

Effects of etifoxine on stress-induced hyperthermia, freezing behavior and colonic motor activation in rats

Marc Verleye*, Jean-Marie Gillardin

Laboratoires Biocodex, Département de Pharmacologie, Zac de Mercières, Chemin d'Armancourt, 60200 Compiègne, France

Received 19 December 2003; received in revised form 30 June 2004; accepted 29 July 2004

Abstract

Anxiety disorders are often associated with autonomic symptoms, including heart palpitations, sweating, elevation of body temperature and alterations of gastrointestinal motility. Some of the alterations observed in animals exposed to stress are analogous to changes in a number of physiological and endocrine parameters observed in anxious patients. With the purpose to guide further clinical studies in subtypes of anxious patients, etifoxine, a nonbenzodiazepine anxiolytic compound, was evaluated in two rat models of anxiety with measures of physiological manifestations: stress-induced hyperthermia (SIH) and conditioned-fear-stress-induced freezing behavior and activation of colonic motility. The sequential handling of animals induced a rise in body temperature attenuated by etifoxine (50 mg/kg IP). The emotional stress induced by fear to receive electric foot shocks is accompanied by freezing behavior and an increase of the frequency of ceco-colonic spike bursts: both parameters were reduced by etifoxine (25–50 mg/kg IP), independently of changes in pain perception and memory-related processes. In response to a stressful event, the stimulation of the corticotropin-releasing hormone (CRH) system is probably involved in the observed modifications of body temperature and colonic motility. It is hypothesized that stress-induced CRH activation is attenuated by the enhancement of the inhibitory GABAergic system activity associated with etifoxine. These findings will guide future evaluation of etifoxine in the treatment of selected anxious patients with altered autonomic symptomatology.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Anxiety disorders; Autonomic symptoms; Stress-induced hyperthermia; Conditioned fear-stress; Rat; Freezing behavior; Colonic motility; Etifoxine; GABAergic transmission

1. Introduction

The pathophysiology of anxiety disorders is thought to involve both endogenous predisposing factors and a dysregulated response to stress [18]. Physical manifestations associated with anxiety states or anxiety attacks include, among others, heart pounding [7], sweating, mild gastrointestinal discomfort and an increase in body temperature [20]. All these body reactions are mediated by the autonomic nervous system and the hypothalamic–pituitary–adrenal axis which are activated after exposure to stressful stimuli (psychological, physical) [14]. Sometimes,

the chronicity of activation of the stress system may lead to a pathologic syndrome such as anxiety states with an unpleasant autonomic hyperfunction. The pharmacological treatment of mood and anxiety disorders is dominated by drugs that directly target monoamine or GABA neurotransmission systems and benzodiazepines are routinely prescribed for anxiety disorders [22]. Moreover, the tolerability of benzodiazepine anxiolytics is reduced by sedation, cognitive impairment and dependence. Attempts are currently being made to develop drugs targeting subtypes of GABA_A receptors to lessen the severity of side effects [22]. Etifoxine (2-ethylamino-6-chloro-4-methyl-4-phenyl-4*H*-3,1-benzoxazine hydrochloride, Stresam[®]), a molecule structurally unrelated to benzodiazepines, has revealed anxiolytic properties in rodents [2,28] and humans [29] without sedative, myorelaxant and mnesic side effects at anxiolytic

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

concentrations [19]. Recent binding and electrophysiological experiments have demonstrated that etifoxine binds to GABA_A receptors via an allosteric site which differs from that of benzodiazepines [32,33].

Interestingly, it was recently shown that etifoxine preferentially acts on GABA_A receptors containing the β_2 or the β_3 subunit [12]. Functionally, this compound increases GABAergic neurotransmission [28]. Clinically, etifoxine shows efficacy in the treatment of adjustment disorder with anxiety in humans [29], an anxiety symptom described in Diagnostic and Statistical Manual of Mental Disorders, fourth edition [1]. Autonomic (dys)functions, such as rise in body temperature and gastrointestinal disturbances, are symptoms in the diagnosis of some anxiety disorders, like generalized anxiety and panic disorders, and the purpose of the present work was to evaluate the effects of etifoxine in animal models of anxiety with measures of autonomic parameters: the rise in body temperature following handling and the activation of ceco-colonic motility during a conditioned fear paradigm in rats. The rise in body temperature called stress-induced hyperthermia (SIH) has been interpreted as being caused by anticipatory fear for an aversive event (handling and disturbance in the cage) whereas the activation of ceco-colonic activity (measured by electromyography) is associated with the freezing reaction of an animal exposed to an environment previously paired with an aversive stimulation (inescapable electric foot shocks). Based on functional similarities between humans and animals, SIH and the conditioned fear stress have been frequently used in preclinical studies of emotional processes [6,9,11,24].

2. Methods

2.1. Animals

Male adult Wistar rats (Janvier; Genêt-St Isle, France), 7–8 weeks of age and weighing 240–300 g at the start of experiments, were housed in groups of six in polypropylene cages (435×435×194 mm) for at least 7 days before study initiation. The housing facility was temperature (22±2 °C) and relative humidity (50±20%) controlled and equipped with artificial illumination (0700 h to 1900 h, lights on). The animals had access to water (tap water) and food (UAR A04-Epinay-France) ad libitum. All procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Stress-induced hyperthermia

The test procedure for stress-induced hyperthermia (SIH) was adapted from the original description by Borsini et al. [3] in the mouse. To validate our protocol in rats, bromazepam, an anxiolytic compound, was chosen as positive control. Briefly, rectal temperature was measured

to the nearest 0.1 °C by a thermometer (Leticia-TMP812 model) via a lubricated thermistor probe (YSI no. 423: 3 mm diameter) inserted 20 mm into the rectum while the rat was hand-held near the base of the tail. The probe was left in place until steady readings were obtained (within 20 s). Experiments were carried out between 1000 h and 1600 h at room temperature (22–23 °C). Six animals were housed per cage. At least 24 h before the experiment, animals within a cage were marked on their fur with colour for later identification. Sixty minutes before taking the rectal temperature, all animals within a given cage were consecutively treated at 2-min intervals with etifoxine (12.5, 25, 50 mg/kg IP), bromazepam (0.25 mg/kg IP) or vehicle. Exactly 60 min later, the rats were consecutively removed from the cage (again at 2-min intervals) and rectal temperature was determined and noted. Once temperature had been recorded, the animals were placed in a different (adjacent) cage. The dependent variable, i.e., SIH, was defined as the difference between the rectal temperature of the sixth removed rat and that of the first removed rat within a cage. The rectal temperature of the first animal was used to evaluate the compound's potential effect on basal body temperature per se. Data were given as means±S.E.M. The rectal temperature of the first animals and the SIH values were compared using a Kruskal–Wallis one-way analysis of variance (ANOVA) followed post hoc by the Dunn test with vehicle group as reference. $p<0.05$ was considered as statistically significant (SigmaStat, v3.0; SPSS).

2.3. Schedule for conditioned fear stress

2.3.1. Motility recordings

Animals under anaesthesia by intraperitoneal administration of pentobarbital sodium (60 mg/kg) were surgically prepared with nickel/chrome electrodes (80 μ m in diameter) implanted into the muscular layers of the proximal colon at 2 and 4 cm distal from the ileo-colonic junction according to a previously described method [27]. The free ends of the electrodes were brought subcutaneously to the back of the neck where they were linked to a connector attached to the skin. Bipolar recordings of myoelectric activity (time constant, 0.02 s; high pass filter, 300 Hz) commenced 5 days after surgery with an electromyographic recorder (Sefram-8800-Emka technologies—France). Rats were fasted 12 h before each experiment.

2.3.2. Conditioned fear stress procedure

It corresponded to the displacement of each animal from its home cage to a test cage where it had previously received electric foot shocks [9]. Briefly, each rat was placed in the test cage (Plexiglas cage: 300×200×200 mm) that can be freely explored for 60 min. At the end of this exploration phase, it received inescapable electric foot shocks [intensity, 1 mA; duration, 1 s with a mean intershock interval of 45 s (5–60 s) during 10 min]. At this end of this session, the animal was returned to its home cage. The placement of the

rat 2 h later in the same cage without electric shock administration was considered as a conditioned fear stress or an emotional stress [9].

2.3.3. Experimental design

In a first experiment, the effects of etifoxine at 50 mg/kg (the highest anxiolytic dose, see Ref. [28]) were studied in the nonshocked animal to establish the lack of effect of this compound. Etifoxine or vehicle were administered by intraperitoneal route 30 min before recordings. In a second experiment, etifoxine at 12, 25 or 50 mg/kg or the vehicle were administered 30 min before placing rats into the test cage for an emotional stress session lasting 30 min. During the first 10 min of observation, the duration of freezing behavior (s) was recorded. Freezing was defined as the absence of all observable movement of the skeleton and the vibrissae except those related to respiration.

2.3.4. Data analysis

The frequency of ceco-colonic spike bursts expressed by the mean number of spike bursts (\pm S.E.M.) occurring in 10-min periods was determined by counting spike bursts on direct electromyographic records during a 30-min period in the basal condition (before the application of electric shocks) and for 30 min after the beginning of the stress session.

The counted spike bursts were characterized by duration and amplitude ranging from 10 to 15 s and from 100 to 150 μ V, respectively. The mean values obtained during the basal state and during the emotional stress were compared using Student's paired *t*-test. The comparison between the values observed before and after the vehicle or etifoxine administration in animals under basal conditions were performed by using Student's paired *t*-test or Student's *t*-test. The effects of etifoxine on the stress-induced colonic activation or freezing behavior were analyzed by a two-way analysis of variance (ANOVA) followed post hoc by the Student Newman–Keuls procedure (SNK) or by a one-way ANOVA followed post hoc by the Dunnett's test, respectively. Differences were considered significant at $p < 0.05$ (SigmaStat, v3.0; SPSS).

2.4. Passive avoidance experiments

Considering the delay between exposure to stressful events and the induction of the conditioned emotional stress, an additional experiment using the classical passive avoidance task in rats [16] was utilized to investigate the effects of etifoxine on memory. The test chamber consisted of two compartments separated by a door, which could be raised. One compartment (360×330×240 cm) was "lighted", the other (160×140×240 cm) was "dark". An electric shock could be delivered through the steel grid floor. On the first experimental day, animals were placed in the lighted compartment and given access to the dark compartment after 10 s by raising the door. The rats were allowed to

freely explore the two compartments for 3 min (training phase). Twenty-four hours later, the acquisition trial was run. After 10 s in the lighted compartment, the door was raised and the latency (s) to enter the dark compartment was measured. This measurement ensured a high degree of homogeneity among the groups of animals before the treatment. When the rats entered the dark compartment, the door was closed and the rats received an inescapable foot shock (0.8 mA) for 2 s. After the foot shock, they were removed 10 s later from the dark compartment and returned to their home cage (acquisition phase). Two hours after the acquisition trial, the rats were placed in the lighted compartment and the same procedure previously described was applied except that no electrical shock was delivered (retention phase). The latency to enter the dark compartment was recorded up to a maximum cutoff time of 3 min. Etifoxine at 50 mg/kg or vehicle was intraperitoneally administered 30 min before the retention trial. Data were expressed as means \pm S.E.M. Comparisons between groups were performed by two-way ANOVA followed post hoc by the Student Newman–Keuls test (SNK procedure). A *p* value ≤ 0.05 was considered significant (SigmaStat, v3.0; SPSS).

3. Drugs

Etifoxine (Biocodex; batches 114 and 196) and bromazepam (Francochim, batch 5788) were suspended in a saline solution (0.9% NaCl in distilled water, *p/v*) with 1% Tween 80 (*v/v*). Control animals received an equivalent volume of vehicle (5 ml/kg).

4. Results

4.1. Stress-induced hyperthermia

None of the treatments (bromazepam or etifoxine) significantly affected basal core body temperature, $H(4) = 0.678$, $p = 0.954$ (Fig. 1A). In the vehicle-treated cages, stress-induced hyperthermia was quantitatively comparable to the values reported in literature (+0.6 °C, Fig. 1B).

The difference in rectal temperatures, between last and first animal removed, was significantly reduced after treatment with bromazepam or etifoxine, $H(4) = 14.44$, $p = 0.006$. Post hoc comparisons showed that the effect of etifoxine appeared dose dependent with a significant effect at 50 mg/kg dose ($p < 0.05$; Dunn test) whereas bromazepam (0.25 mg/kg), used here as a positive control, significantly attenuated stress-induced hyperthermia ($p < 0.05$; Dunn test; Fig. 1B).

4.2. Conditioned fear stress

The effects of etifoxine in the conditioned-fear-stress-induced freezing are shown in Fig. 2. Freezing behavior was

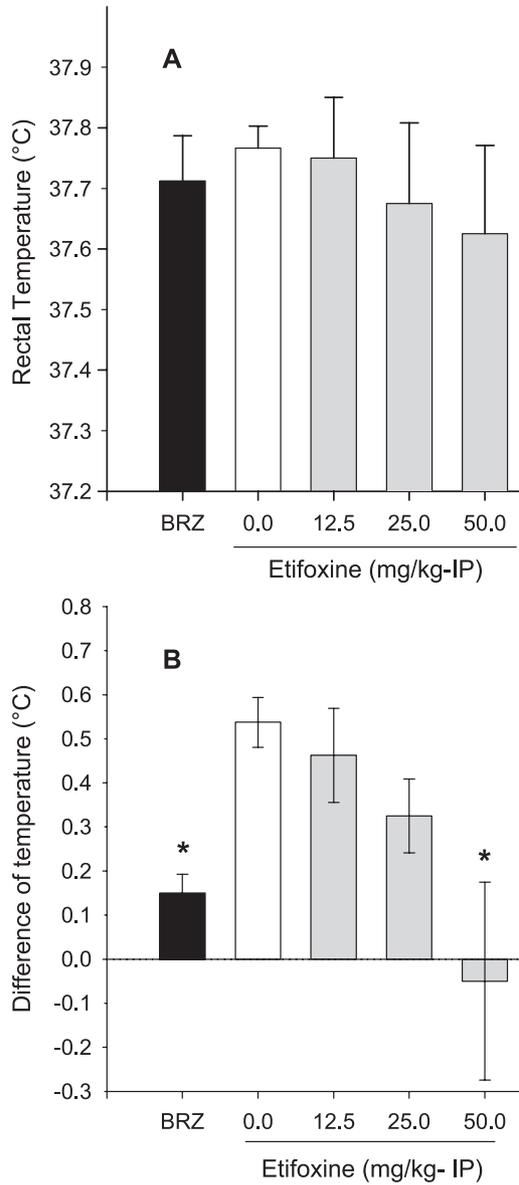


Fig. 1. Stress-induced hyperthermia. (A) Bars represent the mean rectal temperature (with S.E.M.) of the first rat within the cage 60 min after treatment with etifoxine ($n=8/\text{dose}$), vehicle ($n=24$) or bromazepam (BRZ, 0.25 mg/kg; $n=8$). (B) Bars represent the mean (\pm S.E.M.) of the difference of the rectal temperature (between the last and the first animal) per cage of 6 rats 60 min after treatment with etifoxine ($n=8/\text{dose}$), vehicle ($n=24$) or bromazepam (BRZ, 0.25 mg/kg; $n=8$). * $p<0.05$ compared to vehicle group.

absent in the nonshocked animals treated with vehicle or with etifoxine (12.5, 25, 50 mg/kg). At similar doses, etifoxine produced a dose-dependent reduction in freezing behavior in the shocked animals, $F(3,51)=7.87$, $p<0.001$, and the difference was statistically significant at 50 mg/kg ($p<0.05$; Dunnett test).

During the basal period in a pilot study, the myoelectrical activity of the proximal colon exhibited spike bursts at a rhythm of 5.9 ± 0.3 bursts/10 min ($n=10$) at 2 cm from the ileo-colonic junction and at a rhythm of 6.3 ± 0.3 bursts/10 min ($n=10$) at 4 cm from the ileo-colonic junction. No significant differences ($p=0.193$; Student's paired t -test)

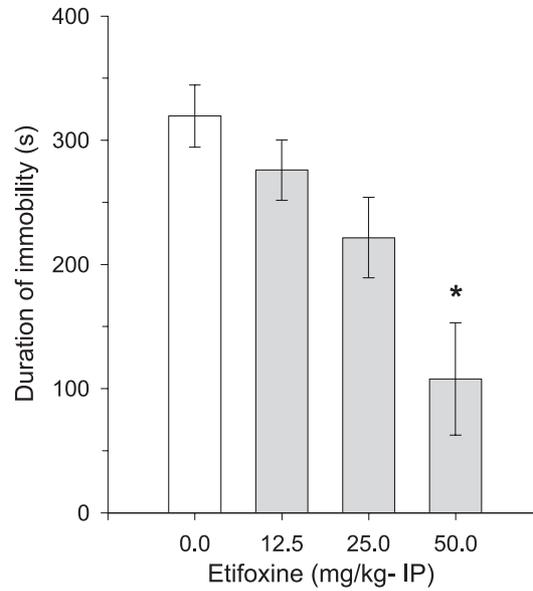


Fig. 2. Effects of etifoxine on the conditioned-fear-stress-induced freezing behavior in rats. Each column represents the mean \pm S.E.M. $n=27$ control animals and $n=9-10$ animals treated with etifoxine. * $p<0.05$ compared to vehicle group.

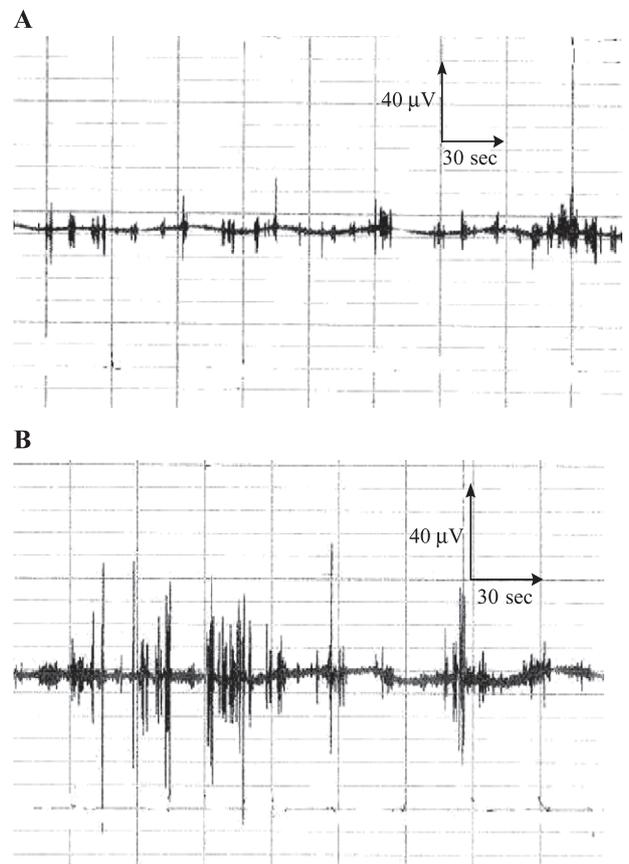


Fig. 3. (A) Examples of ceco-colonic electromyographic recordings at +2 cm from the ileo-colonic junction in the basal conditions and (B) in the same animal during the emotional stress.

Table 1
Effects of etifoxine on the colonic spike burst activity in rats

	Dose (mg/kg)	Number of spike bursts/10 min ^a		
		Before administration	After administration	Difference
Vehicle	0	7.1±0.5	8.0±0.4	0.9±0.5
Etifoxine	50	7.1±0.4	7.6±0.4	0.5±0.4

^a Measured for 30 min in the basal conditions without treatment and 30 min after etifoxine administration. Each value represents the mean±S.E.M. $n=10$ animals/group. No statistical differences between the two groups.

were observed between the two sites investigated. Consequently, only the values obtained at 2 cm from the ileo-colonic junction were considered for the following experiments. This frequency of colonic spike bursts, illustrated in Fig. 3, was significantly increased by 108% during the 30 min of emotional stress compared with corresponding basal values: The number of colonic spike bursts significantly increased from 5.9 ± 0.3 to 12.3 ± 0.6 spikes/10 min during this period ($p<0.001$; Student's paired test). Etifoxine, at 50 mg/kg dose had no effect ($p>0.05$; Student's test) per se on colonic spike bursts frequency in the animal under basal conditions (Table 1). The two-way ANOVA (emotional stress×treatment) on the frequency of colonic spike bursts revealed highly significant effects on the factor emotional stress, $F(1,102)=32.2$, $p<0.001$, on the factor etifoxine treatment, $F(3,102)=10.3$, $p<0.001$, and also on the interaction emotional stress×treatment, $F(3,102)=5.9$, $p<0.001$. Post hoc comparisons indicated that placing the animals in the test cage significantly increased the number of colonic spike bursts in the vehicle group ($p<0.001$, SNK procedure), as previously observed, and that etifoxine at 25 and 50 mg/kg reduced significantly the increase in colonic spike bursts frequency produced by emotional stress ($p<0.05$, SNK procedure; Table 2).

4.3. Passive avoidance performance

Acquisition and retention performance of rats treated with etifoxine (50 mg/kg) or vehicle are shown in Fig. 4. The two-way ANOVA (session×treatment) on the latency to

Table 2
Effects of etifoxine on the increase of colonic spike burst activity induced by emotional stress in rats

	N	Dose (mg/kg)	Number of spike bursts/10 min ^a	
			Basal	Emotional stress
Vehicle	27	0	7.6±0.4	12.2±0.5*
Etifoxine	9	12.5	8.4±0.3	12.5±0.7*
		25	8.1±0.7	8.9±0.7 [#]
		50	6.7±0.5	7.7±0.7 [#]

^a Measured for 30 min in the basal conditions and 30 min from the beginning of the emotional stress. Etifoxine was administered by an intraperitoneal route 30 min prior to the emotional stress. Each value represents the mean±S.E.M. N =number of animals used.

* $p<0.05$ compared with basal values.

[#] $p<0.05$ compared with corresponding vehicle values.

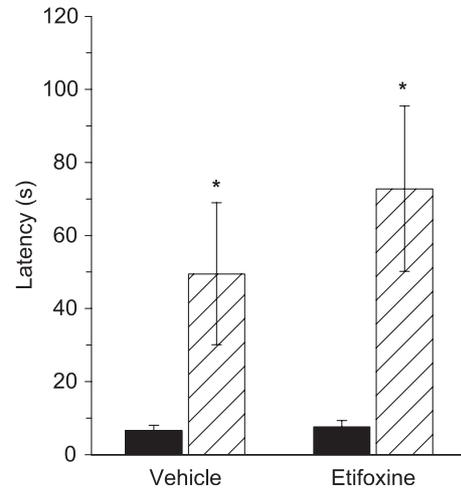


Fig. 4. Effects of etifoxine (50 mg/kg IP) on retention (hatched bars) performance of a passive avoidance task in rats. Black bars represent the performance during the acquisition session. Results are expressed as mean latency (\pm S.E.M.) to enter the dark compartment. $n=10$ animals/group. * $p<0.05$ compared to the nonshocked group (acquisition session).

enter showed a significant effect on the factor session, $F(1,36)=13.08$, $p<0.001$, but neither on the factor treatment with etifoxine, $F(1,36)=0.66$, $p=0.42$, nor on the interaction session×treatment, $F(1,36)=0.56$, $p=0.46$. The post hoc statistical analysis (SNK procedure) revealed that latencies to enter the dark compartment between the acquisition and retention sessions significantly differed in the vehicle group ($p=0.05$) and in the etifoxine-treated group ($p=0.004$). The latency to enter the dark compartment was not statistically different between vehicle- and etifoxine-treated groups during the acquisition session and during the retention session ($p=0.96$ and $p=0.28$, respectively).

5. Discussion

Strong indications, that stress-induced hyperthermia in man uses physiological and endocrinological mechanisms similar to those shown in animals, have been reported [17]. The rise in body temperature, or the fact that the animals removed last always have higher rectal temperature than those removed first, has been interpreted as being caused by anticipatory fear of an aversive event (handling and disturbance in the cage). The stress-induced hyperthermia, also called “psychogenic fever” [23], appears to be a universal phenomenon occurring in many species, including humans [13,17,25]. The present results of decrease in body temperature difference between last and first animal removed suggest strong anxiolytic-like effect for etifoxine and bromazepam (positive control). It appears that these two compounds selectively counteracted the anxiety-dependent variable because they did not affect the basal temperature per se: the rectal temperature of the first animals remained unchanged after treatment with etifoxine or bromazepam. In the conditioned fear stress paradigm, the freezing behavior

has been interpreted as evidence that fear has been conditioned by the context surrounding the stressful event [6]. Thus, consistent with this interpretation, rodents exhibited a response characterized by a period of complete immobility when tested in the same environment where they had been previously exposed to aversive stimuli, such as the inescapable electric foot shocks used in this study. The present results indicated that etifoxine reduced the fear-stress-induced freezing behavior in rats, an index of anxiety in an apparent dose-dependent fashion.

Under similar experimental conditions, this stress-induced freezing behavior was attenuated by both benzodiazepine and nonbenzodiazepine anxiolytics [15]. This etifoxine effect is probably not related to a stimulatory effect of spontaneous motor activity because sedative effects have been observed at similar doses in rats [2]. Concomitantly with the freezing behavior, emotional stress activates colonic activity as shown by an increase of the spike bursts frequency. Furthermore, in humans, recordings of colonic motility during stress sessions show a stimulation related to the nature of the stressful event [21]. Etifoxine, administered alone at the highest anxiolytic dose (50 mg/kg) in the nonstressed animals did not affect the colonic spike activity, indicating a lack of prokinetic or spasmolytic activity for this compound. When administered at similar doses in the stressed animals, etifoxine reduced the increase in colonic spike burst frequency produced by emotional stress. This effect was selectively related to the decrease in fear-evoked behavior independent of changes in pain perception (etifoxine was administered after the electric shocks session) and in memory-related processes. In fact, etifoxine did not disrupt the passive avoidance retention response: the animals did not forget the electric shock previously received in the dark compartment. It is to be noted that the magnitude of the stress-induced colonic motility activation in the present study is similar to that measured in other studies [8–10]. In addition, anxiolytic compounds, like benzodiazepines, showed identical effects in the same experimental paradigm [8]. It is well known that exposure of animals to stressful stimuli, like handling or conditioned fear stress, activates the hypothalamic–pituitary–adrenal axis and the sympatho–adrenal–medullary system [14]. Corticotropin-releasing hormone (CRH), the first released factor after stimulation of the hypothalamic–pituitary–adrenal axis, has been reported to possess thermogenic effects which were dependent on sympathetic activation of brown adipose tissue [26]. As the hypothalamic–pituitary–adrenal axis is stimulated during the stress-induced hyperthermia procedure [24,26], the increase in rectal temperature can be a direct effect of hypothalamic–pituitary–adrenal stimulation. Additionally, it was shown that the increase in colonic motility associated with fear of receiving electric foot shocks is related to the central release of CRH because it is blocked by a CRH antagonist and mimicked by intracerebroventricular administration of CRH in rats [9]. Interestingly, previous studies have shown

that inhibitory endogenous GABA acts on GABA_A receptors in discrete area of the central nervous system, like the hypothalamus, to regulate the level of experimental anxiety in rats [5,30]. It was demonstrated that etifoxine enhances the inhibitory effects of endogenous GABA after binding to a site near or on the chloride channel coupled to the GABA_A receptor complex [28,33]. In addition, GABAergic neurons are considered to inhibit CRH secretion tonically in the hypothalamus [4,31]. Thus, it is tempting to hypothesize that the effects of etifoxine on the expression of the two autonomic components, body temperature and colonic activity, are related to inhibition of CRH release by activation of the inhibitory GABAergic system. To this effect, further biochemical and binding studies in relation to CRH binding protein and to subtypes of CRH receptors are needed to elucidate the nature of interactions between etifoxine and the CRH system. It is interesting to note that the specific binding of etifoxine on the subtypes β_2 or β_3 of the GABA_A receptor complex [12] might explain why this compound is denuded of unwanted side effects, like dependence and cognitive impairment [19]. While additional studies are needed to evaluate the anxiolytic efficacy of etifoxine on other stress-induced changes in physiological parameters (e.g., tachycardia), the present results constitute a basis for therapeutic evaluation of this compound in selected anxious patients in whom autonomic symptoms constitute very disturbing life events.

Acknowledgements

The authors would like to thank Pr. René Levy (University of Washington, Seattle, USA) for carefully reading the manuscript. We are also grateful to Ingrid Lorette, Isabelle Heulard and Nathalie André for their excellent technical assistance.

References

- [1] American Psychiatric Association. Diagnostic and statistical manual of psychiatric disorders. (DSM-IV) 4th ed. Washington, DC: APA Press; 1995.
- [2] Boissier JR, Simon P, Zaczinska M, Fichelle J. Experimental psychopharmacologic study of a new psychotropic drug, 2-ethyl-amino-6-chloro-4-methyl-4-phenyl-4H-3,1-benzoxazine. *Therapie* 1972;27:325–38.
- [3] Borsini F, Lecci A, Volterra G, Meli A. A model to measure anticipatory anxiety in mice? *Psychopharmacology (Berl)* 1989; 98:207–11.
- [4] Cullinan WE. GABA(A) receptor subunit expression within hypothalamic CRH neurons: a dual hybridization histochemical study. *J Comp Neurol* 2000;419:344–51.
- [5] DiMicco JA, Samuels JA, Zaretskaia MV, Zaretsky DV. The dorsomedial hypothalamus and the response to stress: part renaissance, part revolution. *Pharmacol Biochem Behav* 2002;71:469–80.
- [6] Fanselow MS, Helmstetter FJ. Conditional analgesia, defensive freezing, and benzodiazepines. *Behav Neurosci* 1988;102:233–43.

- [7] Friedman BH, Thayer JF. Autonomic balance revisited: panic anxiety and heart rate variability. *J Psychosom Res* 1998;44:133–51.
- [8] Gue M, Alary C, Rio-Lacheze CD, Junien JL, Bueno L. Comparative involvement of 5-HT₁, 5-HT₂ and 5-HT₃ receptors in stress-induced colonic motor alterations in rats. *Eur J Pharmacol* 1993;233:193–9.
- [9] Gue M, Junien JL, Bueno L. Conditioned emotional response in rats enhances colonic motility through the central release of corticotropin-releasing factor. *Gastroenterology* 1991;100:964–70.
- [10] Gue M, Tekamp A, Tabis N, Junien JL, Bueno L. Cholecystokinin blockade of emotional stress- and CRF-induced colonic motor alterations in rats: role of the amygdala. *Brain Res* 1994;658:232–8.
- [11] Habib KE, Gold PW, Chrousos GP. Neuroendocrinology of stress. *Endocrinol Metab Clin N Am* 2001;30:695–728.
- [12] Hamon A, Morel A, Hue B, Verleye M, Gillardin JM. The modulatory effects of the anxiolytic etifoxine on GABA(A) receptors are mediated by the beta subunit. *Neuropharmacology* 2003;45:293–303.
- [13] Hasan MK, White AC. Psychogenic fever: entity or nonentity? *Postgrad Med* 1979;66:152–4.
- [14] Henry JP. Biological basis of the stress response. *Integr Physiol Behav Sci* 1992;27:66–83.
- [15] Inoue T, Tsuchiya K, Koyama T. Serotonergic activation reduces defensive freezing in the conditioned fear paradigm. *Pharmacol Biochem Behav* 1996;53:825–31.
- [16] Kameyama T, Nabeshima T, Kozawa T. Step-down-type passive avoidance- and escape-learning method. Suitability for experimental amnesia models. *J Pharmacol Methods* 1986;16:39–52.
- [17] Marazziti D, Di Muro A, Castrogiovanni P. Psychological stress and body temperature changes in humans. *Physiol Behav* 1992;52:393–5.
- [18] McEwen BS. Allostasis and allostatic load: implications for neuropsychopharmacology. *Neuropsychopharmacology* 2000;22:108–24.
- [19] Micallef J, Soubrouillard C, Guet F, Le Guern ME, Alquier C, Bruguerolle B, et al. A double blind parallel group placebo controlled comparison of sedative and amnesic effects of etifoxine and lorazepam in healthy subjects. *Fundam Clin Pharmacol* 2001;15:209–16.
- [20] Murphy DL, Lesch KP, Aulakh CS, Pigott TA. Serotonin-selective arylpiperazines with neuroendocrine, behavioral, temperature, and cardiovascular effects in humans. *Pharmacol Rev* 1991;43:527–52.
- [21] Narducci F, Snape Jr WJ, Battle WM, London RL, Cohen S. Increased colonic motility during exposure to a stressful situation. *Dig Dis Sci* 1985;30:40–4.
- [22] Nemeroff CB. Anxiolytics: past, present, and future agents. *J Clin Psychiatry* 2003;64(Suppl. 3):3–6.
- [23] Oka T, Oka K, Hori T. Mechanisms and mediators of psychological stress-induced rise in core temperature. *Psychosom Med* 2001;63:476–86.
- [24] Olivier B, Zethof T, Pattij T, van Boogaert M, van Oorschoot R, Leahy C, et al. Stress-induced hyperthermia and anxiety: pharmacological validation. *Eur J Pharmacol* 2003;463:117–32.
- [25] Reeves DL, Levinson DM, Justesen DR, Lubin B. Endogenous hyperthermia in normal human subjects: experimental study of emotional states (II). *Int J Psychosom* 1985;32:18–23.
- [26] Rothwell NJ. Central effects of CRF on metabolism and energy balance. *Neurosci Biobehav Rev* 1990;14:263–71.
- [27] Rukebusch M, Fioramonti J. Electrical spiking activity and propulsion in small intestine in fed and fasted rats. *Gastroenterology* 1975;68:1500–8.
- [28] Schlichter R, Rybalchenko V, Poisbeau P, Verleye M, Gillardin J. Modulation of GABAergic synaptic transmission by the non-benzodiazepine anxiolytic etifoxine. *Neuropharmacology* 2000;39:1523–35.
- [29] Servant D, Graziani PL, Moysse D, Parquet PJ. Treatment of adjustment disorder with anxiety: efficacy and tolerance of etifoxine in a double-blind controlled study. *Encephale* 1998;24:569–74.
- [30] Shekhar A, Hingten JN, DiMicco JA. GABA receptors in the hypothalamus regulate experimental anxiety in rats. *Brain Res* 1990;512:81–8.
- [31] Tsagarakis S, Grossman A. Central neuroregulation of hypothalamic corticotropin-releasing hormone (CRH-41) secretion. *J Endocrinol Invest* 1990;13:765–75.
- [32] Verleye M, Pansart Y, Gillardin JM. Effects of etifoxine on ligand binding to GABA(A) receptors in rodents. *Neurosci Res* 2002;44:167–72.
- [33] Verleye M, Schlichter R, Gillardin JM. Interactions of etifoxine with the chloride channel coupled to the GABA(A) receptor complex. *NeuroReport* 1999;10:3207–10.