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Analgesic and anti-edemic properties of etifoxine in models of inflammatory sensitization

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ABSTRACT

Inflammatory processes are critical promoting factors of chronic pain states, mostly by inducing peripheral and central sensitization of the nociceptive system. These processes are associated with a massive increase in glutamatergic transmission, sometimes facilitated by spinal disinhibition. In this study, we used etifoxine, a non-benzodiazepine anxiolytic known to amplify inhibition mediated by gamma-aminobutyric acid type A ($GABA_A$) receptors in pain processing regions, either directly (through allosteric modulation) or indirectly (through the synthesis of endogenous neurosteroids). We used different models of local inflammation to evaluate the possible direct action of etifoxine on analgesia and edema. Pain symptom and edema measurements were performed after intraplantar carrageenan injection or after topical ear inflammation. We found that etifoxine treatment was associated with reduced plantar surface temperature 24 h after intraplantar carrageenan injection. In this model, etifoxine also alleviated thermal hot and mechanical hyperalgesia. A similar finding was observed while analyzing pain symptoms in the late phase of the formalin test. In a model of ear inflammation, etifoxine appeared to have a moderate anti-edemic effect after topical application. This slight action of etifoxine on the limitation of inflammatory processes could be mediated in part by cyclo-oxygenase 1 activity inhibition. Etifoxine appears as a promising therapeutic tool contributing to the limitation of inflammatory pain symptoms. Since etifoxine is already prescribed as an anxiolytic in several countries, it could be a good candidate for the prevention of inflammatory-driven edema and hyperalgesia, although the precise mechanism of action relative to its anti-inflammatory potential remains to be elucidated.

1. Introduction

Inflammatory processes are critical promoting factors of chronic pain states, mostly by inducing peripheral and central sensitization of the nociceptive system. Central sensitization in the spinal cord is known to involve a long-term amplification of N-methyl-D-aspartate (NMDA)-type glutamate receptor function and this phenomenon is likely to be prolonged by peripheral inflammation (McMahon et al., 2015). These excitatory processes are sometimes exacerbated by local disinhibition, as demonstrated in the spinal cord for glycinergic inhibition when prostaglandin E_2 (PGE_2) is released by microglial cells (Harvey et al., 2004). Inflammation may also increase the expression of the Na^+-K^+-2Cl^- cotransporter NKCC1, further limiting the efficacy of chloride-mediated inhibition mediated by gamma-aminobutyric acid type A receptor ($GABA_A$ receptor) and glycine receptor-channels (Morales-Aza et al., 2004; Valencia-de Ita et al., 2006). Facilitating this remaining inhibitory neurotransmission by positive allosteric modulators has thus been proposed as a therapeutic strategy to compensate for this disinhibition (Zeilhofer et al., 2012).

Following a similar strategy, the non-benzodiazepine anxiolytic etifoxine successfully reduced and sometimes fully alleviated pain symptoms in several animal models of inflammatory and neuropathic pain (Aouad et al., 2009, 2014a, 2014b). Etifoxine is generally used to treat adjustment disorders with anxiety and exhibits a limited set of adverse effects (Micallef et al., 2001; Nguyen et al., 2006; Servant et al., 1998; Stein, 2015). Indeed, prolonged exposure to etifoxine is associated with a limited functional tolerance (Servant et al., 1998) and doesn't induce any effects on vigilance, psychomotor or cognitive performance (Micallef et al., 2001). Contrary to benzodiazepines, etifoxine exerts a positive allosteric modulation of $GABA_A$ receptors after binding to the β subunits (β2 > β3 > β1), i.e. at a site distinct from that of benzodiazepines (Hamon et al., 2003). Moreover, etifoxine potentiation

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of $GABA_A$ receptor function can be synergistically amplified by the local synthesis of neuroactive steroids, such as allopregnanolone, which are the most potent endogenous modulators of $GABA_A$ receptors (Schlichter et al., 2000). This indirect action on $GABA_A$ receptors is long-lasting and has been shown to result from the binding of etifoxine on mitochondrial translocator protein complexes (TSPO) (Verleye et al., 2005; Wolf et al., 2015). Interestingly, this mechanism seems to prevail for the long-term anxiolytic and analgesic actions of etifoxine (Poisbeau et al., 2014; Ugale et al., 2007).

Apart from these properties, a growing number of scientific reports mention the neuroprotective and neuroregenerative role of etifoxine in inflammatory-driven pathologies. This includes brain trauma (Girard et al., 2009; Li et al., 2017; Simon-O'Brien et al., 2016), nerve lesions (Girard et al., 2012), multiple sclerosis (Daugherty et al., 2013; Ravikumar et al., 2016) and experimental monoarthritis (Aouad et al., 2014b). In the latter study for example, long-lasting etifoxine analgesia in monoarthritic rats was associated with a reduced expression of several pro-inflammatory mediators and of cyclo-oxygenase 2 (COX-2) (Aouad et al., 2014b). Considering the aforementioned evidence of etifoxine efficacy on persistent inflammation, we sought to characterize its potential as an anti-inflammatory component, after an acute or chronic treatment.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (Janvier, France), CD1 mice (Charles River, France) and NMRI mice [Janvier, France (Fig. 2)], weighing 220–300 g and 25–35 g respectively, were used for this study. Animals were housed in a temperature (22 °C) and humidity (50 ± 20%) controlled room, under a 12 h light-dark cycle (lights on at 7:00 a.m.), with ad libitum access to food and tap water. All procedures were conducted in accordance with EU regulations and approved by the regional ethical committee (authorization number D6015904 and AL11-14-03-04).

2.2. Pharmacological treatments

Etifoxine was kindly provided by Biocodex laboratories (2-ethylamino-6-chloro-4-methyl-4-phenyl-4H-3,1-benzoxazine hydrochloride, batches 406, 439, 562 and 653; Biocodex, Gentilly, France) and prepared in saline (NaCl 0.9% in distilled water) containing 1% Tween 80 (v/v; Sigma-Aldrich). To study the acute effect of etifoxine on inflammation (Fig. 1), this solution was administered in a final volume of 0.5 ml/100 g (intraperitoneal – IP) or 1 ml/100 g (*per os*). In this case, treatment (IP or *per os*) was co-administered with carrageenan, or administered (IP only) after the onset of inflammation, 2.5 h after carrageenan injection. Since carrageenan-induced inflammation develops progressively and lasts several hours, this specific time point was chosen for treatment to be administered while inflammation was at its maximum. In the formalin test (Fig. 2), etifoxine (50 mg/kg IP or 150 mg/kg *per os*) or ibuprofen (400 mg/kg, subcutaneous injection) were administered 30 min prior to formalin injection. To study the effect of etifoxine on inflammatory pain symptoms (Fig. 3), five consecutive daily IP injections (50 mg/kg in a final volume of 1 ml/kg) were given to rats prior to intraplantar carrageenan. Control animals received an equivalent volume of vehicle. To assess the topical effect of etifoxine on phorbol 12-myristate 13-acetate (PMA)-induced ear edema (Fig. 4), etifoxine was applied locally 30 min prior to PMA administration. When administered topically on mice ear surface, etifoxine was solubilized in 50 µl (etifoxine 1 mg) or 100 µl (etifoxine 2 mg) acetone.

2.3. Inflammatory pain model

Inflammation was induced by a subcutaneous injection (100 µl) of λ-carrageenan [2% (Fig. 1) or 3% (Fig. 3) in NaCl 0.9%; Sigma-Aldrich, Saint Louis, MO, USA] into the plantar surface of the right hind paw of conscious rats (left paw being used as internal control) (Winter et al., 1962).

2.4. Nociceptive tests

Unless indicated, nociceptive tests were performed prior to the daily injection of etifoxine.

2.4.1. Hargreaves' plantar test

Thermal heat nociceptive response was assessed using Hargreaves' method (Hargreaves et al., 1988) using a standard apparatus (Ugo Basile, Italy). Briefly, a thermal radiant stimulus was applied with an infrared heat lamp underneath the rat's targeted hind paw. Paw withdrawal latency was automatically measured, with a cutoff set at 30 s to avoid tissue damage. Withdrawal responses were determined three times for each paw and values were averaged.

2.4.2. Calibrated forceps

Mechanical sensitivity was measured using a calibrated forceps (Bioseb, France) previously validated (Luis-Delgado et al., 2006). Briefly, the tips of the forceps were placed at each side of the paw of a loosely restrained rat. A gradually increasing force was then applied, and the pressure producing withdrawal of the paw was measured. Noxious pressure thresholds were assessed three times for each hind paw and values were averaged. Cutoff value was set at 1000 g but was never reached in this study.

2.4.3. Formalin test

The formalin test was performed following the method described by Dubuisson and Dennis (Dubuisson and Dennis, 1977). Formalin (5%, 20 µl) was injected subcutaneously in the right hind paw of restrained mice. Time spent licking the injected paw was measured during 5 min periods 30–50 min after injection (late phase corresponding to inflammatory pain symptoms).

2.5. Thermography

Hind paw surface temperature was measured using an infrared camera (ThermoVision™ A-series camera) on animals briefly anesthetized with isoflurane 2–3%. Data were analyzed using the FLIR Systems imaging software (ThermoVision™ SDK 2.5 SR-1, FLIR Systems, France).

2.6. Edema measurements

Edema formation in the rat was assessed by changes in paw volume after carrageenan injection. To measure volume of the edema, the paw was immersed in a cuvette filled with water (density 1 mg/ml) and placed on an electronic scale. The extra weight displayed by the scale was assumed to represent paw volume. Edema volume was obtained by subtracting the volume of the left paw from the volume of the right carrageenan-injected paw.

Topical inflammation was induced by the application of 2.5 µg PMA solubilized in 20 µl acetone on the right ear of mice, as described by Passos and collaborators (Passos et al., 2013). The left ear was used as control and received the vehicle only (acetone). Etifoxine was administered locally 30 min before PMA application. Ear thickness was measured before PMA application, and every hour for 9 h, and after 24 h, with a micrometer (Mitutoyo, ref 293–145, Japan). Mice were lightly anesthetized with isoflurane before measurements.

2.7. In vitro pharmacology

Experiments were undertaken by Cerep (Celle l'Evescault, France).

Briefly, human COX-1 and COX-2 were expressed in human recombinant Sf9 cells which were submitted to 2–4 µmol arachidonic acid. PGE_2 levels, reflecting COX activity, were measured after a 5 min incubation at room temperature. Measurement was made by fluorimetry (HTRF™ Cisbio), in accordance with Glaser and collaborators (Glaser et al., 1995). IC_{50} values were determined by non-linear regression analysis of the inhibition/concentration-response curves generated with mean replicate values. Results showing an inhibition higher than 50% were considered to represent significant effects.

2.8. Statistical analysis

Statistical analysis was performed using the GraphPad Prism 6 software (LaJolla, CA, USA). Data are expressed as mean ± standard error of the mean (S.E.M.). For each experiment, normal distribution of values was verified with the Shapiro-Wilk normality test before performing parametric analysis. One-way analysis of variance tests (ANOVA) were used for Figs. 1, 2 and 4B. Two-way (treatment x time) ANOVAs, with repeated measures for the time variable (RM twANOVA), were used for Figs. 3 and 4A. Holm-Sidak's post hoc multiple comparison test was used between groups when the main effect was considered to be statistically significant ($P < 0.05$).

3. Results

3.1. Analgesic effects of etifoxine after IP or oral administration

Etifoxine-mediated analgesia was investigated in the carrageenan inflammatory pain model following different protocols of etifoxine administration. Intraplantar carrageenan is known to induce thermal hot hyperalgesia, shown here with Hargreaves' plantar test (Fig. 1). Paw withdrawal latency is of 19.1 ± 0.9 s for control animals and significantly drops to 8.2 ± 0.9 s for carrageenan-injected animals (Fig. 1A; ANOVA: $F_{(4,71)} = 15.22$; $P < 0.0001$; $n = 11–21$). Hyperalgesia is present 2 h after injection and remains after 5 h, therefore all measurements were performed in this time window. IP injection of etifoxine concomitant with carrageenan injection alleviates thermal hot hyperalgesia 2 h after co-administration at a dose of 50 mg/kg, since latency to withdraw is not statistically different between controls and carrageenan-injected animals who received 50 mg/kg etifoxine (16.0 ± 2.4 s). However, etifoxine does not induce analgesia at doses of 10 or 30 mg/kg. Etifoxine administered orally (Fig. 1B) induces analgesia at a dose of 100 mg/kg 2 h after co-administration with carrageenan (ANOVA: $F_{(4,69)} = 16.11$; $P < 0.0001$; $n = 10–21$), but not at 50 mg/kg nor at the higher dose of 150 mg/kg, even though a tendency towards analgesia is observed for the latter.

The anti-inflammatory properties of etifoxine were then assessed when inflammation was already present, through administration of etifoxine 2.5 h after carrageenan injection (Fig. 1C). IP injection of etifoxine at doses of 30 mg/kg or 50 mg/kg induced strong thermal hot analgesia (ANOVA: $F_{(4,55)} = 15.45$; $P < 0.0001$; $n = 12$) 30 min after etifoxine treatment (thus 3 h after carrageenan injection). The smaller dose of 10 mg/kg did not alleviate hyperalgesia.

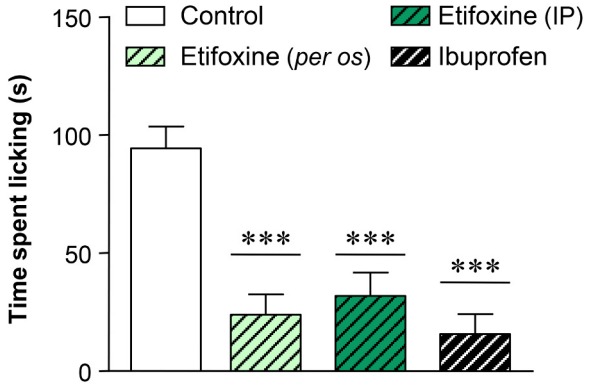

Fig. 2. Effect of etifoxine compared to ibuprofen in the second phase of the formalin test. All drugs were administered 30 min prior to formalin injection, at a dose of 150 mg/kg (etifoxine per os), 50 mg/kg (etifoxine IP injection) and 400 mg/kg (ibuprofen subcutaneous injection). Time spent licking was significantly reduced with the three treatments. Statistical significance was assessed with Holm-Sidak multiple comparison test, illustrated as follows: $P < 0.001$ (***).

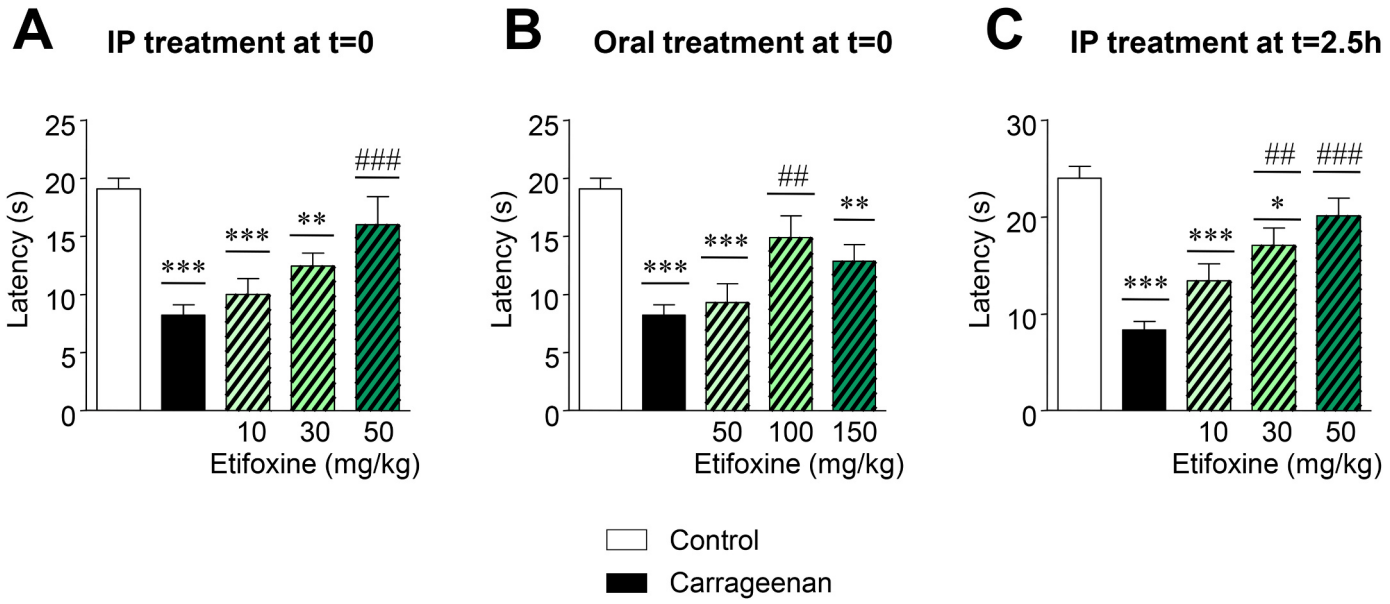

Fig. 1. Etifoxine effect on paw withdrawal latency with the plantar test after carrageenan administration. Etifoxine is administered simultaneously with carrageenan by IP injection at a dose of 10, 30 or 50 mg/kg (**A**; n = 11–21), or *per os* at a dose of 50, 100 or 150 mg/kg (**B**; n = 10–21). 50 mg/kg IP etifoxine and 100 mg/kg oral etifoxine exert an analgesic effect 2 h after co-administration with carrageenan. Etifoxine IP injection 2.5 h after carrageenan administration (**C**; n = 12) produces analgesia at 30 and 50 mg/kg as soon as 30 min after etifoxine injection. Statistical significance was assessed with Holm-Sidak multiple comparison test, illustrated as follows: $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) vs control and $P < 0.01$ (##) or $P < 0.001$ (###) vs carrageenan.

Analgesic properties of etifoxine were then assessed in the late phase of the formalin test and compared with ibuprofen (Fig. 2). Both oral and intraperitoneal etifoxine significantly reduced pain behavior (ANOVA $F_{(3,44)} = 16.11$; $P < 0.0001$), since time spent licking was of 94.3 ± 9.3 s (in non-treated controls; n = 22) and reduced to 23.92 ± 8.62 s (etifoxine *per os*; n = 8) and 31.84 ± 10.04 s (etifoxine IP; n = 8). This reduction in pain behavior was similar to that observed after 400 mg/kg subcutaneous ibuprofen (15.7 ± 8.5 s; n = 10).

3.2. Etifoxine treatment alleviates hyperalgesia resulting from intraplantar carrageenan injection

Considering aforementioned results, etifoxine effect was investigated in the carrageenan inflammatory model after 5 consecutive daily IP injections at a dose of 50 mg/kg. Mechanical sensitivity was assessed using a calibrated forceps (Fig. 3A). Carrageenan injection in the right paw induced a significant decrease in the mechanical threshold (478.9 ± 31.78 g in the control Vehicle-Left group vs 211.4 ± 24.89 g in the Vehicle-Right group), which lasted at least seven days (501.6 ± 13.26 g in the control Vehicle-Left group vs 267.5 ± 32.38 g in the Vehicle-Right group). Etifoxine treatment alleviated the carrageenan-induced mechanical hyperalgesia at day two, and the analgesic effect persisted until day 7 (RM twANOVA, time x treatment: $F_{(3,36)} = 36.62$; $P < 0.0001$; *n = 10*; 267.5 ± 32.38 g in the Vehicle-Right group vs 436.5 ± 45.08 g in the Etifoxine-Right group).

Intraplantar carrageenan also resulted in thermal hot hyperalgesia (Fig. 3B), illustrated by a severe decrease in the withdrawal latency during Hargreaves' plantar test that persisted at least seven days. Etifoxine treatment alleviated carrageenan-induced thermal hot hyperalgesia from day 1 (RM twANOVA, time x treatment: $F_{(3,36)} = 18.19$; $P < 0.0001$; 8.75 ± 1.09 s in the Vehicle-Right group vs 14.34 ± 1.20 s in the Etifoxine-Right group) until day 7 (12.68 ± 1.16 s in the Vehicle-Right group vs 18.1 ± 1.54 s in the Etifoxine-Right group). It is worth noting that the latency withdrawal response of carrageenan-injected treated animals was not significantly different from that of the control animals from day 1 onwards. Etifoxine did not influence neither the mechanical nor the thermal hot threshold of the control left paw.

3.3. Effect of etifoxine on carrageenan-induced edema and plantar surface skin temperature

Intraplantar carrageenan injection drastically increased hind paw surface temperature (Fig. 3C), measured 7 h after injection (RM twANOVA, time x treatment: $F_{(1,9)} = 5.122$; $P = 0.0499$). Etifoxine treatment significantly reduced plantar surface temperature, thermography showing no significant difference between baseline values and etifoxine-treated animals after 24 h.

As expected, carrageenan injection also induced an edema (Fig. 3D), brought out by the increase in paw volume 7 h after injection, both in

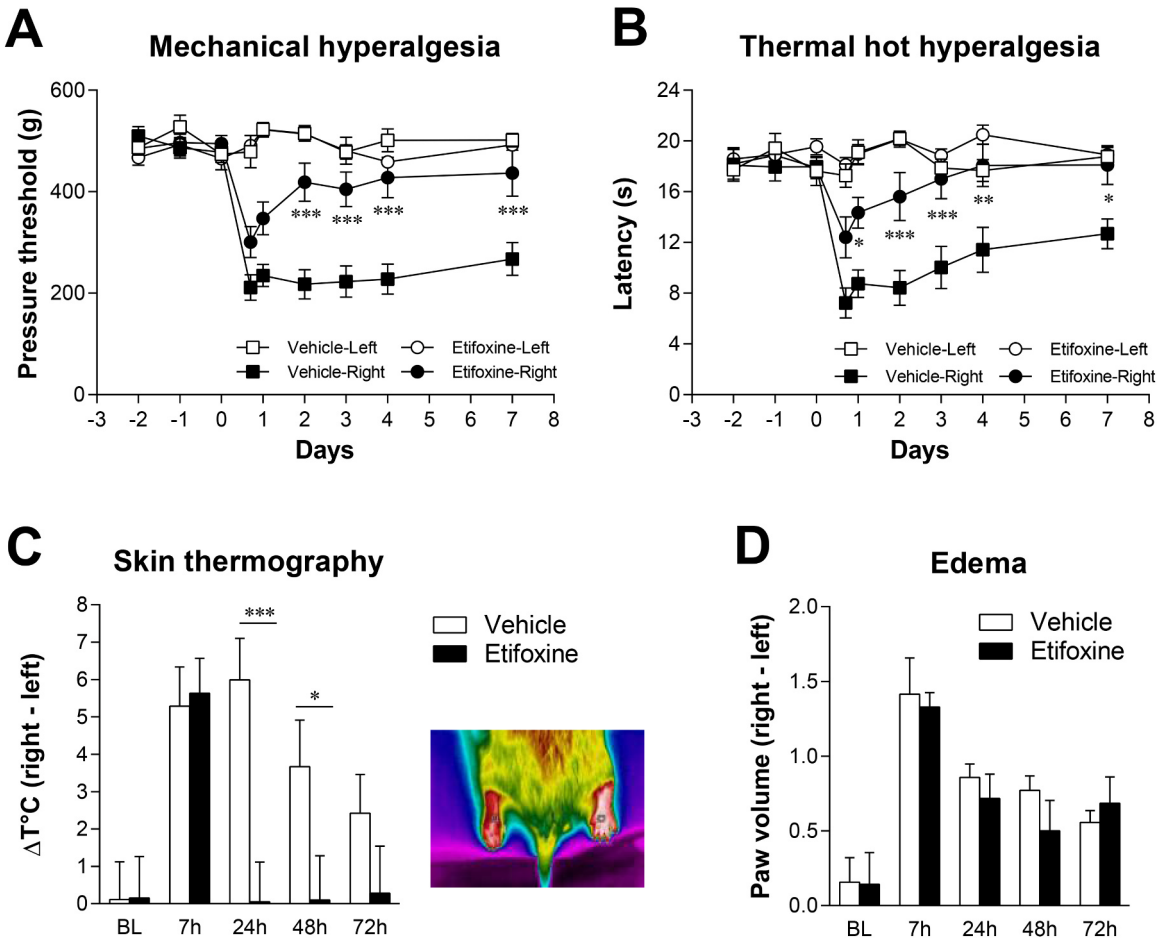

Fig. 3. Effect of 5 consecutive daily injections of 50 mg/kg etifoxine on intraplantar carrageenan inflammation. Etifoxine alleviated mechanical (**A**; n = 10) and thermal hot (**B**; n = 10) hyperalgesia, as illustrated by the increased threshold with the calibrated forceps and latency withdrawal during Hargreaves' test. Etifoxine also reduced hind paw surface skin temperature 24 h after carrageenan injection (**C**; n = 10), but had no effect on edema volume (**D**; n = 7). The region of interest used for thermography measurements is marked as a square on the thermal image. BL stands for baseline measures prior to carrageenan inflammation. Statistical significance was assessed with Holm-Sidak multiple comparison test, illustrated as follows: $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) vs carrageenan (Vehicle-Right).

etifoxine-treated (1.33 ± 0.1 ml, relative to baseline) and vehicle-treated animals (1.41 ± 0.24 ml, relative to baseline). However, despite reducing surface skin temperature, etifoxine had no effect on paw volume during the three days following carrageenan injection (RM twANOVA, time x treatment: $F_{(1,6)} = 0.655$; $P = 0.449$).

3.4. Etifoxine treatment delays PMA-induced ear edema

Considering the lack of efficacy of etifoxine in reducing paw edema in the carrageenan model, the topical anti-inflammatory effect of etifoxine was assessed after PMA-induced ear edema in mice (Fig. 4A). A dose of 2.5 µg of PMA induced a rapid edema that was detected after 2 h and lasted for at least 24 h (RM twANOVA, time x treatment: $F_{(5,70)} = 83.71$; $P < 0.0001$), which is consistent with previous reports (Passos et al., 2013). Topical application of 1 or 2 mg etifoxine alone had little effect on ear thickness. In mice that received PMA, etifoxine administration 30 min prior to PMA application significantly delayed edema progression. Etifoxine's effect was not pronounced at a dose of 1 mg, whereas 2 mg etifoxine application partially reduced ear swelling.

6 h after application, PMA induced a 125% increase in ear thickness (Fig. 4B), from 272 ± 5 µm for the left control ear receiving acetone to 611 ± 29 µm for the right ear receiving PMA. A 2 mg dose of etifoxine elicited a significant 29% decrease of the right ear thickness (ANOVA: $F_{(5,70)} = 76.70$; $P < 0.0001$; n = 10–20).

3.5. Effect of etifoxine on COX activity

Considering aforementioned results, we used in vitro pharmacology to test the effect of etifoxine on COX activity. We failed to reveal that etifoxine had direct binding on COX-2 ($IC_{50} > 100$ µM with ≈ 25% inhibition at 100 µM), but etifoxine successfully inhibited COX-1 activity. Indeed, the production of PGE_2 was significantly inhibited with a mean IC_{50} value of 81.6 ± 8.5 µM, averaged from three independent assays.

4. Discussion

This study provides new data highlighting the anti-inflammatory properties of etifoxine. Intraperitoneal administration of etifoxine at a dose of 50 mg/kg successfully alleviated carrageenan-induced thermal hot hyperalgesia, both when administered before the onset of inflammation and when inflammation was already present. This result confirms the anti-hyperalgesic effect of etifoxine shown in other models (Aouad et al., 2009, 2014a, 2014b). This therapeutic dose was therefore used for posterior experiments, from which we reported etifoxine-mediated temperature reduction of hind paw plantar surface after carrageenan administration. However, etifoxine did not reduce edema volume during the three days following carrageenan injection. Interestingly, etifoxine had a moderate anti-edemic effect in the mouse model of ear inflammation. This topical effect needs to be further evaluated in other models of local inflammation.

Corroborating our data, Daugherty and collaborators demonstrated

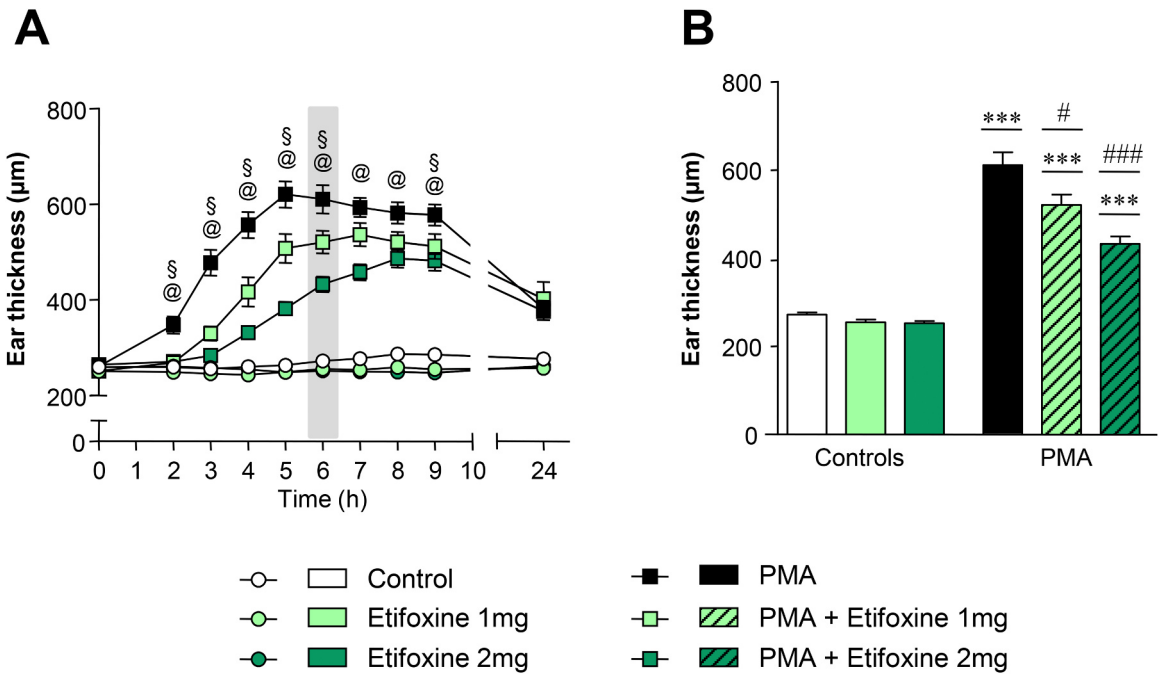

Fig. 4. Effect of etifoxine on ear thickness after PMA-induced edema. **(A)** Ear thickness was measured every hour for 9 h and 24 h after PMA application alone (n = 16), following etifoxine application (n = 10 for each dose), or etifoxine application alone (n = 10 for each dose). The left ear was used as control (n = 16). Etifoxine slowed down PMA-induced edema progression, as shown by the delayed ear thickening. For clarity, the same code was used to show statistical differences for $P < 0.05$ and below, by comparing PMA and PMA + etifoxine 1 mg (§) or PMA and PMA + etifoxine 2 mg (@). **(B)** 6 h after PMA application, when its effect is maximal, etifoxine significantly reduced ear thickness. Statistical significance was assessed with Holm-Sidak multiple comparison test, illustrated as follows: $P < 0.001$ (***) vs control and $P < 0.05$ (#) or $P < 0.001$ (###) vs PMA.

the benefits of etifoxine in an experimental model of multiple sclerosis (Daugherty et al., 2013), in which treatment led to a decrease in immune cell infiltration in the spinal cord. Etifoxine also reduced proinflammatory cytokine production after lesion of the rat sciatic nerve (Girard et al., 2012), and reduced cortical levels of proinflammatory cytokines as well as astrogliosis and microglial activation after traumatic brain injury (Simon-O'Brien et al., 2016). A recent study also revealed the reduced perihematomal brain edema and production of proinflammatory factors mediated by etifoxine treatment in a model of intracerebral hemorrhage (Li et al., 2017). Taken together, these studies suggest anti-inflammatory properties of etifoxine, which contribute to the limitation of pain symptoms.

Etifoxine is known to act as a positive allosteric modulator of the $GABA_A$ receptor and to potentiate 3α-reduced neurosteroid production after binding to the mitochondrial translocator protein complexes TSPO. However, the mechanism of action of etifoxine relative to its anti-inflammatory potential remains to be elucidated. Since some neurosteroids such as progesterone, as well as their metabolites, demonstrate anti-inflammatory properties, the analgesic and slight anti-edemic effect of etifoxine could be mediated by the production of 3α-reduced compounds (Giatti et al., 2012). For instance, in models of intracerebral hemorrhage, progesterone exerted a neuroprotective effect in male and ovariectomized mice, as revealed by a decrease in brain edema and improved neurobehavioral recovery, which was mediated in part by the modulation of the inflammatory response (Jiang et al., 2016; Lei et al., 2016). Another hypothesis would take into account the interaction between etifoxine and COX-1 activity demonstrated in this study. Although our result supports the moderate effect of etifoxine in acute inflammation, inhibiting COX-1 activity cannot account for the beneficial effect of etifoxine in persistent inflammation, which may involve a plethora of pathways. Indeed, it has been shown that COX-1 inhibitor intrathecal treatment does not alleviate pain symptoms consecutive to peripheral inflammation, although the same study also showed a reduction of carrageenan-induced thermal hyperalgesia after oral administration of a COX-1 inhibitor (Yaksh et al., 2001). Nonetheless, COX-1, but not COX-2, inhibitors have been shown to reduce paw incision-induced mechanical hypersensitivity (Zhu et al., 2003). This result is in line with the reduction of thermal and mechanical hypersensitivity observed after intrathecal ketorolac (COX-1 preferring antagonist) administration in models of neuropathic pain (Ma et al., 2002; Parris et al., 1996), supporting a role for COX-1 in some pain processing mechanisms.

We have provided evidence indicating the moderate topical anti-inflammatory action of etifoxine on mouse ear inflammation, its preventive and curative properties in the carrageenan model as well as its beneficial effect in the late phase of the formalin test. Taken together, our data suggest that etifoxine may be a good candidate for clinical studies on the prevention of inflammatory-driven edema and hyperalgesia in humans.

CRediT authorship contribution statement

Géraldine Gazzo: Writing - original draft, Writing - review & editing, Visualization, Formal Analysis. **Philippe Girard:** Investigation, Formal analysis, Methodology, Writing - review & editing. **Nisrine Kamoun:** Investigation. **Marc Verleye:** Conceptualization, Project administration, Writing - review & editing. **Pierrick Poisbeau:** Supervision, Resources, Writing - review & editing.

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Declaration of interest

PP and NK received funding from Biocodex to study the mechanism of action of etifoxine. PG and MV are researchers from Biocodex department of pharmacology.

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