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Fast non-genomic effects of progesterone-derived neurosteroids on nociceptive thresholds and pain symptoms

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Abstract

Fast Inhibitory controls mediated by glycine (GlyRs) and GABAA receptors (GABAARs) play an important role to prevent the apparition of pathological pain symptoms of allodynia and hyperalgesia. The use of positive allosteric modulators of these receptors, specifically expressed in the spinal cord, may represent an interesting strategy to limit or block pain expression. In this study, we have used stereoisomers of progesterone metabolites, acting only via non-genomic effects, in order to evaluate the contribution of GlyRs and GABAARs for the reduction of mechanical and thermal heat hypernociception. We show that 3α neurosteroids were particularly efficient to elevate nociceptive thresholds in naive animal. It also reduced mechanical allodynia and thermal heat hyperalgesia in the carrageenan model of inflammatory pain. This effect is likely to be mediated by GABAA receptors since 3β isomer was inefficient. More interestingly, 3α5β neurosteroid was only efficient on mechanical allodynia while having no effect on thermal heat hyperalgesia. We characterized these paradoxical effects of 3α5β neurosteroid using the strychnine and bicuculline models of allodynia. We clearly show that 3α5β neurosteroid exerts an antinociceptive effect via a positive allosteric modulation of GABAARs but, at the same time, is pronociceptive by reducing GlyR function. This illustrates the importance of the inhibitory amino acid receptor channels and their allosteric modulators in spinal pain processing. Moreover, our results indicate that neurosteroids, which are synthesized in the dorsal horn of the spinal cord and have limited side effects, may be of significant interest in order to treat pathological pain symptoms.

Keywords: Spinal cord; Neurosteroid; Progesterone; Allopregnanolone; Mechanical allodynia; Thermal heat hyperalgesia; GABAA receptor; Glycine receptor; Carrageenan; Inflammatory pain; Spinal inhibition

1. Introduction

Neuroactive steroids might be of significant interest to treat severe neuropathologies [10,11,30] associated with neuronal hyperexcitability. Non-genomic rapid effects of neurosteroids are particularly efficient while using allopregnanolone (3α5αTHP: 3α,5α-tetrahydro-progesterone) or pregnanolone (3α5βTHP: 3α,5β-tetrahydro-progesterone), two metabolites of progesterone which acts at nanomolar concentration on GABAA receptors (GABAARs) and potentiate their inhibitory function in the central nervous system [16,35]. Interestingly, changes in the level of endogenous 3α-neurosteroids have been reported in many physiological [3,21,24,26] and pathological situations [2,7,8,11,12,30,35]. Recently, the neurosteroid binding sites on GABAARs have been identified and characterized [17]. Depending on the subunit composition of GABAARs [16], a positive allosteric modulation is achieved by 3α-neurosteroids binding in a cavity within in the transmembrane domains of the α subunit, whereas a direct activation of the receptor is possible if the neurosteroids bind to interfacial residues between α and β subunits. This indicates that neurosteroids preferentially access the receptor from the

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intracellular compartment or alternatively by lateral membrane diffusion [1]. Apart from GABAARs, other receptor-channels were shown to be modulated by exogenously-applied steroids [33], mostly used at high concentrations (>10 μM). Among them, however, a recent study indicated that pregnanolone significantly reduced glycine receptor (GlyR) function [19]. The reduction of glycinergic current was seen at micromolar concentrations and was more pronounced if the channels were composed of the α3 subunit which is strongly expressed in the spinal nociceptive system [15]. The level of spinal inhibition is a key element to prevent the apparition of pathological pain symptoms [5,6,15]. This is well illustrated by intrathecal injection of the GABAAR antagonist or the GlyR antagonist, which both trigger the apparition of a short-lasting allodynia [18,37]. Recently, we have demonstrated that the elevated concentration of neurosteroids seen in inflammatory pain states significantly reduced thermal heat hyperalgesia [32].

The aim of this work was to characterize the rapid (receptor-channel mediated) effects of different reduced metabolites of progesterone (3α5αTHP, 3α5βTHP, and 3β5βTHP) on nociceptive thresholds and pathological pain symptoms. We focused our attention on their possible spinal action since some of them are synthesized locally [21,27,32,35] and others, from peripheral origin [4,25,26], likely reach the spinal cord after crossing the blood brain barrier. More precisely, we have analyzed the action of the three stereoisomers on mechanical and thermal heat nociception. This led us to dissect the relative antinociceptive contribution of 5αTHP and 5βTHP based on their allosteric modulation of GABAARs and/or GlyRs.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (300–450 g; Janvier, Le Genest St. Isle, France) were used for this study. They were housed by a group of four under standard conditions (room temperature, 22 °C; 12/12 h light/dark cycle) with *ad libitum* access to food and water. All animals were manipulated and habituated to the tests and to the room for at least 1 week. All behavioral tests were done during the light period (i.e., between 7:00 and 19:00). All the procedures were performed in accordance with European committee council Direction of November 24, 1986 (86/609/EEC), authorization from French Department of Agriculture (license number 67-116 to PP) and from the regional ethic committee.

2.2. Behavioral testing

2.2.1. Mechanical allodynia

In all the experimentations, to test the animal mechanical sensitivity, we used a calibrated forceps (Bioseb, Chaville, France) previously developed in our laboratory [23]. Briefly, the habituated rat is loosely restrained with a towel masking the eyes in order to limit stress by environmental stimulations. The tips of the forceps are placed at each side of the paw and a graduate force is applied. The pressure producing a 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 allodynia/hyperalgesia

To test the animal heat sensitivity, two different tests were used. We used the Plantar test with Hargreaves method (Ugo Basile, Comerio, Italy) to compared the response of each hindpaw [14] when we tested healthy animals and animals having received unilateral intraplantar carrageenan injection. Exposed to a radiant heat, the latency time of paw withdrawal was measured. Hot plate (Bioseb, Chaville, France) set at a fixed temperature of 52 °C allowed us to measure the paw withdrawal latency of rats quickly after the induction of a transient allodynia resulting from the intrathecal injection of bicuculline or strychnine.

2.2.3. Drugs

Neurosteroids were injected at 1 μM and 10 μM, at concentrations compatible with their known allosteric modulation of glycine and GABAA receptor channel-function. Two stock solutions of pregnanolone (5β-pregnan-3α-ol-20-one), allopregnanolone (5α-pregnan-3α-ol-20-one), and epipregnanolone (5β-pregnan-3β-ol-20-one) were prepared in ethanol 96% at concentrations of 1 mM and 10 mM, respectively. The day of the experiment, stock solution were diluted a thousand times in NaCl 0.9% to obtain the injectable solution. The final solution of ethanol was ineffective to change the nociceptive thresholds as well as the pain symptoms. Moreover, it allowed a rapid increase in the spinal concentration of neurosteroid, a prerequisite to study the fast allosteric modulation of spinal receptor-channels. All drugs or vehicle were administered intrathecally (i.t.) on animals lightly anaesthetized with halothane 3%. Acute intrathecal injection was realized by puncture through the intervertebral space between L5 and L6 using a 50-micromolar Hamilton microsyringe with a 26-gauge needle. A characteristic tail flick indicated penetration of the needle in the intrathecal space and ensures success for the delivery of the drug. After injection of either 10 μL (majority of experiments) or 20 μL (strychnine and bicuculline experiments) of the drug solution or vehicle, the animal was immediately recovered from halothane anesthesia and was placed in the testing chamber. In order to estimate the possible spinal effect of the neurosteroids, rats were tested for mechanical thermal nociception immediately after the

intrathecal injection (i.e 1 h, 4 h) and at 24 h, to check for recovery. In the case of strychnine- or bicuculline-induced allodynia, animals were tested at the post-injection time of 15 min and 30 min, respectively, due to the short-lasting effect of these compounds.

2.2.4. Carrageenan model of inflammatory pain

In order to induce a peripheral inflammation, 150 μl of λ-carrageenan (Sigma, St. Louis, MO), prepared at 3% in NaCl 0.9%, was injected in the right hindpaw of the rat. All carrageenan injections were performed under light halothane anesthesia (3%). Animals were used 24 h after the paw injection, a period during which animals exhibited a clear mechanical allodynia and thermal heat hyperalgesia.

2.2.5. Strychnine- and bicuculline-induced allodynia

Strychnine or bicuculline were used to block spinal glycine and GABAA receptors, a procedure which produces a transient mechanical and thermal allodynia [18,37]. In our experiment, rats were intrathecally injected with 20 μl of NaCl 0.9 % containing 20 μg of strychnine or 1 μg of bicuculline. We tested mechanical and thermal sensitivities 15 min or 30 min after i.t. injection of strychnine or bicuculline, respectively.

2.2.6. Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). Statistical tests were performed with Statistica 5.1 (Statsoft, Tulsa, Oklahoma, USA) using repeated-measures two-way ANOVA, with the following factors: *treatment* (between), and *time* (within). When the ANOVA test was significant, the Tukey test was used for post-hoc multiple comparisons between individual groups. Results were considered to be statistically significant if p values were below 0.05 (∗), 0.01 (∗∗), and 0.001 (∗∗∗).

3. Results

3.1. Antinociceptive action of neurosteroids in healthy rats

In this study, we aimed at evaluating the efficacy of spinal neurosteroids, described as allosteric modulators of inhibitory amino acid receptor-channels (i.e. GlyRs, GABAARs), to control mechanical and thermal nociception. We first measured the nociceptive thresholds to heat and to a mechanical stimuli in healthy rats having received an intrathecal injection of pregnanolone (3α5βTHP) or allopregnanolone (3α5αTHP), two neurosteroids which have an equal efficacy to potentiate GABAAR function at low micromolar concentrations. Epipregnanolone (3β5βTHP) has no described effect on these receptor-channels and was used to evaluate possible non-specific effect resulting from changes in the membrane properties. Thresholds were measured 1, 4, and 24 h after the initial injection and compared to the control, *i.e.* threshold measured before the injection.

3α5αTHP and 3α5βTHP (both at 1 and 10 μM) significantly and dose-dependently increased the mechanical thresholds whereas 3β5βTHP (10 μM) and vehicle were ineffective (Fig. 1, panels A1 and A2). Four hours after i.t. injection, 3α5αTHP and 3α5βTHP had a similar antinociceptive potency which ranged from $4.88 \pm 0.51\%$ to $12.09 \pm 0.84\%$ using 1 and 10 μM of each, respectively ($n = 6$ per group and concentration; ANOVA, $p < 0.001$). For example, in the case of 3α5αTHP (10 μM), the mean threshold was increased from a value of 410.96 ± 2.41 g to a maximum value of 460.10 ± 2.02 g at 4 h ($n = 6$, ANOVA, $p < 0.001$). Twenty-four hours after i.t. injection, thresholds were back to the control values (407.60 ± 1.36 g; $n = 6$). A similar observation was made for all neurosteroids tested indicating that these compounds were no more efficient to modulate nociceptive thresholds 24 h after i.t. injection.

A slightly different situation was observed while testing the animals with thermal heat stimulus. At both concentrations (1 and 10 μM), 3α5αTHP increased the hindpaw latency time thus confirming its antinociceptive action for thermal heat (Fig. 1, panels B1 and B2). The lowest concentration of 3α5αTHP (1 μM) strongly increased the withdrawal latency from 7.17 ± 0.41 s to 8.87 ± 0.36 s at 4 h ($n = 6$; ANOVA, $p < 0.001$). This corresponded to a $32.48 \pm 6.36\%$ change in latency time. Interestingly, used at 10 μM, 3α5αTHP had a lower efficacy ($14.90 \pm 5.06\%$ change, $n = 6$; ANOVA, $p > 0.05$) but still had a significant antinociceptive effect at 4 h. With this thermal heat modality, we failed to reveal any antinociceptive effect after injection of 3α5βTHP, 3β5βTHP or of the vehicle (Fig. 1, panel B2). Similarly to what observed with the mechanical modality, all values were back to control after 24 h.

3.2. Antinociceptive action of neurosteroids in carrageenan-treated rats

In order to evaluate the antinociceptive role of neurosteroids in a pathophysiological pain situation, we used the λ-carrageenan model of inflammatory pain to measure nociceptive thresholds in animals before and after being intrathecally injected with neurosteroids (Fig. 2). As expected, 24 h after the injection of λ-carrageenan in the animal hindpaw, we found very low nociceptive threshold values for mechanical (carrageenan: 227.98 ± 12.92 g vs. control: 418.67 ± 2.92 g, $n = 36$; ANOVA, $p < 0.001$) and thermal modalities (carrageenan: 5.69 ± 0.74 s vs. control: 9.35 ± 1.49 s, $n = 36$; ANOVA, $p < 0.001$).

Mechanical allodynia was efficiently reduced using 3α5αTHP or 3α5βTHP, injected at 1 μM and 10 μM

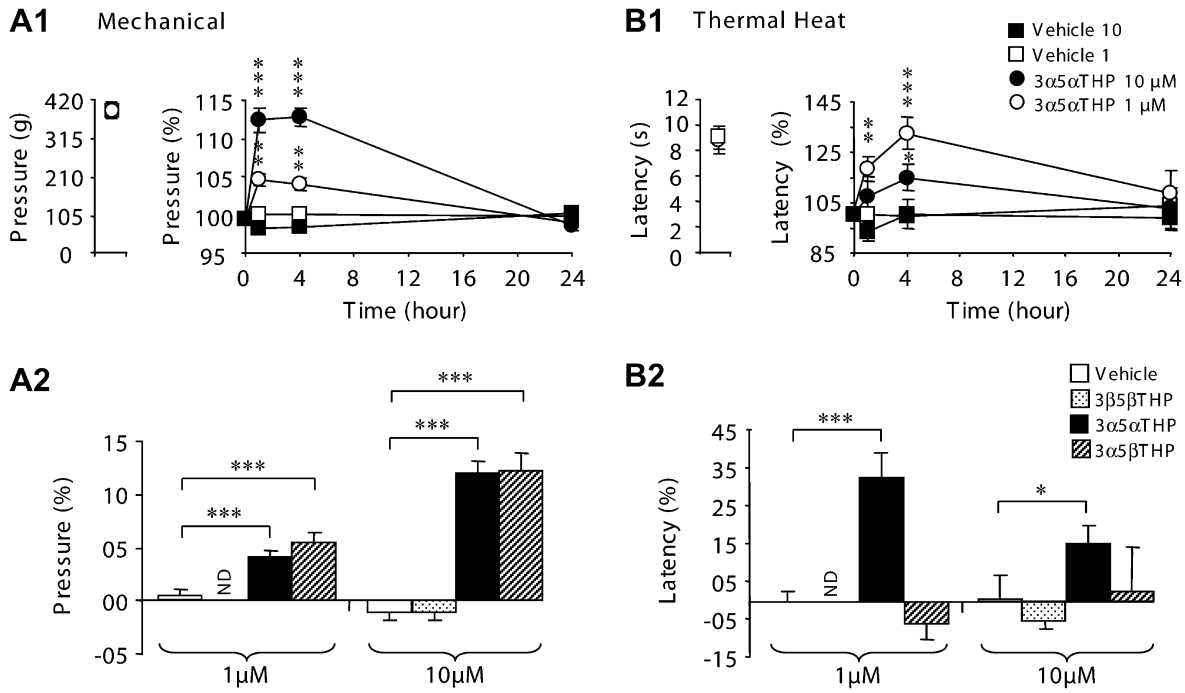

Fig. 1. Effects of progesterone metabolites 3α5αTHP, 3α5βTHP, and 3β5βTHP on mechanical and thermal heat nociceptive thresholds exhibited by naive animals. Top graphs show the time course of the change in pressure threshold (A1) and latency time (B1) after the intrathecal injection of 3α5αTHP (1 μM and 10 μM). The histograms below (A2, B2) summarize the changes observed in pressure thresholds (A2) and latency times (B2) 4 h after the intrathecal injections of 3α5αTHP, 3α5βTHP, and 3β5βTHP. ND, Not determined. Asterisk indicates statistical significance ($^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$, $n = 6$ per group) using ANOVA (A1 and B1, compared to pretest; A2 and B2 compared to vehicle).

(Fig. 2A). The reduction in allodynia obtained with 3α5αTHP was already maximal (42.83 ± 13.67%; from 200.56 ± 21.85 g to 275.06 ± 17.32 g 4 h after i.t. injection, $n = 6$; ANOVA, $p < 0.001$) using the lower concentration of 1 μM. This was not the case for 3α5βTHP which produced a dose-dependent effect, ranging from 24.58 ± 5.15% to 40.28 ± 5.94% using 1 or 10 μM, respectively. Injection of the vehicle had no effect.

Similarly to what was observed in naive animals, 3α5αTHP reduced thermal heat hyperalgesia whereas 3α5βTHP and vehicle had no effect (Fig. 2B). Moreover, the increase in paw latency following injection of 3α5αTHP was maximal using 1 μM (at 4 h 65.6 ± 10.44% increase from 3.91 ± 0.36 s to 6.53 ± 0.83 s in 3α5αTHP, $n = 6$; ANOVA, $p < 0.001$). Using a higher concentration (10 μM), 3α5αTHP had only a modest non-significant analgesic effect (25.83 ± 11.37% hat 4 h), a phenomenon which was already seen for naive animals.

3.3. Respective contributions of GABAAR and GlyR mediated inhibition on pregnanolone antinociception

One of the surprising results obtained in the first part of this study was the absence of effect of 3α5βTHP on thermal heat nociception although 3α5βTHP is a positive allosteric modulator of the GABAARs like 3α5αTHP. Indeed, 3α5αTHP reduced both mechanical and thermal nociceptive thresholds and this effect is likely to be mediated mainly by a potentiating effect on GABAARs. Because 3α5βTHP has been shown to have, in addition to its positive GABAARs modulatory effect, a negative modulatory action on GlyRs, we tried to better dissect the 3α5βTHP effects on spinal nociceptive circuits by dissociating the glycinergic and GABAergic contribution on the two sensory modalities, *i.e.* mechanical and thermal heat. To do so, we realized intrathecal coinjections of 3α5βTHP (1 μM) with either bicuculline (1 μg in 20 μl) or strychnine (20 μg in 20 μl) in order to observed its effect on a short term allodynia due to the blockade of spinal GABAARs or GlyRs, respectively.

Spinal administration of the two antagonists induced a transient allodynia which is seen as a reduction in the mechanical threshold from 418.44 ± 1.02 g to 274.31 ± 2.34 g (30 min after i.t. injection) and from 424.60 ± 2.01 g to 283.43 ± 6.82 g (15 min after i.t. injection) for bicuculline and strychnine, respectively ($n = 8$ per group; ANOVA, $p < 0.001$). Thermal allodynia was also observed at same time points after the injection. Withdrawal latencies were reduced from 11.63 ± 0.59 s to 7.48 ± 0.29 s and from 11.65 ± 0.37 s to 8.72 ± 0.32 s for bicuculline and strychnine, respectively ($n = 8$ per group; ANOVA, $p < 0.001$).

While measuring mechanical thresholds in allodynic animals (Fig. 3), 3α5βTHP had a significant analgesic

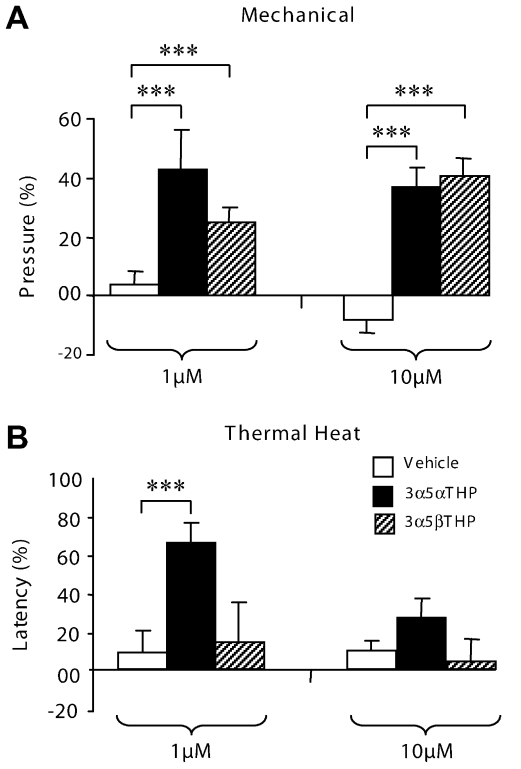

Fig. 2. Analgesic effects of the progesterone metabolites 3α5αTHP and 3α5βTHP in carrageenan-inflamed rats. Histograms show the mean changes observed on mechanical (A) or thermal heat thresholds (B) at 4 h after injection of 3α5αTHP and 3α5βTHP. Asterisk indicates statistical significance ($^{***}p < 0.001$, $n = 6$ per group) using ANOVA.

effect only in animals displaying a strychnine-induced allodynia (strychnine: 274.31 ± 2.34 g; strychnine + 3α5βTHP: 337.19 ± 7.31 g; $n = 8$; ANOVA, $p < 0.001$). This corresponded to an analgesic potency of $18.96 \pm 2.57\%$. No changes were noted in mechanical allodynia triggered by spinal bicuculline if 3α5βTHP was added in the injection solution (Fig. 3A).

While measuring thermal heat thresholds to heat stimulus, we still found that 3α5βTHP reduced strychnine allodynia (strychnine: 8.72 ± 0.32 s; strychnine + 3α5βTHP: 11.00 ± 0.17 s; $n = 8$; ANOVA, $p < 0.001$) of $26.07 \pm 1.93\%$, but surprisingly, 3α5βTHP reinforced the allodynia to thermal heat (Fig. 3B) induced by bicuculline (bicuculline: 7.48 ± 0.29 s; bicuculline + 3α5βTHP: 5.96 ± 0.15 s; $n = 8$). This reinforcement of thermal heat allodynia was of $20.37 \pm 2.06\%$ and was statistically significant (ANOVA, $p < 0.05$, Fig. 3B).

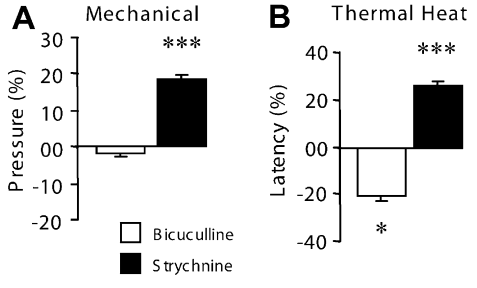

Fig. 3. Effects of 3α5βTHP on strychnine- and bicuculline-induced allodynia. The histograms illustrate the changes in mean pressure threshold (A) or latency time (B) if the injection of bicuculline (white bar) or strychnine (black bar) was realized in the simultaneous presence of 3α5βTHP (1 μM). Asterisk indicates statistical significance ($^{***}p < 0.001$; $^{*}p < 0.05$; $n = 8$ per group) using ANOVA.

4. Discussion

In this study, we characterized the modulatory role of reduced metabolites of progesterone on spinal nociception in healthy animals and in a model of inflammatory pain. These compounds (3α5αTHP and 3α5βTHP), used at concentration ranging from 10 nM to 10 μM, are known to modulate the inhibitory tone mediated by spinal GABAARs [21,32,38] and GlyRs [19]. We showed that 3α-reduced neurosteroids (but not 3β), injected intrathecally at low micromolar concentrations in the lumbar region of the spinal cord, produced a transient analgesia in naive and carrageenan-inflamed animals. 3α5αTHP was analgesic on both mechanical and thermal heat nociceptive modality. This was not the case for 3α5βTHP which produced antinociception on mechanical reflex thresholds but had no apparent effect on thermal heat. By blocking the fast glycinergic (strychnine-induced allodynia) or GABAergic inhibitory controls (bicuculline-induced allodynia), we found that this analgesic effect was mainly mediated by a potentiation of GABAAR function control in the spinal cord. In bicuculline-induced allodynia, we also show that 3α5β significantly decrease threshold to thermal heat thresholds (proalgesia), a result which is in good agreement with the recently described decrease of GlyR function [19]. This phenomenon is likely to explain the absence of apparent analgesia seen with thermal heat nociception because 3α5β had opposite effects of GlyR- and GABAAR-mediated spinal inhibition.

In our study, the beneficial effects of neurosteroids on pain thresholds in naive and carrageenan-inflamed animals were mainly seen while using 3α5αTHP, 3α5βTHP but not 3β5βTHP. This result is in good agreement with a positive allosteric modulation of the inhibitory currents mediated by extrasynaptic and synaptic GABAARs seen in various structure of the CNS [16] but also in the spinal cord [21,29,32,35,36,38]. GABAAR function was never seen to be modulated by 3β5βTHP [16,22,31] and this compound, infused at 10 μM, represents a very important control in order to eliminate possible non-specific (and non-GABAAR-mediated) membrane effects resulting in analgesia. So far very few data are available regarding the use of neurosteroids to prevent pain symptoms in physiological and pathological situations. In male mice, intracerebroventricular

administration of 3α5αTHP significantly and dose-dependently increased the pain thresholds to heat stimulus [20], an effect which was mediated by GABAARs (i.e. bicuculline and picrotoxin sensitive). In this study, progesterone was less potent than 3α5αTHP and, interestingly, 3α5βTHP was without effect. This is also the case in our hands but we clearly show that 3α5βTHP is analgesic for mechanical nociceptive thresholds, a modality which was not tested in the work of Kavaliers and Wiebe. Moreover, we obtained a dose-dependent increase in the mechanical threshold using 3α5αTHP and 3α5βTHP. The analgesic response for thermal heat, seen only for 3α5αTHP, was also dose-dependent but the maximal effect was seen at the lowest concentration of 3α5αTHP (1 μM). It is interesting to note that such a difference in the efficacy of progesterone metabolites, administered at the periphery or centrally, was also observed while measuring thermal heat nociception using the tail flick test [9]. At the higher concentration (10 μM), 3α5αTHP had a lower efficacy to produce analgesia. This has been seen previously while studying the potentiation of GABAAR function by increasing concentrations of 3α-neurosteroids in frog melanotrophs [22]. A reduction in the amplitude of GABAAR total current is seen only when they are induced by micromolar concentration of extracellular GABA co-applied with high concentration of pregnanolone (10 μM). Using lower concentration of GABA, this negative effect is never seen. Based on this result, we can speculate that thermal nociceptive thresholds are placed under a tonic control realized by GABAARs, a situation rendering them more sensitive to neurosteroid modulation. This seems actually to be the case since we showed recently that endogenous neurosteroids are produced in the dorsal horn of the spinal cord in rats exhibiting inflammatory pain symptoms [32]. In this pathological situation, the local production of neurosteroids reduced thermal heat hyperalgesia and not mechanical allodynia [32]. It should be noted here that the local production of neurosteroids is realized in the most superficial layers of the spinal cord, including lamina II, an area containing a dense population of GABAergic interneurons and receiving an important number of thermal sensory inputs [13,28].

On the other hand, we failed to reveal any effects of 3α5βTHP on thermal heat nociception in naive and carrageenan animals. The absence of effect might result from differences in the efficacy of the two different isomers (5α vs. 5β). In particular, we cannot exclude a loss of specificity (other membrane protein affected) or a change in the allosteric modulation of the amino acid receptor-channels (positive, silent or negative) while using micromolar concentrations [33,38]. Alternatively and since 3α5βTHP has the same potency than 3α5αTHP on GABAAR function [19,22], we hypothesized that 3α5βTHP might have a complex effect acting positively on GABAARs and negatively in GlyRs [19]. The sum of these two opposite modulations might explain the apparent absence of effect on thermal heat nociception. To test this hypothesis we used the model of allodynia triggered by the intrathecal injection of the GABAAR antagonist bicuculline or the GlyR antagonist strychnine [18,37]. This allowed us to evaluate how the modulation of GABAARs and GlyRs by 3α5βTHP could contribute to limit thermal heat hyperalgesia and mechanical allodynia. After blockade of GlyRs by i.t. injection of strychnine, we found that 3α5βTHP produced an efficient transient reduction of mechanical allodynia and thermal heat hyperalgesia. This result illustrates the analgesic contribution of 3α5βTHP through its action on GABAARs. On the other hand, 3α5βTHP applied after blockade of spinal GABAARs (bicuculline-induced allodynia) had no effect on mechanical threshold and, moreover, was proalgesic for thermal heat. This fast effect, compatible with the modulation of a receptor-channel, is likely to be explained by a negative modulation of spinal GlyR, although we can not exclude another modulatory action on a still unidentified receptor [33]. Because 3α5βTHP may act differentially on GlyRs and GABAARs, we should keep in mind that these effects might also have functional consequences on the modulation of locomotor activity or anxiety/depression (not restrictive), two behaviors which were not evaluated in this study but received attention previously [10].

In conclusion, this study illustrates the importance of the modulation of fast inhibitory transmission during spinal pain processing in physiological and pathological situations. It brings novel arguments characterizing the respective roles of GlyR and GABAAR-channels in the control of pain symptoms triggered by mechanical or thermal heat stimulus. We show for the time that stereoisomer reduced metabolites of progesterone can be used to finely modulate the expression of pain symptoms. The use of these metabolites, which are devoid of any genomic effects [33,34], may be of significant interest in order to develop novel strategies, with synthetic or naturally present neurosteroids, in order to treat pathological pain symptoms with respect to their sensory modality.

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