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Differential expression of $GABA_{B(1b)}$ receptor mRNA in the thalamus of normal and monoarthritic animals

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Abstract

$GABA_B$ receptors have been implicated in the plastic changes occurring in the spinal cord during the development of chronic inflammatory pain. In this study, we evaluated whether the expression of $GABA_{B(1b)}$ receptor mRNA is regulated supraspinally, namely in the thalamus, as part of the response to chronically enhanced noxious input arising from experimental monoarthritis (MA).

In situ hybridization with [^{35}S]-labelled oligonucleotide probes was performed in sections of control, 2, 4, 7 and 14 days MA rats' brains (n = 6/group). The distribution of $GABA_{B(1b)}$ mRNA was determined bilaterally in the ventrobasal complex (VB), posterior (Po), centromedial/centrolateral (CM/CL) and reticular (Rt) thalamic nuclei. The amount of $GABA_{B(1b)}$ mRNA was expressed as times fold of background values.

In normal animals, values of mRNA expression were very similar in VB, Po and CM/CL, ranging from 2.2 ± 0.2 to 2.7 ± 0.4 (mean ± S.E.M.) times higher than background levels. No expression of $GABA_{B(1b)}$ mRNA was found in the Rt of control or MA animals. A significant decrease of 26% at 4 days, and 37% at 7 days of MA, was observed in the VB contralateral to the affected joint. On the contrary, in the Po there was a significant bilateral increase at 2 days (38% contralaterally, 25% ipsilaterally), returning to basal levels at 4 days MA. No significant changes were observed in CM/CL.

These results suggest that the expression of $GABA_{B(1b)}$ in the VB and Po is regulated by noxious input, and might contribute to the functional changes that occur in the thalamus during chronic inflammatory pain.

Keywords: $GABA_{B(1b)}$; Monoarthritis; In situ hybridization; Thalamus; Chronic inflammatory pain; Neuroplasticity

1. Introduction

The perception of somatic pain involves the stimulation and activation of peripheral neurons, designated nociceptors, which then activate spinal cord neurons that modulate and transmit the nociceptive information to higher centers of the central nervous system (CNS). Supraspinal regions, including thalamic areas, act as recipients of ascending nociceptive transmission and also as origins of descending modulatory pathways [1–4].

Tissue injuries leading to the development of chronic inflammatory pain produce long-lasting changes in the function and activity of the CNS [5,6]. A widely used model of chronic inflammatory pain is monoarthritis (MA), induced by intraarticular injection of complete Freund's adjuvant (CFA) in the rat tibiotarsal joint [7]. This causes a stable and prolonged (weeks to months) inflammatory monoarthritis that produces hyperalgesia and allodynia of the affected joint [7,8].

In previous studies in MA rats, increased neuronal activity was found in several areas of the CNS related to pain processing mechanisms, including a number of thalamic nuclei [9]. Such increases had a non-linear time profile, possibly reflecting the existence of modulatory mechanisms that facilitate and/or inhibit the nociceptive input arising from MA. Moreover, changes in the mRNA expression of several metabotropic glutamate receptors (mGluRs) subtypes were observed in some thalamic nuclei of MA rats at different time points of inflammation [10].

The thalamus plays an important role in controlling and analysing the visual, auditory and somatosensory information, before relaying it to the cerebral cortex [11]. It contains three broad classes of neurons, relay neurons, local GABAergic interneurons and GABAergic neurons in the reticular nucleus (Rt) of the thalamus [12]. A number of

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neurotransmitters, comprising acetylcholine, noradrenaline, serotonin, γ-amino butyric acid (GABA) and L-glutamate (L-Glu) are released by these neurons and modulate the action of the thalamic nuclei, contributing to activity-dependent changes during nociception and showing altered expression levels in response to chronic noxious input [13].

GABA is the most abundant inhibitory neurotransmitter in the mammalian CNS and plays important roles in regulating neuronal activity, plasticity and pathogenesis. It exerts its activity mainly through two receptor subtypes, one ionotropic ($GABA_A$) and the other metabotropic G-protein coupled ($GABA_B$). $GABA_A$ receptor mediated inhibition occurs through largely postsynaptic mechanisms, via fast inhibitory postsynaptic potentials by activation of Cl^- conductance [14,15]. $GABA_B$ metabotropic receptors mediate slow, long-lasting inhibitory responses, through presynaptic inhibition of Ca^{2+} channels, causing suppression of excitatory neurotransmitters release, and/or via inhibitory postsynaptic potentials by increasing neuronal K^+ conductance and inhibiting neuronal excitability [16–20]. Some authors proposed the existence of an additional ionotropic receptor, $GABA_C$, whose response, like $GABA_A$, seems to be mediated by a Cl^- current [21].

$GABA_B$ receptors have been cloned and two highly conserved splice variants have been identified, $GABA_{B(1a)}$ and $GABA_{B(1b)}$, of 130 and 100 kDa, which differ only in the length of their N-terminal regions, and appear to be associated with post- and pre- synaptic elements in the thalamus, respectively [22,23]. Subsequently, a new subunit of the $GABA_B$ polypeptide family was described, $GABA_{B(2)}$, that shows sequence homology with the $GABA_{B(1b)}$ subunit [24–26]. Several lines of evidence suggest that $GABA_{B(2)}$ must be co-expressed with $GABA_{B(1)}$ to form a dimeric $GABA_B$ functional receptor [24–26]. These subunits seem to form a tightly associated heterodimer throughout an interaction of coiled-coil domains in the C-terminal tails [26,27]. However, it is possible that they can function as monomers distinct from each other and from the heterodimer [27,28]. Subsequent studies identified two novel splice variants ($GABA_{B(1c)}$ and $GABA_{B(1d)}$) of the $GABA_{B(1)}$ in the rat [29,30].

$GABA_{B(1)}$ subunits have been associated with receptor binding of the dimeric $GABA_B$ functional receptor [22], and in the thalamus it has been reported that $GABA_{B(1b)}$ receptor splice variant is widely expressed [28,31]. Therefore, in the present study, the levels of $GABA_{B(1b)}$ mRNA expression were analysed in four thalamic regions known to play an important role in nociception, the ventrobasal complex (VB, ventroposterolateral and ventroposteromedial nuclei), the posterior (Po), the centromedial-centrolateral (CM-CL) and the reticular (Rt) nuclei. Different time points of MA were studied, in order to investigate whether these changes would follow a specific time course, as observed for the metabolic activity changes [9] and for some of the mGluRs subtypes mRNA expression [10].

2. Materials and methods

2.1. Animals

Adult male Wistar rats (IBMC, Porto, Portugal) weighing between 250 and 300 g were used. Animals were housed in cages with food and water *ad libitum* and kept at a constant temperature of 22 °C and controlled lighting (12 h light/ 12 h dark cycle). Monoarthritis was induced by intraarticular injection of CFA into the left tibiotarsal joint according to the method described by Butler et al. [7]. Briefly, CFA was prepared by mixing 60 mg of killed and desiccated *Mycobacterium butyricum* (Difco Laboratories, Michigan, USA) with 6 ml of paraffin oil, 4 ml of saline and 1 ml of Tween 80. This suspension was then autoclaved at 120 °C for 20 min and 0.05% (w/v) of sodium azide was added. Under brief halothane anaesthesia, 50 μl of CFA was intracapsularly injected and the animals were sacrificed 2, 4, 7 and 14 days later ($n = 6$ per time point). A control group of rats ($n = 6$) was similarly injected with saline and sacrificed after 2 days. The evolution of the inflammatory reaction was monitored using a subjective scoring, where 0 means no inflammatory signs and 4 means severe inflammation with repercussion over the motor activity of the animal [32]. The experiments were performed in accordance with the ethical guidelines for the study of experimental pain in conscious animals [33], as well as the European Communities Council Directive 86/609/EEC.

2.2. In situ hybridisation

For tissue preparation, rats were decapitated and brains quickly dissected, frozen on dry ice and stored at −80 °C. Serial coronal sections (14 μm thick) were cut on a cryostat at −20 °C, thaw-mounted on poly-L-lysine coated glass slides and then briefly fixed in 4% paraformaldehyde at 4 °C, washed in 1 × phosphate buffer saline (PBS), dehydrated and stored in 98% ethanol at 4 °C until hybridisation. Radioactive in situ hybridisation was performed as previously described [34,35]. Specific synthetic oligonucleotide probes were used, 30-mers complementary to $GABA_{B(1b)}$ rat sequences in the 87 bp region downstream the start codon [36]. Probes were labelled at the 3′-end with [α-^{35}S]-dATP (1250 Ci/mmol; Perkin-Elmer, Boston, USA) using terminal transferase (Roche Diagnostics GmbH, Germany) and a 30:1 molar ratio of dATP:oligonucleotide.

Sections were removed from ethanol, allowed to dry, and hybridised in a humid chamber at 42 °C for 17 h, in hybridisation buffer containing 50% formamide (Fluka, Switzerland), 4 × saline-sodium citrate (SSC), 10% dextran sulphate (Fluka, Switzerland), 5 × Denhardt's solution (Fluka, Switzerland), 200 μg/ml of salmon sperm DNA (Sigma-Aldrich, Portugal), 100 μg/ml of polyadenylic acid (Sigma-Aldrich, Portugal), 25 mM

of sodium phosphate, 1 mM of sodium pyrophosphate and the labelled probe at a concentration of 1 pg/μl. Sections were then washed sequentially in 1 × SSC at room temperature, 1 × SSC at 56 °C for 30 min, followed by rinses in 1 × SSC, 0.1 × SSC and dehydration in ethanol at room temperature. After dehydration, slides were dipped in NTB2 Kodak photographic emulsion diluted in 0.5% glycerol in distilled water and exposed for 8 weeks in a light-proof box. Sections were developed with D-19 developer (Kodak, USA), fixed and counterstained with thionin for analysis under bright and dark field microscopy. To assess the labelling specificity, some control sections were co-incubated with a 100-fold excess of unlabelled probe with the corresponding ^{35}S-labelled probe. No labelling above background level was detected in these sections.

2.3. Data analysis

Values of mRNA expression were determined in four thalamic regions, namely the ventrobasal complex (VB; ventroposterolateral and ventroposteromedial nuclei), posterior (Po), centromedial/centrolateral (CM/CL) and reticular (Rt) thalamic nuclei, in accordance to their known involvement in nociception [11]. Where possible, each thalamic region was assessed at three different rostro-caudal levels corresponding to interaural values between 4.84 and 5.40 mm for the first level, 6.20 and 6.44 for the second, and 7.20 and 7.60 for the third level. Delimitation of the nuclei was done according to the rat brain atlas of Paxinos and Watson [37]. In accordance with previous studies [35] values of silver grains area over whole cell area in each region were calculated on the sides ipsi- and contralateral to the injected paw. Data were obtained using a computer-assisted image analyser (Optimas-Bioscan, USA) equipped with a Leica Axioplan microscope and a Sony Hyper HAD Digital colour video camera. When the selected nucleus was too large for the image size given by the microscope, more than one image (2–5) of the same nucleus at each rostro-caudal level where acquired. All images of the same nucleus at the different rostro-caudal levels were then analysed and the results pooled and averaged for each animal. Background levels were determined in the neuropile where, most probably, no specific labelling occurred. Subsequently, the individual average values were divided by background levels and expressed as ''times background''. Mean group values of mRNA expression for each region were obtained by averaging the values of each animal. Grain density values below 1.5 times background were considered to be in the background range. To compare the expression of mRNA in each thalamic region of control, 2, 4, 7 and 14 days MA rats, a one-way analysis of variance (ANOVA) was performed for each sampled region, followed by post hoc LSD test. A level of significance of 0.05 was accepted.

3. Results

Control animals that received an intraarticular saline injection showed no inflammatory signs and had a normal behaviour (inflammatory score 0). CFA injection caused physiopathological and behavioural responses that were similar and followed a time course equivalent to what has been previously described [9]. MA animals showed a remarkable inflammatory reaction restricted to the injected paw, with intense swelling and avoidance of passive movements at 2 days after CFA injection (score 2 or 3). In the 4, 7 and 14 days MA groups, the animals showed more severe signs of inflammation, as well as a guarding ''behaviour'', with persistent flexion of the injected joint (score 3 or 4) (Fig. 1).

The $GABA_{B(1b)}$ mRNA expression was investigated in the VB, Po, CM/CL and Rt of control and MA rats at 2, 4, 7 and 14 days after CFA injection. In all cases, mRNA expression was apparently restricted to neuronal cells, as judged by the Nissl counterstaining (Fig. 2).

In the VB, Po and CM/CL, the intensity of $GABA_{B(1b)}$ mRNA labelling was very similar in control animals, values ranging from 2.2 ± 0.2 to 2.7 ± 0.4 (mean ± S.E.M.) times higher than background level. No marked differences were found throughout the rostro-caudal extensions of the regions studied and no statistically significant differences between ipsilateral and contralateral sides were observed (Fig. 3). In contrast, no expression could be detected in the Rt of control animals throughout its rostro-caudal extension.

In monoarthritic rats, statistically significant changes were observed in the $GABA_{B(1b)}$ mRNA expression in some of the nuclei analysed, which were time- and region-dependent. Hence, as disease progressed, a decrease in $GABA_{B(1b)}$ mRNA expression was observed in the VB at different time points of inflammation, as compared to controls. However, these changes were restricted to the

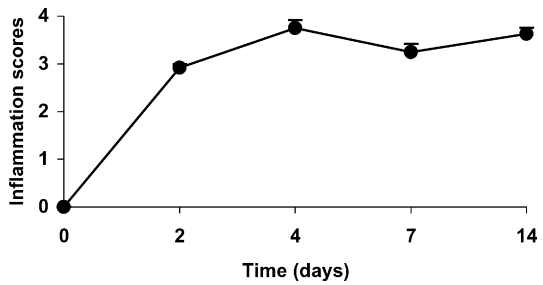

Fig. 1. Inflammation scores (mean ± S.E.M.) of MA rats before CFA injection (time 0) and at 2, 4, 7 and 14 days of inflammation ($n = 6$ per time point). Scoring was evaluated, according to Castro-Lopes et al. [32], at each time point before sacrificing the animals, 0 meaning no inflammatory signs and 4 severe inflammation with repercussion over the motor activity of the animal. At 2 days after CFA injection all rats showed a marked inflammatory reaction restricted to the injected joint. At days 4, 7 and 14 of MA, the animals displayed severe inflammatory signs with avoidance of passive movements and ''guarding'' behaviour, showing average scores close to the maximum.

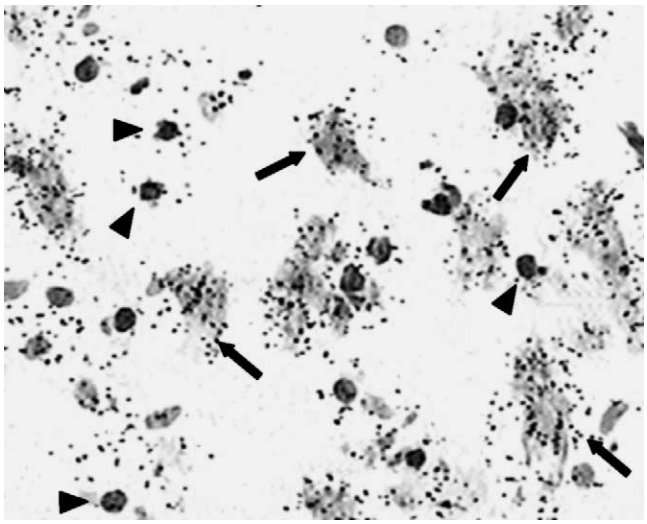

Fig. 2. High power bright field photomicrograph of emulsion-dipped section showing cellular expression of $GABA_{B(1b)}$ mRNA in neurons (arrows); no expression is detected in glial cells (arrowheads).

side contralateral to the injected joint. While, at 2 days of MA, mRNA expression was in the range of the controls, a statistically significant decrease of 26% ($P < 0.05$) was detected at 4 days of MA, and of 37% ($P < 0.01$) at 7 days MA (Fig. 4A). In the contralateral VB of 14 days MA rats, the values of $GABA_{B(1b)}$ mRNA expression were still significantly decreased in comparison to controls ($P < 0.05$), being in the range of the 7 days MA (Fig. 4A and Fig. 5). In the ipsilateral side, $GABA_{B(1b)}$ mRNA expression was in the range of the controls at all time points of inflammation studied (Fig. 4A).

In the Po, the pattern of $GABA_{B(1b)}$ mRNA expression during the development of MA was distinct from that observed at the VB. A bilateral statistically significant increase of $GABA_{B(1b)}$ mRNA expression was observed at 2 days of MA (25% and 38% for the ipsi- and contralateral sides, respectively, $P < 0.05$) (Fig. 4B and Fig. 6). At 4 days, however, mRNA expression returned to basal levels, both ipsi- and contralaterally. In the 7 days MA rats,

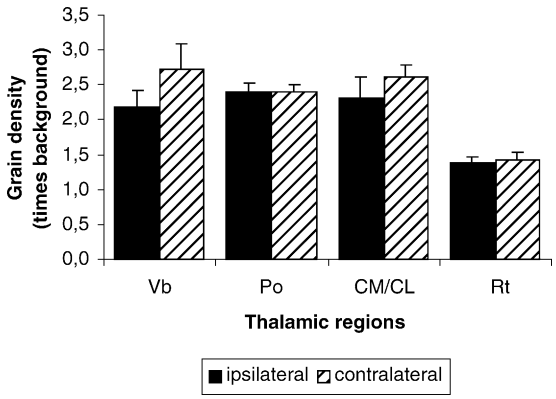

Fig. 3. Quantification (mean grain density ± S.E.M.) of $GABA_{B(1b)}$ mRNA expression in the thalamic regions analysed in control animals ($n = 6$) on the side ipsilateral and contralateral to the paw injected with saline.

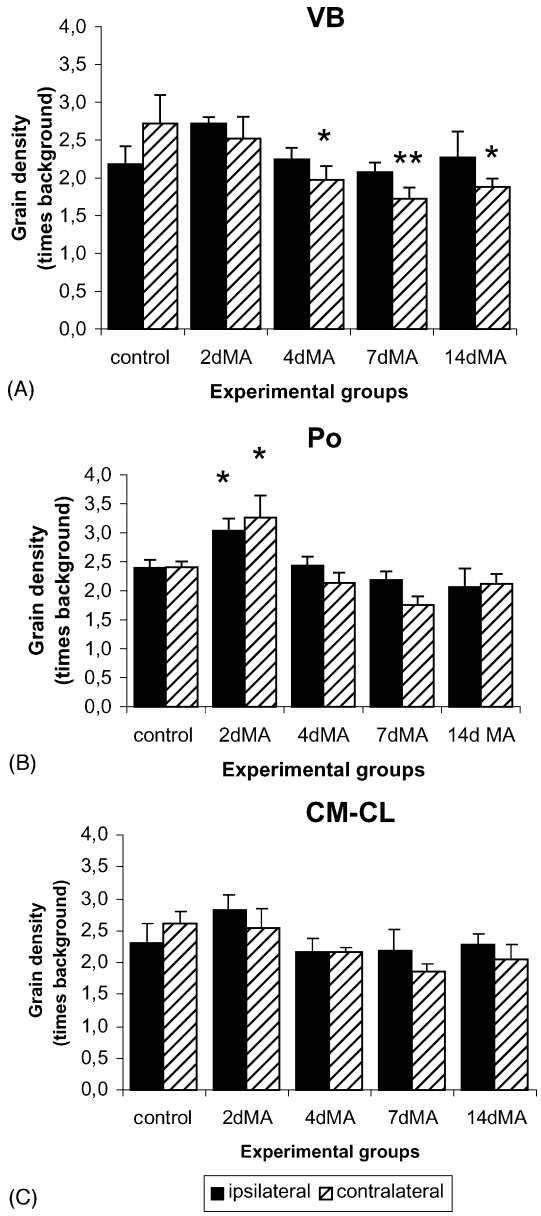

Fig. 4. Quantification (mean grain density ± S.E.M.) of $GABA_{B(1b)}$ mRNA expression in control, 2, 4, 7 and 14 days MA ($n = 6$ per time point), on the ipsi- and contralateral sides. (A) In the VB, the mRNA expression on the contralateral side was statistically significantly decreased at 4, 7 and 14 days MA rats. (B) In the Po, a statistically significant bilateral increase was observed at 2 days of MA ($P < 0.05$). (C) No statistically significant changes were observed in the CM/CL. *Significantly different from controls ($P < 0.05$, ANOVA plus LSD post hoc test); **significantly different from controls ($P < 0.01$, ANOVA plus LSD post hoc test).

a slight decrease in the contralateral side was detected, though non-statistically significant, whereas no changes were observed at 14 days MA (Fig. 4B).

In the CM/CL no differences in the $GABA_{B(1b)}$ mRNA expression were observed ipsilaterally as the disease progressed, but in the contralateral side there was a slight decrease, althought not reaching the statistical significance, that was more pronounced at 7 days of the disease (Fig. 4C).

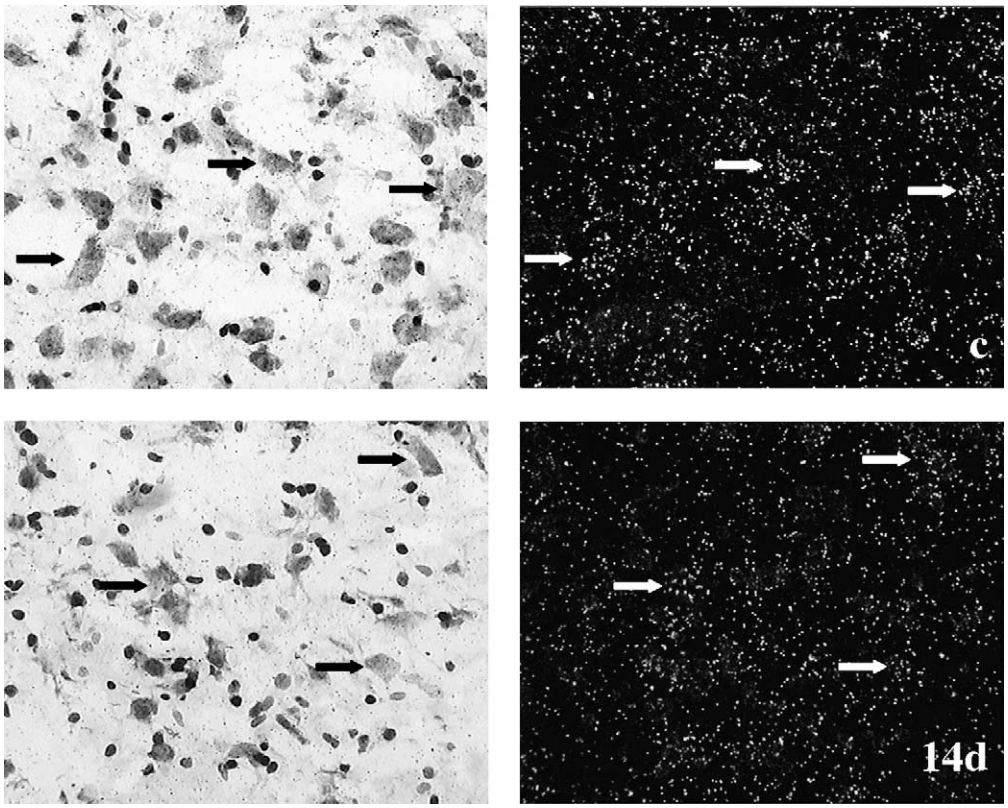

Fig. 5. High power bright field (left column) and corresponding dark field (right column) digital images of emulsion-dipped sections illustrating $GABA_{B(1b)}$ mRNA expression on the contralateral VB of control animals (c) and 14 days MA (14d). Arrows point to neurons expressing $GABA_{B(1b)}$.

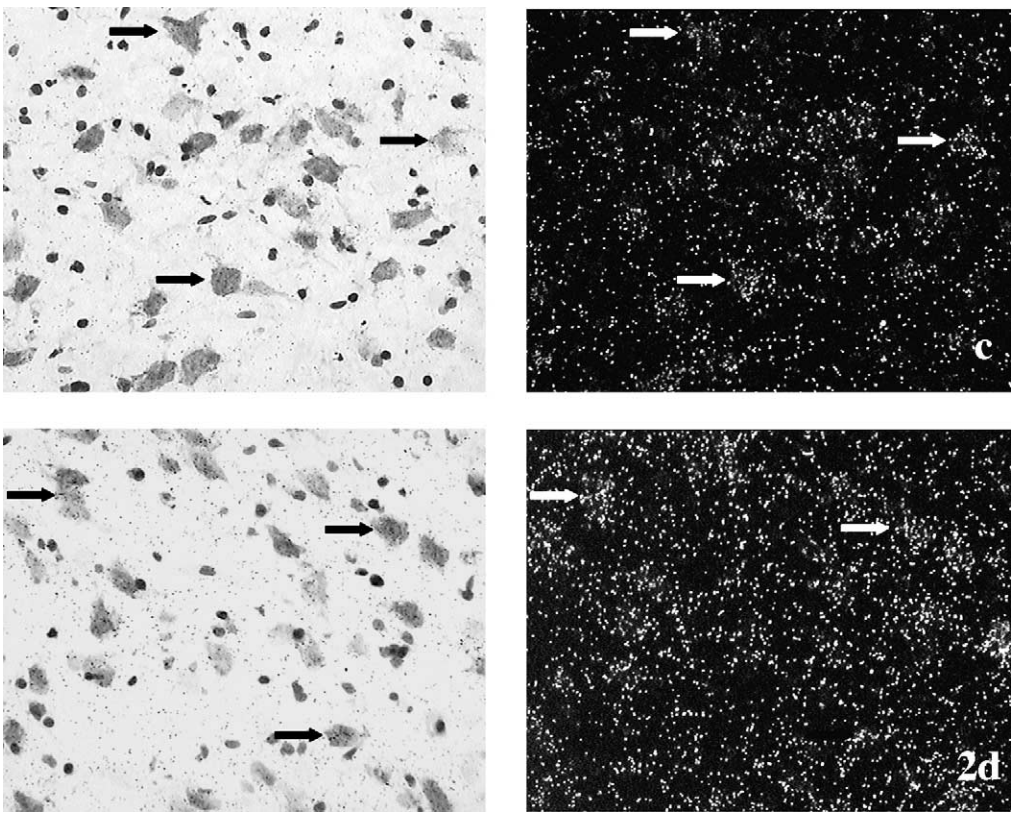

Fig. 6. High power bright field (left column) and corresponding dark field (right column) digital images of emulsion-dipped sections illustrating $GABA_{B(1b)}$ mRNA expression on the contralateral Po of control animals (c) and 2 days MA (2d). Arrows point to neurons expressing $GABA_{B(1b)}$.

No expression of the $GABA_{B(1b)}$ mRNA was found in the Rt nuclei at any time point of MA.

4. Discussion

Inflammatory nociceptive lesions induce dramatic alterations in the somatosensory system, leading to a reduction in pain threshold (allodynia) and an increased response to noxious stimuli (hyperalgesia). At early stages, peripheral neural mechanisms, such as nociceptor sensitization and activation of several inflammatory mediators, are likely to contribute to pathological pain [6,38–40]. However, the persistence of this condition is believed to result not only from a reduction in the threshold of nociceptors, but also from an enhanced excitability of CNS neurons implicated in pain transmission, which involves among others, changes in the expression of neurotransmitters and its receptors [5,38].

The thalamus plays an important role in the pathophysiology of chronic pain. In the present study, supraspinal $GABA_{B(1b)}$ mRNA expression was analysed in four thalamic regions, chosen considering their known involvement in thalamic nociceptive transmission, of which the VB, Po and CM/CL receive the major ascending input from the spinothalamic tract [2,11]. But while the VB and Po seem to be implicated in the sensory-discriminative aspect of nociception [2,41–43], the CM/CL were proposed to play a role in the motivational-affective component of pain [42]. The Rt nucleus is suggested to act as a ''gate'', filtering thalamo-cortico-thalamic information transfer, including nociceptive input [44]. It should be noted that previous studies showed increased neuronal activity in most of those thalamic regions in MA rats [9].

The intensity of mRNA labelling in control saline-injected animals was moderate and uniformly distributed, being restricted to neuronal cells. No marked differences were found throughout the rostro-caudal extensions of the regions studied as well as no statistically significant differences between ipsilateral and contralateral sides. Of particular note was the absence of basal expression in the Rt throughout its rostro-caudal extension. In the present study we did not use an additional group of naïve control rats to assess the basal mRNA expression of $GABA_{B(1b)}$, since previous in situ hybridisation studies did not show significant differences in the levels of mRNA expression between saline-injected and normal rats [10,35]. Moreover, c-Fos expression in the spinal cord dorsal horn of naïve rats is similar to that of saline-injected animals (unpublished data from our lab). Direct comparison with some in situ hybridisation studies is difficult to accomplish since they cover a large array of brain areas without giving enough quantitative detail about specific thalamic nuclei [45]. However, by using quantitative in situ hybridisation, Bischoff et al. [46], described a similar $GABA_{B(1b)}$ mRNA expression in some of the thalamic nuclei here analysed. Moreover, the present data is consistent with previous immunocytochemistry studies that postulate that $GABA_{B(1b)}$ is the predominant isoform in the thalamus, cerebral cortex and cerebellum in adult brain [47]. Our findings are also in close agreement with an early study by Chu et al. [48], which demonstrated, by [^{3}H]GABA quantitative autoradiography, that in VB, CM and Po, $GABA_B$ binding sites had a moderate to high expression. Also, Margeta-Mitrovic et al. [49] showed high $GABA_{B(1)}$ immunohistochemistry labelling in Po and moderated in the VPM, VPL, CM and CL. Some small differences in relation to our findings were observed, which can be due to differences in the methodology used, namely by the fact that either the active receptor [48] or the two splice variants of the protein subunit [49] were studied in the previous reports as compared to the mRNA of an isoform here analysed. In our study, $GABA_{B(1b)}$ mRNA expression was apparently found only in neuronal cells, corroborating previous in situ hybridisation studies where glial labelling was restricted particularly to the spinal cord white matter [45].

Interestingly, Rt, which has cell bodies of GABAergic projection neurons, did not show any $GABA_{B(1b)}$ mRNA expression in the present study. This is consistent with previous studies reporting no detectable $GABA_{B(1b)}$ in situ hybridisation signal [45,46] or low levels of $GABA_{B(1b)}$ immunoreactivity in this thalamic nucleus [23]. Additionally, weak density for the $GABA_B$ receptor or for the $GABA_{B(1)}$ subunit was also found by using binding studies [46,48], as well as low levels of $GABA_{B(1)}$-like immunoreactivity [49]. Previous studies also found very low levels of co-expression of $GABA_{B(1)}$ and glutamic acid decarboxylase (GAD), a marker of GABAergic cell bodies and terminals, in some regions including the Rt [49]. These findings suggest that $GABA_{B(1)}$ does not function as an autoreceptor in this region [49].

In monoarthritic rats, $GABA_{B(1b)}$ mRNA expression was regulated in the VB and Po thalamic nuclei. Moreover, the alterations observed displayed specific time course patterns during the evolution of the disease. In the VB, $GABA_{B(1b)}$ transcript was significantly decreased at 4, 7 and 14 days of MA, on the contralateral side of the inflamed paw. These changes are consistent with this region being implicated in pain transmission mechanisms. Time-dependent changes were also observed in the mRNA expression of several other metabotropic receptors in this area during CFA-induced inflammation [10]. Electrophysiological studies in arthritic rats suggest that VB neurons have increased activity upon noxious and non-noxious mechanical stimulation of the joint or in the vicinity of the inflamed area [43,50]. Additionally, burst activity was also observed in many VB neurons without any purposed stimulation [50]. Whether the down-regulation of $GABA_{B(1b)}$ mRNA expression contributes to the observed electrophysiological changes remains to be elucidated. In fact, the lack of similar information about the

$GABA_{B(1a)}$, or most importantly, the $GABA_{B(2)}$ genes expression, restricts an accurate evaluation of the impact of $GABA_{B(1b)}$ mRNA changes in the thalamus during MA.

In the Po, a bilateral significant increase was observed, but only at 2 days MA. In previous studies, bilateral statistically significant increases of neuronal activity were found in this region at 2 days of MA [9], confirming the participation of this nucleus in pain processing mechanisms [2,11]. Additionally, changes of mRNA expression for different receptor subtypes, namely of mGluRs, have been already reported in the Po of monoarthritic rats [10].

$GABA_B$ receptors are present in most regions of the mammalian brain, both on presynaptic terminals and on postsynaptic neurons [51]. In the thalamus, $GABA_{B(1b)}$ isoform appears to be predominantly expressed over the $GABA_{B(1a)}$ isoform, as shown by immunocytochemistry [23,31] or by RT-PCR [28]. Although some authors [52] have associated $GABA_{B(1a)}$ and $GABA_{B(1b)}$ isoforms with pre- and postsynaptic events, respectively, other studies suggest that in the thalamo-cortical circuit [23], as well as in other CNS regions [53], $GABA_{B(1b)}$ might form part of the presynaptic receptor modulating GABA neurotransmitter release. In fact, $GABA_B$ autoreceptors seem to be present on GABAergic terminals within thalamic relay nuclei [54]. Furthermore, during sensory stimulation, $GABA_B$ receptor-mediated inhibition seems to be important in controlling the size of the neuronal receptive field in the rat VB, therefore regulating the efficacy of the sensory input [55]. Although it is known that subunit expression is not always accompanied by full functional activity of the receptor, as assessed by binding studies [19,36], some studies report a parallelism between the levels of subunit mRNA and receptor binding in many CNS regions, including thalamic nuclei [46]. Hence, if we assume that a decrease of $GABA_{B(1b)}$ mRNA isoform will eventually lead to a decrease of the functional presynaptic $GABA_B$ receptor in the VB, GABA release in relay thalamic neurons will possibly be disinhibited and, as a consequence, its inhibitory action on postsynaptic receptors will be enhanced. Ultimately, this will cause a reduction on the excitability of thalamic relay neurons, as a response to enhanced nociceptive input arising from the periphery. In the VB, this effect was more pronounced on intermediate and late time points (4, 7 and 14 days) of development of chronic MA, suggesting an inhibitory role of VB neurons. In fact, some authors proposed an antinociceptive mechanism operating in the thalamus, namely in the VPL, under visceral painful stimulation [56]. On the contrary, assuming the same presynaptic mechanism, the $GABA_{B(1b)}$ mRNA increase found in the Po at 2 days MA, would most likely result in an enhanced excitability of thalamic relay neurons at this time point of the disease. This suggests that the Po might play a role in facilitatory mechanisms during the initial phase of chronic inflammatory pain conditions.

In the CM/CL, only a slight decrease in $GABA_{B(1b)}$ mRNA labelling, not statistically significant, was found contralaterally at 4 days MA. The intralaminar CM/CL neurons receive many projections from brainstem nuclei previously implicated in pain, mainly of visceral origin [11,57], and project to cerebral cortical regions associated with the motivational-affective aspects of pain, such as the rostral agranular insular cortex [11,58]. Our data suggests that peripheral chronic inflammatory pain does not affect significantly the expression of $GABA_{B(1b)}$ isoform in the CM/CL neurons.

In the Rt, no expression of $GABA_{B(1b)}$ mRNA was observed in control animals, and this did not alter during inflammation. Has previously discussed, some authors propose that no functional $GABA_B$ autoreceptors are present in the Rt [49]. In the rat thalamus, GABAergic neurons were present mainly in the Rt and to a lesser extent in the lateral geniculate nucleus (LG), accounting less than 1% in the other thalamic relay and intralaminar nuclei [59]. Additionally, it is known that Rt cells receive information from thalamocortical and corticothalamic axons, exerting their inhibitory input into thalamic relay neurons [11] and into its own neurons [60,61]. GABAergic effects from Rt neurons on those regions are probably mediated through postsynaptic GABA receptors. Inhibitory interactions between Rt neurons, in particular, seem to be dependent on $GABA_A$ receptor activation, as discussed by some authors [61,62]. Conversely, other $GABA_{B(1)}$ splice variants might be present and form functional heterodimers with $GABA_{B(2)}$. However, although in some studies the highest level of $GABA_{B(1a)}$ immunoreactivity was found in the Rt, in comparison with other thalamic nuclei [23], the mRNA distribution of $GABA_{B(2)}$ was the lowest in that nucleus [63]. Therefore, assembly of the two subunits to form functional heterodimers is unlikely to occur.

The changes reported in the present study during inflammatory chronic pain add to the plastic events that occur in nociception at thalamic level, induced and maintained by the continuous nociceptive input from the periphery. However, the molecular mechanisms that operate to produce these alterations are unknown. As changes in the mRNA expression levels of the $GABA_{B(1b)}$ isoform do not prove that either the $GABA_{B(1b)}$ protein levels or receptor function have changed, immunocytochemistry and functional assays are required, as well as studies concerning other $GABA_{B(1)}$ isoforms and the $GABA_{B(2)}$ subunit, in order to better understand the role of metabotropic $GABA_B$ receptor in the functional changes underlying thalamic processing of chronic inflammatory pain. In conclusion, the present data suggests that the expression of $GABA_{B(1b)}$ mRNA is regulated in the VB and Po by noxious peripheral input. A presynaptically $GABA_B$ receptor-mediated mechanism may lead to an inhibitory or facilitatory action of the VB or Po, respectively, at different time points of the disease.

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