Reduction and prevention of vincristine-induced neuropathic pain symptoms by the non-benzodiazepine anxiolytic etifoxine are mediated by 3α-reduced neurosteroids

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ABSTRACT

The central processing of peripheral nociceptive messages is highly controlled by the activity of local inhibitory networks in the spinal cord and supraspinal centers. Recently, it has been shown that endogenous 3α-reduced neurosteroids (3αNS) exert a significant spinal antinociception by potentiating GABA$_A$ receptor function. Because endogenous 3αNS can be produced in many relay structures of the nociceptive system, we tested the potential analgesic efficacy of promoting the production of neurosteroids by using etifoxine (ETX, 50 mg/kg i.p.). This prescribed non-benzodiazepine anxiolytic was shown previously to stimulate neurosteroidogenesis in its early step after binding to the mitochondrial translocator protein complex (TSPO). Using an animal model of generalized neuropathic pain resulting from a 2-week treatment with the antitumoral agent vincristine sulfate (VCR, 0.1 mg/kg i.p.), we show that injections of ETX (50 mg/kg i.p.) given every day reduced the VCR-induced mechanical and thermal pain symptoms but also prevented their appearance, if used in prophylaxia 1 week before VCR. Both the curative and preventive effects of ETX on pain symptoms were mediated by the production of 3αNS as demonstrated in animals treated with the enzymatic inhibitor provera (6-medroxyprogesterone acetate; 20 mg/kg s.c.). Altogether, this study shows for the first time that promoting 3αNS could be a possible therapeutic strategy to treat neuropathic pain symptoms. Since ETX is already available as an anxiolytic, its use in humans, provided that its analgesic properties are confirmed, could be rapidly considered.

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1. Introduction

The control of neuronal excitability in the nociceptive system has a major impact on pain perception and expression. Fast GABAergic inhibition plays an important role in this process. For example, intrathecal perfusion of the GABA$_A$ receptor (GABA$_A$R) antagonist bicuculline is known to be responsible for the appearance of pain symptoms [6,15,30]. Using animal models of pathological pain, plastic alterations of these inhibitory controls have been identified [8,10] and may be the substrate to explain the severe neuropathic pain symptoms expressed in response to mechanical and cold stimulations. The protection or amplification of GABA$_A$R channel function might therefore be a good strategy in pathological (e.g. neuropathic) pain situations [10]. This could be achieved by using positive allosteric modulators of GABA$_A$Rs, such as 3α-reduced neuroactive steroids (3αNS) which are particularly potent to reduce pain symptoms [6,12,25]. Apart from the design of exogenous modulators targeting specific GABA$_A$Rs within the nociceptive system [19], there are growing lines of evidence indicating that the antinociceptive properties of local endogenous neuroactive neurosteroids could be used as an alternative strategy [28]. Neurosteroids are initially synthesized from cholesterol in the mitochondria and further converted into active metabolites upon the action of various enzymes which are widely expressed in the central and peripheral nervous systems [7,21]. For example, synthesis of the extremely potent GABA$_A$R modulator allopregnanolone is achieved by successive reductions of cholesterol-derived neuroprogesterone by a 5α reductase (5αR) and a 3α-hydroxysteroid oxydoreductase (3αHSOR). The presence of allopregnanolone-synthesizing enzymes in the spinal cord has been well characterized and our laboratory has demonstrated the functional importance of the local endogenous production of allopregnanolone-like neurosteroids (also referred to as 3αNS below) during the postnatal development of inhibitory synaptic controls [18] and in tonic inflammatory pain situations [27]. This spinal antinociceptive control, which relies on an increase of fast GABAergic inhibition by the concomitant local production of 3αNS, was further extended recently in a neuropathic pain model [22].

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To evaluate the antinociceptive efficacy of promoting the production of endogenous 3αNS, we chose to use etifoxine (ETX), a well-characterized non-benzodiazepine anxiolytic [29]. ETX exerts a positive allosteric modulation of GABA$_A$R function after binding to β subunits, a direct effect rapidly reversed upon removal of the drug. ETX also potentiates the longer lasting production of 3αNS after binding to a translocator protein complex (TSPO; previously called peripheral benzodiazepine receptor) of the outer mitochondrial membrane which initiates neurosteroidogenesis by facilitating the incorporation of cholesterol [23]. This indirect effect leads to a prolonged increase in synaptic and extrasynaptic inhibition mediated by GABA$_A$Rs.

In the present work, we aimed at evaluating the possible use of ETX in both curative and/or preventive treatments in the vincristine sulfate (VCR) model of chemotherapy-induced neuropathy in rats [1,38]. Similar to what is seen in humans, VCR treatment is associated with the appearance of pathological pain symptoms such as mechanical and thermal cold hyperalgesia/allodynia. We characterized the effect of ETX on these long-lasting symptoms and identified its mechanism of action.

2. Materials and methods

2.1. Animals

In the present study, Sprague–Dawley rats (250–300 g; Janvier, Le Genest St. Isle, France) were housed in groups of four (cage size in cm: $L\ 40 \times l\ 40 \times h\ 20$) under standard conditions (room temperature: 22 °C; 12/12 h light/dark cycle) with *ad libitum* access to food and water. This work was done exclusively with adult male rats to avoid any sex-specific bias due to differences in hormonal status which are known to affect neurosteroidogenesis. All animals were habituated to the room and to the tests at least 1 week before starting the experiments. All procedures were performed in accordance with the recommendations of the European Committee Council Direction of November 24, 1986 (86/609/EEC) and received authorization from the French Department of Agriculture (licence number 67-116 to PP) and from the regional ethic committee (CREMEAS AL/12/15/03/07).

2.2. Behavioral testing

2.2.1. Mechanical hyperalgesia
Mechanical sensitivity was measured using a calibrated forceps (Bioseb, Vitrolles, France) previously validated in our laboratory [20]. Briefly, the habituated rat was loosely restrained with a towel masking the eyes in order to limit stress by environmental stimulations. The tips of the forceps were placed at each side of the paw and a gradually increasing force was applied. The pressure producing withdrawal of the paw, or in some rare cases a vocalization of the animal, corresponded to the nociceptive threshold value. This manipulation was performed three times for each hindpaw and the values were averaged.

2.2.2. Thermal cold allodynia
Thermal cold allodynia was characterized by scoring the aversive behaviors of rats using the acetone test [11]. Rats were placed on a wire mesh delimited by a Plexiglas cage and allowed to accommodate for at least 15 min. A drop of acetone was then placed on the ventral side of the hindpaw producing a non-noxious decrease in temperature. The rat behavioral response was scored during 20 s following acetone application as follows: 0, no response of the animal; 1, quick withdrawal, flick or stamp of the paw; 2, prolonged withdrawal or repeated flicking of the paw; and 3, repeated flicking of the paw with licking of the paw. The

manipulation was performed three times for each paw and the values were added (maximal score: 18).

2.3. Drugs

Vincristine sulfate (VCR: Sigma, St. Louis, MO, USA) was dissolved in saline (NaCl 0.9% in distilled water) and stored at 4 °C at a stock concentration of 1 mg/l. VCR was injected i.p. at a final concentration of 0.1 mg/kg/day (volume injected: 1 ml/kg) during two cycles of five consecutive working days (i.e. days 1–5 and days 7–11 with 2 days off) as previously described [38]. Etifoxine (2-ethylamino-6-chloro-4-methyl-4-phenyl-4*H*-3,1-benzoxazine hydrochloride; Stresam™, Biocodex, Gentilly, France) was prepared in saline (NaCl 0.9% in distilled water) containing 1% Tween 80 (v/v; Sigma, St. Louis, MO, USA) and injected i.p. (50 mg/kg in a final volume of 1 ml/kg). We chose this concentration for the following reasons: 50 mg/kg treatment was used to characterize the synthesis of 3αNS in male rat brain [35]. At this concentration, it has been shown in vitro to potentiate GABA$_A$ receptor function through neurosteroid action [29] and to have anxiolytic properties in vivo. To study the effect of etifoxine on pain symptoms, five injections were given every day during the week following vincristine treatment (i.e. days 14–18). Prophylactic ETX treatment started 1 week before the vincristine treatment and consisted of five i.p. injections with 2 days off every week. Provera (6-medroxyprogesterone acetate; Steraloid, London, England) is an inhibitor of the 3α-hydroxysteroid oxydoreductase (3αHSOR), the enzyme responsible for the production of the 3α-reduced neuroactive steroids (3αNS) [16,26]. Provera was suspended in olive oil (20 mg/ml) and injected s.c. in the subscapular area at a dose of 20 mg/kg. In every experiment, control animals received an equivalent volume of vehicle.

2.4. Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). One-, two- or three-way repeated-measures analysis of variance (ANOVA) and Tukey's post hoc multiple comparison tests, performed with Statistica (StatSoft, Tulsa, OK, USA), were used to analyze the effects on mechanical sensitivity of treatments (VCR, ETX, provera; *between* factors) and of time (*within* factor). To analyze the ordinal data from the acetone scoring test, non-parametric tests (Friedman test followed by Steel test, or Wilcoxon test) were used (KyPlot, KyensLab, Tokyo, Japan); scores were also expressed as means ± SEMs in figures for a better readability of graphs. Differences were considered to be statistically significant for $P < 0.05$. Significance codes: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

3. Results

The effects of ETX were characterized in the rat model of generalized neuropathic pain following a 2-week treatment with vincristine sulfate (VCR). Neuropathic pain symptoms (hyperalgesia/allodynia) induced by VCR treatment were seen as a reduction in the mechanical nociceptive thresholds sufficient to induce a pain reflex and by an increase in the behavioral score to non-nociceptive paw application of acetone (Fig. 1, circles). During the course of the VCR treatment (Fig. 1, grey period), a statistically significant mechanical hyperalgesia ($P = 0.000025$; comparison with initial basal value) was seen in VCR-treated rats (Fig. 1A1 and B1, circles) from day 3 of treatment. In VCR-treated animals that did not receive the ETX treatment (open circles), mechanical hyperalgesia reached its maximum at the end of the second week of treatment (between day 8 and day 20) and gradually returned to baseline 35–42 days after the beginning of the treatment. The time course of cold allodynia (Fig. 1A2 and B2) was similar to that of mechan-
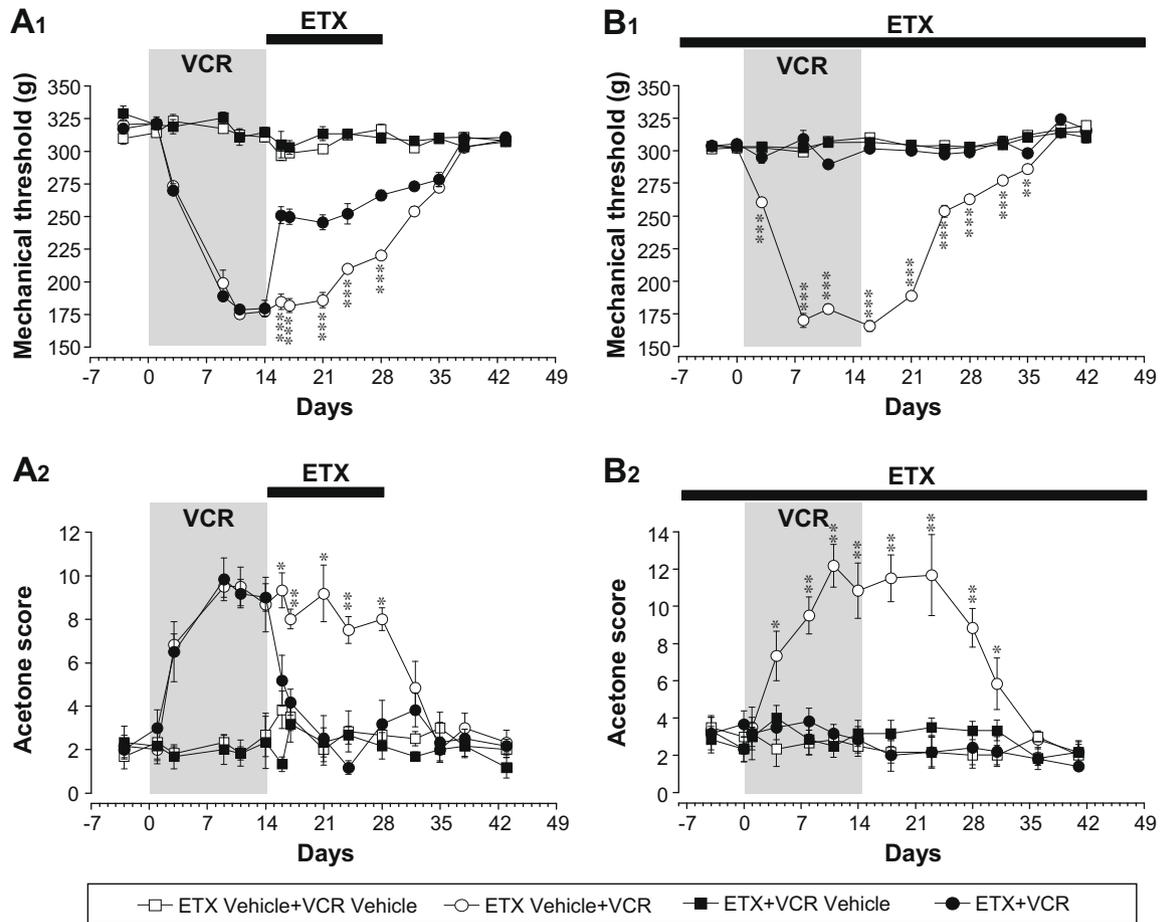
Fig. 1. Time course of mechanical thresholds (A1 and B1) and behavioral scores following acetone hindpaw application (A2 and B2) of rats ($n = 6$ animals per group) treated with 10 injections of vincristine sulfate (VCR; grey period; circles) or VCR vehicle (squares) every day. Etifoxine (ETX, black symbols) or ETX vehicle (open symbols) was administered during 5 consecutive days (from day 14 to 18) for the curative treatment (A1 and A2) and over 5 weeks for the prophylactic treatment (B1 and B2) as indicated by the black bars. (A1) Three-way repeated-measures ANOVA on mechanical sensitivity showed a significant $ETX \times VCR \times time$ effect ($F_{14,280} = 11.02$, $P < 0.0001$). From day 16 to day 28, threshold values were significantly larger ($P = 0.000025$; *) in ETX-treated animals than in ETX vehicle-treated animals, though significantly smaller than in VCR vehicle controls. (A2) VCR treatment also induced significant changes (Friedman test) with time in the acetone score for animals further treated with ETX vehicle ($\chi^2(14) = 62.23$, $P < 0.0001$) or with ETX ($\chi^2(14) = 56.68$, $P < 0.0001$). No such change was observed in VCR vehicle controls, whether treated with ETX ($\chi^2(14) = 12.14$, $P = 0.595$) or with ETX vehicle ($\chi^2(14) = 13.73$, $P = 0.470$). ETX- and ETX vehicle-treated allodynic animals had significantly different scores (*, Wilcoxon test) from day 16 to day 28. (B1) In the preventive ETX treatment experiment, three-way repeated-measures ANOVA revealed a significant $ETX \times VCR \times time$ effect ($F_{12,240} = 40.65$, $P < 0.0001$) on mechanical sensitivity threshold. The same significant decrease of threshold as in A1 was observed in VCR-treated animals not treated with ETX from day 3 to day 35 (*, comparison with initial value). No significant change with time was displayed by the three other groups, i.e. ETX treatment totally prevented VCR-induced hypersensitivity. (B2) Significant changes of the acetone score with time were observed only for VCR-treated animals without preventive ETX treatment (Friedman, $\chi^2(12) = 59.32$, $P < 0.0001$). ETX vehicle-treated animals (open circles) had significantly higher scores than ETX-treated ones (black circle) from day 4 to day 31 (*, Wilcoxon test). Significance code: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

ical hyperalgesia. The emergence of nociceptive behaviors to non-noxious cold stimulation was rapidly seen after 3 days of VCR treatment (circles). It rapidly reached a maximum value during the second week of treatment before coming back to a baseline behavioral score between day 28 (Fig. 1 A2) and day 31 (Fig. 1 B2). For control animals, treated with the vehicle of VCR (open squares), no significant change in mechanical or thermal responses occurred with time and baseline values were retained during the 7 weeks of experiment, whether rats received ETX curative treatment or ETX vehicle.

3.1. Curative and preventive effects of etifoxine on mechanical hyperalgesia and cold allodynia

In a first experiment, analgesic effects of etifoxine (ETX) were characterized in neuropathic rats, i.e. after the 10 days of VCR treatment (Fig. 1A1 and A2, circles). While testing VCR-treated animals (black circles) before every i.p. injection of ETX (days 14–18) given every day, we observed a significant and persistent reduction

(~50–60%) in mechanical hyperalgesia (Fig. 1A1, black circles) starting on day 16, i.e. after 2 days of ETX treatment, whereas VCR-treated animals receiving the vehicle of ETX (open circles) remained hyperalgesic for more than 2 weeks. ETX analgesia persisted until day 28 despite stopping the ETX treatment on day 18. In comparison to VCR-treated rats receiving the vehicle of ETX (Fig. 1A2, open circles), a persistent reduction in nociceptive behavioral score to cold acetone stimulation was also seen 2 days after starting the ETX treatment (Fig. 1A2, black circles). Moreover, VCR-treated animals receiving ETX were undistinguishable from those having received the vehicle of VCR (open and black squares) after the five injections of ETX and until the end of the experiment (up to 49 days).

In a second set of experiments, we evaluated the interest of using ETX to prevent the appearance of VCR-induced hyperalgesia/allodynia (Fig. 1B1 and B2). ETX (50 mg/kg) or its vehicle was administered to animals ($n = 6$ rats per group) a week before VCR chemotherapy and was maintained for the following six weeks. No differences in basal mechanical nociception and thermal cold

behavioral score to acetone were observed between rats treated with ETX (black symbols) or its vehicle (open symbols) before VCR treatment (from day −7 to day 0). Interestingly, we failed to observe any sign of mechanical hyperalgesia or cold allodynia in ETX-treated animals during or after VCR treatment (Fig. 1B1 and B2, black circles) while these symptoms were systematically present in rats injected with the vehicle of ETX (Fig. 1B1 and B2, open circles).

Altogether, these results strongly suggest that ETX possesses interesting analgesic properties and might be used to prevent the development of neuropathic pain symptoms induced by vincristine sulfate.

3.2. Mode of action of etifoxine

With respect to the previous characterization of ETX mode of action and to the long-lasting effects seen in this study, we checked whether ETX action was achieved via an increased production of 3α-reduced neuroactive steroids (3αNS). To test this hypothesis, animals were treated with provera, an inhibitor of 3αHSOR, the enzyme which synthesizes 3αNS. In the following experiments all groups were treated with VCR (Figs. 2 and 3).

Focusing our attention first on the development of pain symptoms during the VCR treatment (Fig. 2A, $n = 12$ per group), we found that provera-treated animals (squares), which cannot synthesize 3αNS, exhibited more intense pain symptoms. In comparison with rats possessing an intact neurosteroidogenesis, mechanical thresholds (Fig. 2, A1) were significantly lower from the second day of VCR treatment (day 15) and throughout the treatment period. Animals treated with provera also displayed a significantly higher behavioral score following acetone stimulation (Fig. 2 A2) as compared to the control group. This first observation suggested that endogenous 3αNS are produced in significant amounts during the development of hyperalgesia/allodynia induced by vincristine and that they indeed limit the intensity of painful symptoms.

In the following experiments, the two groups of animals, treated or not with provera, were subdivided ($n = 6$ per group) to study the effect of ETX (or its vehicle). In animals producing 3αNS (Control: not treated with provera; Fig. 2B, graphs on the left-hand side), effects similar to those described in Fig. 1 were observed. ETX (50 mg/kg, i.p.; black symbols) still reduced mechanical hyperalgesia (Fig. 2 B1 left; ANOVA $ETX \times time$, $F_{22,220} = 8.52$, $P < 0.0001$) and cold allodynia (Fig. 2B2, left). In sharp contrast, while testing provera-treated animals (Fig. 2B, right graphs) no effect of ETX was noted on mechanical thresholds (ANOVA, $F_{1,10} = 0.02$, $P = 0.892$) and on acetone behavioral scores after day 14 (Friedman, $\chi^2(13) = 12.05$, $P = 0.524$ for ETX-treated and $\chi^2(13) = 8.36$, $P = 0.819$ for vehicle ETX-treated). This strongly suggested that the anti-hyperalgesic/allodynic effects of etifoxine were due to an increased production of 3αNS.
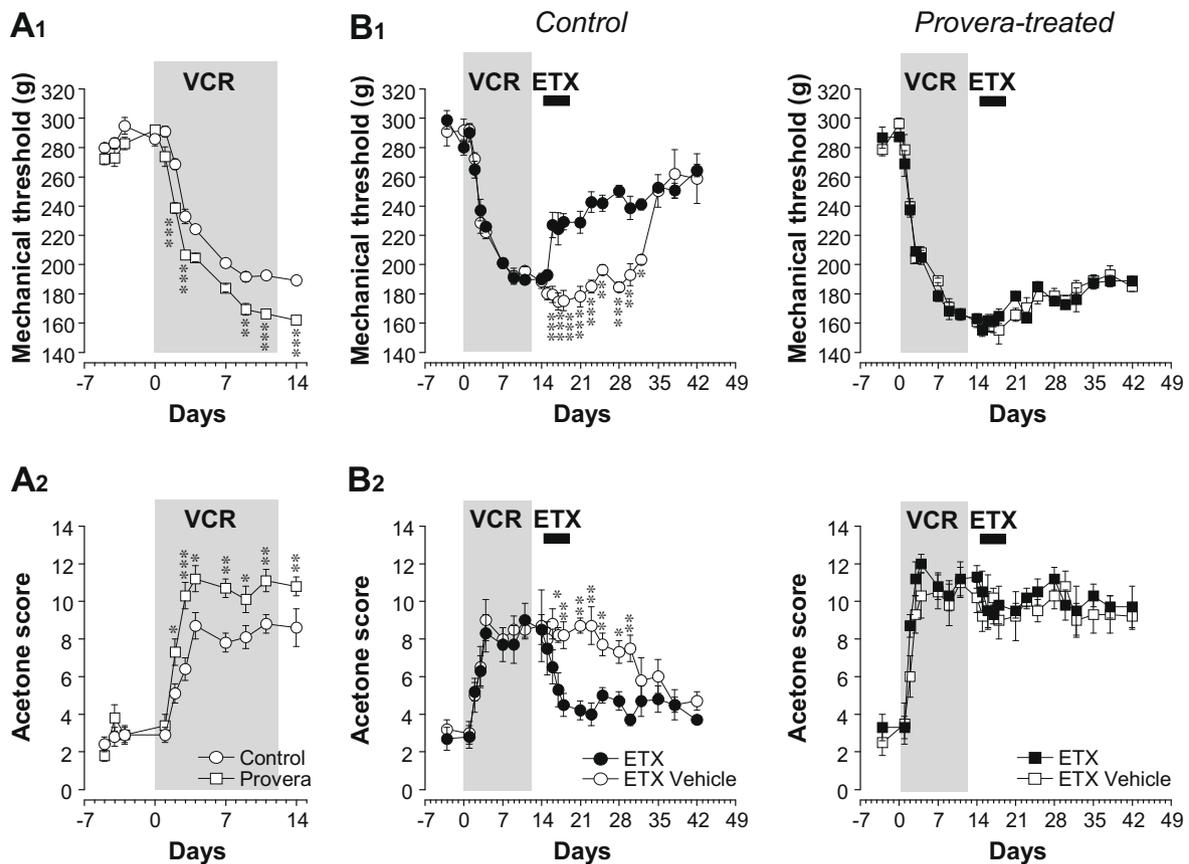

Fig. 2. Onset of VCR-induced neuropathy in provera-treated animals and efficacy of curative ETX treatment. (A) Onset of mechanical hyperalgesia (A1) and thermal cold allodynia (A2) in VCR-treated animals ($n = 12$ per group) with intact endogenous production of 3αNS (Control; s.c. olive oil injections were given every day starting from day −7; circles) or not (s.c. injection of provera starting from day −7; squares). A1. Two-way repeated-measures ANOVA on mechanical sensitivity showed a significant effect of provera on the VCR-induced decrease of mechanical threshold ($provera \times time$, $F_{11,242} = 4.67$, $P < 0.0001$). A2. The acetone score was similarly significantly increased in the presence of provera, as soon as day 2 (Wilcoxon test, $P = 0.0168$). (B) Changes in hyperalgesic mechanical thresholds (B1) and allodynic behavioral scores to acetone (B2) during and after a 5- day treatment with ETX (50 mg/kg; black symbols) or its vehicle (open symbols) in VCR-treated rats ($n = 6$ per group). *Graphs on the left-hand side:* data from rats with intact endogenous production of 3αNS (Control; s.c. olive oil injections given every day from day −7 to day 49; circles) *Graphson the right-hand side:* data from rats treated with the 3αHSOR inhibitor provera (s.c. injection from day −7 to day 49; squares). Significance code: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
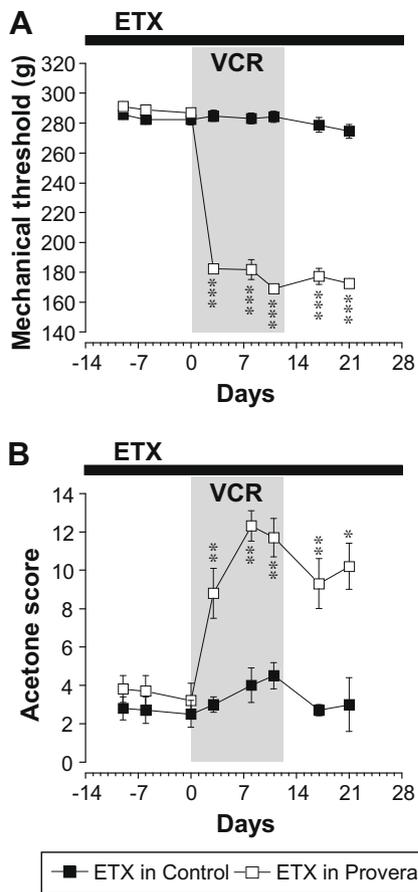
Fig. 3. ETX stimulation of 3αNS synthesis prevents the appearance of VCR-induced pain symptoms. ETX changes in mechanical thresholds (A) and behavioral scores to non-noxious cold stimulation (B) before, during and after VCR treatment (grey period). Rats (n = 6 per group) received a prophylactic etifoxine treatment (from day −7 to day 28), with intact production of 3αNS (black squares; s.c. injection of olive oil every day from day −14 to day 28) or not (open squares; s.c. injections of provera every day from day −14 to day 28). Provera-treated animals (open squares) rapidly exhibited VCR-induced pain symptoms that were absent in those receiving the vehicle of provera (black squares) both in mechanical (A; ANOVA provera × time, $F_{7,70}$ = 138.7, $P < 0.0001$) and in thermal tests (B; Friedman, $\chi^2(7)$ = 8.0, P = 0.333 for controls and $\chi^2(7)$ = 34.76, $P < 0.0001$ for provera). Significance code: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Eventually, we also characterized the efficacy of a preventive ETX treatment in provera-treated animals to test whether the production of 3αNS was also involved in its preventive effect. As illustrated in Fig. 3 and in agreement with our previous finding (Fig. 1B), animals with intact neurosteroidogenesis (black symbols) and receiving a preventive ETX treatment did not show any sign of mechanical hyperalgesia (Fig. 3A) or cold allodynia (Fig. 3B) during or after VCR treatment. This was not the case for provera-treated animals receiving ETX and submitted to VCR chemotherapy (open symbols), which rapidly developed abnormal low mechanical thresholds and elevated behavioral scores to acetone. In addition to the curative effect seen above, these results indicated that ETX prevention of neuropathic pain symptoms was mediated by the stimulated production of 3αNS.

4. Discussion

In this work, we showed that the non-benzodiazepine anxiolytic ETX has strong anti-hyperalgesic and anti-allodynic properties. Within 2 days of treatment, ETX reduced already installed chemotherapy-induced neuropathic pain symptoms but also prevented their apparition. We also showed that this effect is mediated by the production of allopregnanolone-like steroids as demonstrated with provera-treated animals.

ETX is presently prescribed as an anxiolytic and its mechanisms of action have been previously well described. Several studies have shown that this molecule interacts with the GABA$_A$ receptor (GABA$_A$R) by binding preferentially to β subunits [14], which leads to the potentiation of GABAergic inhibition [29,37]. A significant part of its anxiolytic effect [3,36] is also mediated through its binding to the TSPO, which initiates the synthesis of endogenous neurosteroids [34,35], including allopregnanolone-like steroids, the most potent allosteric modulators of GABA$_A$Rs [2]. At a reference dose of 50 mg/kg, plasmatic and total brain allopregnanolone concentrations were 2–4 times more important 1 h after i.p. injection in naïve male rats [35]. Because the long-lasting analgesic effect we observed required at least 2 days for being statistically significant, it is unlikely that ETX exerted its effect directly by potentiating GABA$_A$R function. Similar to what was seen for ETX anxiolysis [34,36], we favoured the hypothesis of a steroid-mediated action. Moreover, there is no known antagonist for the ETX binding site on GABA$_A$Rs (or for the neurosteroid binding site), which makes the direct effect hypothesis difficult to be tested experimentally. In allodynic animals, we found that the analgesic effect of ETX was mediated by the production of 3αNS because ETX was ineffective in provera-treated animals. A similar conclusion was reached while examining the mechanism of ETX prevention of VCR-induced neuropathic pain.

Multiple sources of 3αNS could account for the observed anti-hyperalgesic/allodynic effects of ETX, but at this stage it is difficult to define whether ETX action is mediated by an increased gonadal neurosteroidogenesis or by the local production of steroidogenic cells at various levels of the nervous system processing nociceptive inputs. Indeed, ETX treatment is associated with massive increases of endogenous allopregnanolone in brain and plasma [35]. We cannot exclude at this point a supraspinal action affecting the emotional status (i.e. anxiolysis), which is known to modulate pain processing and expression [9]. It is also well documented now that endogenous spinal 3αNS significantly reduce mechanical or thermal nociception in inflammatory [27] or neuropathic pain situations [22]. A recent study has also demonstrated the efficacy of an ETX-based neuroprotective strategy after cryolesion of the rat sciatic nerve [13]. Although this study does not report changes in nociceptive thresholds, ETX did improve the regeneration of peripheral axons and accelerated the functional recovery of sensorimotor reflexes. It is then tempting to speculate that ETX antinociception might primarily act on peripheral nerves since they are morphologically and functionally affected by VCR treatment. VCR-associated disorganization of microtubule assembly [31] is accompanied by an alteration of anterograde and retrograde transports along axons [33] and by abnormal electrophysiological properties [5,32] of the nociceptive afferent fibers. In line with our results, a mitochondrial dysfunction in neuropathic pain states has been suggested in two recent studies. First, a newly developed TSPO ligand, TRO19622, significantly improved peripheral nerve conduction and reduced tactile allodynia in the VCR model and in the streptozocin model of diabetic neuropathy [4]. Second, prophylactic treatment with acetyl-L-carnitine, which plays an essential role in transporting long-chain free fatty acids into mitochondria, was recently shown to prevent mechanical pain symptoms in the paclitaxel model of chemotherapy-induced neuropathy [17].

The present study strengthens the idea that protecting mitochondrial functions might be a good therapeutical strategy to limit or prevent pathological pain states. We acknowledge that the mechanism of action of 3αNS, over-produced following ETX action, needs to be better clarified. In particular, further studies are

needed to identify the contribution of ETX-induced stimulation of 3αNS in the potentiation of GABA$_A$R function with respect to its anti-hyperalgesic/allodynic properties [28]. Alternatively, it is possible that the stimulation of neurosteroidogenesis by ETX may also involve a reduction in neuronal excitability in nociceptive pathways by inhibiting transient type voltage-gated calcium channels responsible for the central release of glutamate by primary afferent fibers [24].

In summary, ETX has already shown its neuroprotective efficacy after nerve lesion [13]. Our present study suggests that the preventive and curative ETX actions on neuropathic pain symptoms are mediated by a TSPO-dependent production of allopregnanolone-like steroids (3αNS). Because ETX is already used as a non-benzodiazepine anxiolytic and displays limited side-effects, the encouraging results of the present work suggest that this drug has the clinical potential to be rapidly evaluated for pathological pain states in human.

Disclosure/conflict of interest

The authors state that they have no disclosures or conflict of interest.

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