the $GABA_A$ receptor function. Changes in the hypnotic activity of PTB is associated with alteration in the $GABA_A$ receptor [13].

Male mice of BALB/cByJ (BALB) or C57BL/6J (C57) strain (Charles River Laboratories, France), 7 weeks of age at the time of the experiment, were housed in groups of 10 in a controlled environment (22 ± 2 °C; 50 ± 20% relative

* Corresponding author. Tel.: +33-3-4486-8228; fax: +33-3-4486-8234.
E-mail address: m.verleye@biocodex.fr (M. Verleye).

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humidity) with non-reversed lighting conditions (light period: 07:00–19:00 h). The animals were acclimatized to the animal facility for at least 1 week before the experiments and had free access to food and water. Procedures involving animals and their care were conducted in conformity with European Communities Council Directive of 24th November, 1986 (86/609/EEC).

Sodium pentobarbital (PTB) (Sigma, France) was dissolved in saline (0.9% NaCl) and etifoxine (EFX) (batch 114; Biocodex Laboratories, France) was suspended in saline solution containing 1% Tween-80 (v/v). PTB was injected intraperitoneally (i.p.) at the dose of 40 mg/kg and hypnosis time (s) was measured as the time from which righting reflex was lost until it was regained. The righting reflex was deemed to be lost when an animal did not right itself within 15 s of being placed on its back.

The mice were exposed to a restraint stress which consisted of enclosing the animals in a plastic tube (with a few holes for breathing: 2.7 × 10 cm in diameter) in such a way as to prevent all motion. The animals remained immobilized for 10 min and released immediately prior to the PTB injection.

In a first experiment, the dose-dependent effect of EFX (3.1–25 mg/kg i.p.), administered 40 min prior to PTB, was studied in the naive (unstressed) mice of the two strains. At the doses used, EFX has no hypnotic activity of its own. An EFX sub-active dose or maximal dose without significant effect on the duration of PTB-induced LORR was selected in the two strains.

In a second experiment, the effects of a sub-active EFX dose were studied in the two strains exposed to the restraint stress. Control animals received the same amount of vehicle (0.1 ml/10 g).

Data are presented as mean ± S.E.M. They were analyzed with two-way analyses of variance (ANOVA): treatment with EFX and strain as factors in the first experiment, strain and stress or stress and treatment with EFX as factors in the second experiment. Comparisons between individual groups were then made post hoc with the Student Newman–Keuls test (SNK procedure). Significance was determined at $P \leq 0.05$ (SigmaStat v3.0, SPSS Inc.).

Two way ANOVA revealed highly significant strain and EFX effects on PTB-induced LORR ($F(1,94) = 43.6$, $P < 0.001$ and $F(4,94) = 30.66$, $P < 0.001$, respectively). The strain difference was unrelated to the dose of EFX ($F(4,94) = 0.283$, $P = 0.89$). Post hoc multiple comparisons (SNK procedure) showed that, in the vehicle groups, BALB mice exhibited a shorter duration of LORR than C57 mice ($P = 0.003$). Compared with vehicle, EFX produced a significant dose-dependent increase of PTB-induced LORR in BALB and C37 mice: the lowest doses exhibiting a significant effect were 12.5 and 6.2 mg/kg in C57 ($P < 0.001$) and BALB mice ($P = 0.05$), respectively.

Two-way ANOVA showed significant differences in duration of PTB-induced LORR between BALB and C57 mice, both in the naive group and after the restraint stress (strain × stress interaction, $F(1,96) = 16.88$; $P < 0.001$). The post hoc tests (SNK procedure) indicated that, as seen in Fig. 1, naive BALB mice exhibited a significantly shorter duration of LORR than naive C57 mice ($P < 0.001$). In addition, the restraint stress shortened the duration of LORR ($P < 0.001$) in C57 but had no effect in BALB mice ($P = 0.49$). In C57 mice, the duration of PTB-induced LORR differed significantly with EFX ($F(1,56) = 5.98$, $P = 0.018$) and stress ($F(1.56) = 6.59$, $P = 0.013$). There was not a significant EFX × stress interaction ($F(1,56) = 3.54$, $P = 0.065$). However, as shown in Fig. 2, the post hoc analysis (SNK procedure) revealed that the restraint stress shortened the duration of LORR ($P < 0.001$) and that this effect was reversed ($P = 0.004$) by 6.2 mg/kg EFX, a sub-active dose without effect in unrestrained animals ($P = 0.69$). In BALB mice, the two-way ANOVA on the duration of PTB-induced LORR revealed a significant effect on EFX ($F(1,86) = 4.41$, $P = 0.04$) but neither on stress ($F(1,86) = 0.03$, $P = 0.87$) nor on the interaction EFX × stress ($F(1,86) = 1.04$, $P = 0.32$). The within-group analysis (SNK procedure) indicated that the restraint stress had no effect in the animals treated with vehicle ($P = 0.321$) but that EFX at 3.1 mg/kg, a dose without effect in unrestrained animals ($P = 0.45$), significantly reduced the duration of hypnosis in the stressed mice ($P = 0.03$).

Under basal conditions, BALB mice, when compared to C57 mice, exhibit resistance to the hypnotic action of PTB. Additionally, in BALB mice, no effects on the duration of the PTB-induced LORR are noticed in mice exposed to the

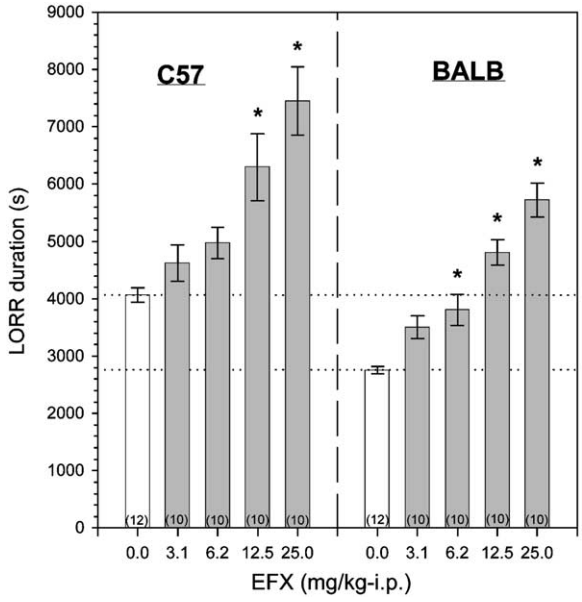

Fig. 1. Dose-dependent effect of etifoxine (EFX) administered i.p. on the duration of the PTB-induced loss of the righting reflex (LORR) in the two inbred strains of mice. Each column represents the mean ± S.E.M. The number of animals used appears in brackets. Within-strain: $^{*}P \leq 0.05$ compared with vehicle (Two-way ANOVA and Student Newman–Keuls test); Between-strain: $^{\S}P \leq 0.05$ compared with the same treatment (Two-way ANOVA and Student Newman–Keuls test).

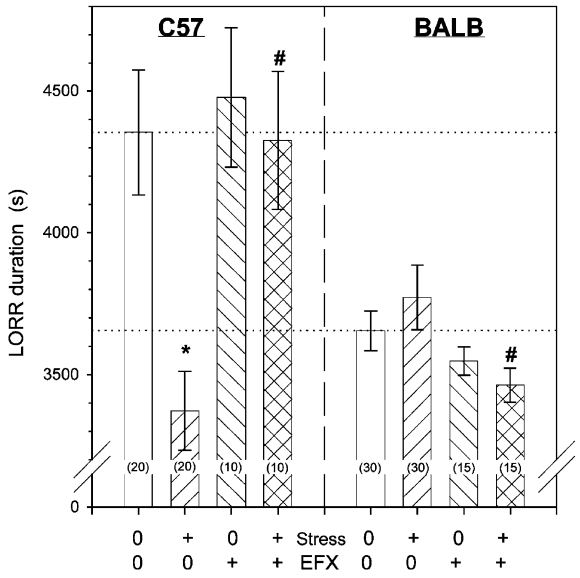

Fig. 2. Effect of etifoxine (EFX; 6.2 mg/kg i.p. in C57 and 3.1 mg/kg i.p. in BALB) on restraint-induced effect of PTB-induced LORR. Each column represents the mean ± S.E.M. The number of animals used appears in brackets. Within-strain: $^{*}P \leq 0.05$ compared with controls; $^{\#}P \leq 0.05$ compared with the stressed-group (two-way ANOVA and Student Newman–Keuls test); Between-strain: $^{\S}P \leq 0.05$ compared with the same treatment (two-way ANOVA and Student Newman–Keuls test).

restraint acute stress whereas a shortening is exhibited in C57 mice subjected to the same stressful event. Various types of stressful manipulations such as restraint have been shown to induce metabolic and/or functional changes in the central nervous system as well as the peripheral system [9]. Furthermore, involvement of the GABAergic system in biochemical and behavioral changes induced by acute stress has also been indicated [17]. Brief exposure to stress elicits changes in the $GABA_A$ supramolecular complex confined to sites associated with the chloride ionophore in the way of a depression of the $GABA_A$ receptor function [4]. Functional changes in the GABAergic system sub-acutely induced by stress may differ between C57 and BALB mice. In C57 mice, shortening of the duration of PTB-induced LORR induced by stress could be partly mediated by attenuation of the GABAergic systems' basal tone. Reported data showed that a high anxiety level exhibited by the BALB mice can be completely abolished by BZD [2], suggesting the involvement of BZD receptors. Furthermore, when compared to C57 mice, BALB mice exhibit a decrease in the number of brain BZD receptors [7,15] and more precisely in the amygdala [11]. Although the brain BZD sites are not always co-localized with the $GABA_A$ receptor/chloride channel complex [12], it is conceivable that the properties of this receptor complex can be altered in discrete regions of the BALB mouse brain and be related to the lower hypnotic effect of PTB at baseline and to the lack of response to an acute stress. The present results show that EFX, which is devoid of hypnotic activity [5], potentiates the hypnotic effect of PTB in the two strains of mice. These findings are consistent with the fact that these two compounds bind to the $GABA_A$ receptor complex and allosterically modulate the $GABA_A$ receptor function in the way of an enhancement of the synaptic transmission [14,19]. The response to EFX after exposure to restraint stress differs in the two strains of mice. In C57 mice, this compound counteracted the effect of the acute stress whereas it tended to 'normalize' the effect of stress in BALB mice by reducing LORR duration. The dysfunction of the GABAergic system in BALB mice, revealed by the lack of response to the acute stress, unlike what was classically observed [4], was somewhat corrected by EFX, a positive allosteric modulator of the $GABA_A$ receptor. However, the present results must be interpreted with caution. For example, the activity of the metabolic enzymes for pentobarbital and the nature of the interaction between stress and the activity of these enzymes could differ between these two strains of mice. Further experiments could help to clarify this possibility. Also, the differences in the level of reactivity between BALB and C57 mice when faced with stress could be related to abnormalities in other neurotransmitter systems such as dopaminergic [10] and opioidergic [1] systems. Further investigations are needed to identify the contributions of neuroanatomical, neurochemical, genetic or environmental factors [3], underlying the strain differences in anxiety-related behavior in mice. It is concluded that in BALB mice, a strain described as 'anxious' compared with the C57 strain, an aberration of the GABAergic system (GABA synthesis, uptake, metabolism or binding to $GABA_A$ recognition sites) is a likely mechanism (not an exclusive one) underlying the absence of reactivity of this neurotransmission system to an acute stress.

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