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Research report

Expression of metabotropic glutamate receptors mRNA in the thalamus and brainstem of monoarthritic rats

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Abstract

Evidence for the involvement of metabotropic glutamate receptors (mGluR) in sensory processing has been emerging. Additionally, the differential distribution of distinct mGluR subtypes mRNA in particular thalamic nuclei of normal rats suggests that they could be involved in the processing of somatosensory information. In the present study, mGluR1, 3, 4 and 7 mRNAs expression was investigated by in situ hybridisation in selected brainstem and thalamic nuclei of adult monoarthritic rats at different time points of the disease (2, 4 and 14 days). Monoarthritic rats displayed behavioural and physical signs of painful arthritis at all time points. At 2 days of monoarthritis the mGluR1 mRNA expression was decreased mainly in the ventrobasal complex (VB) and in the posterior thalamic nuclei (Po) contralateral to the inflamed joint. The mGluR4 mRNA expression was also reduced, but minimum values were found at 4 days of monoarthritis, when no changes could be found in mGluR1 mRNA expression. At 14 days, mGluR4 mRNA expression was similar to controls, while mGluR1 mRNA was again reduced. Similar decreases of mGluR7 mRNA expression in the VB and Po were found at all time points, while mGluR3 mRNA expression was bilaterally increased in the reticular thalamic nucleus (Rt). In the brainstem no changes could be found in the expression of any mGluR subtype mRNA. The reduced expression of mGluR1, 4 and 7 transcripts in VB and Po, and the increases of mGluR3 mRNA in the Rt may contribute to counteract the increased noxious input arising from the periphery.

Theme: Neurotransmitters, modulators, transporters, and receptors

Topic: Excitatory amino acids receptors: structure, function and expression

Keywords: Metabotropic glutamate receptors; Thalamus; Brainstem; Monoarthritis; Pain; In situ hybridisation

1. Introduction

Tissue injuries leading to the development of chronic inflammatory pain produce prolonged changes in the function and activity of the central nervous system (CNS) [14,23] such as expansion of the neuronal receptive fields [21,85,99] and central sensitisation [14,15,61,99]. A wide variety of neurotransmitter and modulatory systems contribute to this activity-dependent plasticity of the nociceptive system, showing altered expression levels in response to chronic noxious input [7,11–14,98].

The excitatory amino acid L-glutamate (L-Glu) is the major excitatory neurotransmitter and has widespread activity in the CNS. There is increasing evidence that it also plays an important role in pain processing [87,96,18,28,33]. The transmitter actions of glutamate are mediated by two main types of receptors, the ionotropic (iGluR) and metabotropic (mGluR) glutamate receptors [67]. The iGluR are directly coupled to selective ligand-gated membrane ion channels and include the *N*-methyl-D-aspartate (NMDA), the α-amino-3-hydroxy-5-metyl-4-isoxazolepropionate (AMPA) and the kainate receptors [5,56,67]. The mGluR belong to the GTP-binding protein (G-protein) coupled seven transmembrane receptor super-

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family and exert their effects through G-protein activation of second messenger cascades. Eight genes coding for mGluR have been cloned so far, of which several have variants generated by alternative splicing at the carboxy-terminal region [16,67,72]. According to their sequence homology, pharmacology and signal transduction mechanisms, mGluR are classified into three groups. Group I, comprising mGluR1 and 5, stimulate phospholipase C (PLC) and Ca^{2+} release from internal stores. In contrast, group II (mGluR2 and 3) and group III (mGluR4, 6, 7 and 8) receptors are negatively coupled to adenylate cyclase reducing the amount of intracellular cyclic-adenosine monophosphate (cAMP) [16,67,72].

Metabotropic glutamate receptors are widely distributed in the CNS [24,37,38,63,65,66,76,86,92], and have been shown to play important roles in brain development and synaptic plasticity, participating in the induction of long-term potentiation (LTP) and long-term depression (LTD), which have been implicated in the mechanisms of learning and memory [75]. Furthermore, they have been associated to L-Glu-induced neurotoxicity and many pathological states, as well as to neuroprotective actions in response to injury [54,62], and their participation in acute and inflammatory pain has been recently reported [7,27,44,51,60,89].

The thalamus plays an important role in the processing of sensory information to the cerebral cortex [48,73,91] and its implication in pain and nociception is well established [4,31,97]. Anatomical and electrophysiological studies suggested that within the thalamic nuclei, the ventrobasal complex (VB), formed by the ventroposterolateral (VPL) and the ventroposteromedial (VPM) nuclei, and the posterior thalamic nucleus (Po), are important relay centres for nociceptive input arising from the spinal cord. They constitute the 'lateral thalamus' responsible for the sensory discriminative aspects of pain [4,32,71]. Also important in nociception are the nucleus submedius (Sub), which is part of the medial thalamus and is probably involved in the motivational-affective dimension of pain [4,20,52], and the parafascicular thalamic nucleus (Pf) [70,74]. A recent detailed analysis of the relative mRNA abundance for mGluR1, 3, 4, 5 and 7 in the various thalamic nuclei showed a distinct and specific distribution pattern for each of these subtypes [58] suggesting that they may play different roles in thalamic nociceptive processing [79].

Many brainstem nuclei have also been involved in nociception [31,83,88,97]. These include, among others, the median (MnR) and dorsal (DR) raphe nuclei, the periaqueductal grey (PAG) and the deep layers of the superior colliculus (dSC), which are mainly related to the affective, fear-motivated and alertness/avoidance reactions upon noxious stimulation [2,31,42,50,83,88,93,95,97]. In the brainstem, levels of mGluR mRNA are heterogeneous, ranging between very low or nil for mGluR4 [63], to weak or moderate in general, for mGluR1, 3 and 7 subtypes [37,65,63,86]. In some particular nuclei, however, high mGluR mRNA labelling was reported, such as in the substantia nigra, interpeduncular and choclear nuclei, and red nucleus for mGluR1 [86], and locus coeruleus for mGluR7 [37,63].

Experimental intraarticular injection of complete Freund's adjuvant (CFA) into the rat tibiotarsal joint causes a stable chronic inflammatory monoarthritis (MA) [8]. Monoarthritic animals display hyperalgesia and allodynia within a few days, and adopt a 'guarding' behaviour for the affected paw [9]. Previous studies in MA rats using the 2-deoxyglucose (2-DG) mapping technique have shown a non-linear biphasic increase of neuronal metabolic activity in spinal [84] and numerous supraspinal regions during the development of the disease, including a large number of thalamic and brainstem nuclei [57]. In the present study, possible changes in mRNA expression levels of mGluR1, 3, 4 and 7 in selected thalamic nuclei (VB nuclei, Po, Sub, Pf and Rt) and brainstem regions (located in the pons and mesencephalon), where increased metabolic activity had been observed [57], have been analysed in MA rats. In order to investigate whether these changes would also follow a specific time course, as observed for the metabolic activity changes, MA rats were studied at different time points of developing monoarthritis (2, 4 and 14 days).

2. Materials and methods

2.1. Induction of chronic inflammation

Adult male Wistar rats (Gulbenkian Institute of Science, Oeiras, Portugal) weighing between 250 and 300 g were used. All 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. [8]. Briefly, CFA was prepared by mixing 60 mg of killed and desiccated *Mycobacterium butyricum* (Difco laboratories) to paraffin oil (6 ml), saline (4 ml) and Tween 80 (1 ml). This suspension was then autoclaved at 120°C for 20 min. Under brief halothane anaesthesia, 50 μl of CFA were intracapsularly injected and the animals were sacrificed 2, 4 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 severe inflammation with repercussion over the motor activity of the animal [12]. The ethical guidelines for the study of experimental pain in conscious animals [103], as well as the European Communities Council Directive 86/609/EEC, were followed.

2.2. In situ hybridisation

Rats were decapitated and brains quickly removed, frozen on dry ice and stored at −80°C. Serial coronal brain sections (14 μm thick) were cut on a cryostat at −20°C and thaw-mounted on poly-L-lysine coated glass slides. The sections were then briefly fixed in 4% paraformaldehyde, washed in 1×phosphate buffer saline (PBS), dehydrated and stored in 96% ethanol at 4°C. Radioactive in situ hybridisation was performed as previously described [58]. Specific synthetic oligodeoxynucleotide probes, 45-mers complementary to sequences encoding 15 amino acids in the region approximately 100 amino acids prior to the first transmembrane domain, were used. The amino acid sequences encoded by each probe have been previously described [7,58]. Probes were labelled with [α-^{35}S]-dATP (1200 Ci/mmol; NEN DuPont) using terminal transferase (Boehringer, Mannheim, Germany) at a 30:1 molar ratio of dATP:oligodeoxynucleotide, and subsequently diluted to a concentration of 1 pg/μl in hybridisation buffer (50% formamide, 4×saline-sodium citrate (SSC) and 10% dextran sulphate). Serial adjacent sets of brain sections of control, 2, 4 and 14 days MA rats were hybridised at 42°C for 17 h and then washed in SSC. After dehydration slides were dipped in NTB2 Kodak photographic emulsion diluted in 0.05% glycerol in distilled water and exposed for 8 weeks. Sections were developed with D-19 developer (Kodak) and counterstained with thionin for analysis under bright and dark field optic microscopy. The specificity of the hybridisation signal was determined by incubating sections with a 100-fold excess of unlabelled probe with the corresponding ^{35}S-labelled probe. No labelling above background was detected in these sections.

2.3. Data analysis

Six nuclei in the thalamus, comprising the ventroposterolateral (VPL), ventroposteromedial (VPM), posterior (Po), submedius (Sub), parafascicular (Pf) and reticular (Rt) thalamic nuclei, were selected for quantitative analysis in accordance to their known involvement in nociception and to the differential expression pattern of mGluR subtypes in those nuclei in normal rats [58]. Where possible, each thalamic nuclei was assessed at three different rostro-caudal levels as previously described [58], based on the rat brain atlas of Paxinos and Watson [68]. Additionally, quantification was also performed in five brainstem nuclei, where increased neuronal metabolic activity had recently been found in MA rats [57]: median (MnR) and dorsal raphe (DR), dorsal (PAGD) and ventrolateral periaqueductal grey (PAGVL), and deep layers of the superior colliculus (dSC). For each animal and each mGluR subtype investigated the ratio between the total area occupied by silver grains overlapping neuronal profiles (and also glial profiles, in the case of mGluR3) and the total area of these profiles was calculated in each nucleus, separately in the sides ipsi- and contralateral to the saline- or CFA-injected paw. Data were obtained in the emulsion-dipped brain sections where the regions could be anatomically identified, using a computer-assisted image analyser (Optimas-Bioscan) fitted with a Leica axioplan microscope and a Sony Hyper HAD Digital colour video camera. In case the selected nucleus was too large for the image size given by the microscope, two or three images of the same nucleus at different places where acquired, analysed and the results pooled and averaged. The mean values of mGluR mRNA expression on the ipsi- and contralateral sides were obtained for each nucleus by pooling and averaging individual values measured at each of the rostro-caudal levels described in detail in Neto et al. [58], except for the Rt where no pooling was performed due to the large differences in mRNA expression found rostro-caudally. Subsequently, these average values were divided by background levels for normalisation. Background levels were determined in the neuropile where, most probably, no specific labelling occurred. Mean group values of mGluR mRNA expression for each nucleus were obtained by averaging the individual normalised values. In the control group, as no differences between the ipsi- and contralateral sides were observed (Student's *t*-test) the values for both sides were pooled and averaged. The values of silver grain density are shown in the tables as the mean±S.E.M. of 'times background'. Values of grain density inferior to 1.5 times background were considered to be in the background range. To compare the expression of each mGluR mRNA in each thalamic and brainstem nuclei of control, 2, 4 and 14 days MA rats, a one-way analysis of variance (ANOVA) was performed for each sampled region, followed by post-hoc Duncan's multiple range test.

3. Results

The physiopathological and behavioural responses of the animals to the CFA injection were similar and followed a time course equivalent to what has been previously described [8,57,84]. Briefly, rats receiving a saline injection showed no inflammatory signs and behaved normally (inflammatory score 0) [12]. On the contrary, CFA injection caused a remarkable inflammatory reaction restricted to the injected paw, with intense swelling and avoidance of passive movements in the 2 days MA rats (score 2–3). In the 4 and 14 days MA groups, the animals showed more severe signs of inflammation and a guarding 'behaviour' with persistent flexion of the injected joint (score 3–4).

The mRNA expression of four metabotropic glutamate receptors, mGluR1, 3, 4 and 7, was analysed in selected thalamic and brainstem nuclei of control and of monoarthritic rats at 2, 4 and 14 days after CFA injection. Table

1 presents the grain density values for the four mGluR in the thalamic nuclei investigated. In general, the mRNA expression for the different mGluR subtypes was differently regulated over time during the evolution of the disease. The signals for the mGluR1, 4 and 7 transcripts all showed decreases in MA, more pronounced in the ventrobasal complex nuclei contralateral to the affected paw, but with distinct time course patterns. On the contrary, the expression of the mGluR3 gene was increased in the Rt of MA animals. In the brainstem, mGlu1, 3, 4 and 7 mRNAs were very weakly expressed or absent in all nuclei analysed in normal control animals and no changes could be found at any time point of monoarthritis (Table 2).

A detailed description of the expression of each mGluR in the thalamic and brainstem nuclei of control and MA animals is given below.

3.1. Thalamus

3.1.1. mGluR1

In order to assess the general time course pattern of mGluR1 mRNA expression changes in the thalamus during monoarthritis, grain density values of both sides of the thalamic regions analysed were pooled and averaged for each time point investigated. In comparison to control rats, a decrease in mGluR1 mRNA expression with a non-linear time course was observed in MA animals (Fig. 1). At 2 days MA rats a decrease of 31% was detected, whereas at 4 days MA animals mGluR1 mRNA expression was in the range of controls. At 14 days MA, mGluR1 mRNA expression was again reduced, though less markedly as compared to 2 days MA animals. At 2 days MA, mGluR1 mRNA expression was significantly reduced contralaterally in the VPL (−37%), VPM (−41%, Fig. 2), Po (−50%) (Table 1) and significant reductions were also observed ipsilaterally in the Pf (48% decrease). No significant changes were detected at 4 days of MA and grain density values overall were very similar to controls for both the ipsi- and contralateral sides. In the 14 days MA animals, a non-significant reduction of mGluR1 mRNA expression was found bilaterally in the ventrobasal complex nuclei and in the Po and Pf (Table 1, Fig. 2). In the submedius nucleus no significant changes were detected at any time point.

3.1.2. mGluR4

Globally, the averaged values of mGluR4 mRNA expression in the thalamic regions analysed showed decreases at 2 and 4 days of MA, reaching a minimum at this later time point, while at 14 days MA the expression levels

Table 1
Grain density values (area grains/cell area) of mGluR1 (Group I), mGluR3 (Group II) and mGluR4 and 7 (Group III) mRNA expression in the thalamic nuclei studied, for each experimental group (values expressed as mean±S.E.M. of 'times background' level)[a]

Thalamic nuclei	Control (c)	2 days MA (2)		4 days MA (4)		14 daysMA (14)		*F*-value/*P*-value in ANOVA (df=23)		Duncan's test	
		ipsi	contra	ipsi	contra	ipsi	contra	ipsi	contra	ipsi	contra
mGluR1											
Ventroposterolateral, VPL	3.5±0.2	3.5±0.4	**2.2±0.2***	3.6±0.5	3.6±0.3	2.8±0.3	2.9±0.3	*F*=1.10/*P*=0.38 n.s.	*F*=5.93/*P*=0.01*	–	2/c, 2/4
Ventroposteromedial, VPM	4.4±0.3	3.4±0.3	**2.6±0.4***	4.3±0.3	4.1±0.2	3.5±0.4	3.8±0.4	*F*=1.99/*P*=0.16 n.s.	*F*=4.35/*P*=0.02*	–	2/c, 2/4, 2/14
Posterior, Po	4.2±0.3	3.7±0.6	**2.1±0.1***	4.5±0.3	4.4±0.6	3.8±0.6	3.4±0.4	*F*=0.53/*P*=0.67 n.s.	*F*=5.18/*P*=0.01*	–	2/c, 2/4
Parafascicular, Pf	5.0±0.4	**2.6±0.3***	2.6±0.3	4.2±0.6	4.5±0.8	3.8±0.4	3.9±0.6	*F*=4.43/*P*=0.02*	*F*=3.08/*P*=0.06 n.s.	2/c, 2/4	–
Submedius, Sub	4.4±0.8	2.9±0.7	3.9±1.0	4.6±1.4	4.3±0.7	3.7±0.7	4.4±0.7	*F*=0.72/*P*=0.55 n.s.	*F*=0.11/*P*=0.95 n.s.	–	
mGluR3											
Reticular, Rt											
caudal	3.6±0.4	4.5±0.5	3.8±0.3	5.1±0.5	3.5±0.3	4.8±0.3	3.4±0.4	*F*=2.11/*P*=0.14 n.s.	*F*=0.22/*P*=0.88 n.s.	–	–
interm.	3.4±0.5	4.9±0.4	4.1±0.4	4.1±0.5	3.6±0.4	3.7±0.5	3.2±0.3	*F*=1.73/*P*=0.20 n.s.	*F*=0.95/*P*=0.44 n.s.	–	–
rostral	1.7±0.2	**3.8±0.3***	**3.2±0.4***	**3.2±0.3***	**3.3±0.3***	**3.0±0.2***	**3.2±0.5***	*F*=7.20/*P*=0.002*	*F*=3.49/*P*=0.04*	2/c, 4/c, 14/c	2/c, 4/c, 14/c
mGluR4											
Ventroposterolateral, VPL	4.5±0.3	**3.5±0.2***	**3.0±0.4***	**3.1±0.2***	**2.8±0.3***	**3.5±0.3***	4.3±0.5	*F*=4.83/*P*=0.01*	*F*=4.74/*P*=0.01*	2/c, 4/c, 14/c	2/c, 4/c, 2/14, 4/14
Ventroposteromedial, VPM	4.7±0.2	4.2±0.4	**3.7±0.4***	3.6±0.1	**2.9±0.4***	4.1±0.3	4.1±0.3	*F*=2.70/*P*=0.08 n.s.	*F*=4.94/*P*=0.01*	–	2/c, 4/c, 4/14
Posterior, Po	4.6±0.3	4.5±0.6	3.7±0.4	4.0±0.3	3.3±0.6	4.6±0.1	5.0±0.3	*F*=0.63/*P*=0.61 n.s.	*F*=3.42/*P*=0.04*	–	4/14, 2/14
Parafascicular, Pf	3.5±0.4	2.8±0.6	2.8±0.6	2.6±0.4	2.3±0.6	3.4±0.5	3.4±0.4	*F*=0.87/*P*=0.48 n.s.	*F*=1.07/*P*=0.39 n.s.	–	–
Submedius, Sub	3.9±0.2	4.5±0.4	4.6±0.5	3.7±0.5	4.4±0.7	5.2±0.5	4.4±0.9	*F*=2.60/*P*=0.08 n.s.	*F*=0.23/*P*=0.87 n.s.	–	–
mGluR7											
Ventroposterolateral, VPL	2.0±0.2	1.2±0.2	1.4±0.2	1.2±0.3	**1.1±0.1***	1.4±0.2	1.4±0.2	*F*=2.74/*P*=0.08 n.s.	*F*=3.29/*P*=0.04*	–	4/c
Ventroposteromedial, VPM	2.0±0.2	**1.2±0.2***	**1.2±0.2***	**1.2±0.3***	**1.0±0.2***	**1.3±0.2***	**1.2±0.2***	*F*=3.31/*P*=0.04*	*F*=5.64/*P*=0.01*	2/c, 4/c, 14/c	2/c, 4/c, 14/c
Posterior, Po	2.0±0.2	1.3±0.2	**1.2±0.2***	1.5±0.3	**1.3±0.2***	1.5±0.2	**1.3±0.2***	*F*=1.94/*P*=0.16 n.s.	*F*=4.31/*P*=0.02*	–	2/c, 4/c, 14/c
Submedius, Sub	1.7±0.1	1.7±0.3	1.6±0.3	1.3±0.2	1.6±0.4	1.6±0.3	2.0±0.6	*F*=0.41/*P*=0.75 n.s.	*F*=0.27/*P*=0.85 n.s.	–	–

[a] Experimental groups are identified by (c), (2), (4) and (14). * Statistically significant *F*-values, i.e. significant differences between the various experimental groups. The two last columns show the experimental groups with significant differences (Duncan's multiple range test, e.g. '4/c' means that group '4 days MA' is significantly different from group 'control'). Grain density values in bold with an * depicts only significant differences with the group 'control'. n.s., not significant.

Table 2
Grain density values (area grains/cell area) of mGluR1 (Group I), mGluR3 (Group II) and mGluR4 and 7 (Group III) mRNA expression in the brainstem regions studied, for each experimental group (values expressed as mean±S.E.M. of 'times background' level)

Brainstem regions	Control (c)	2 days MA (2)		4 days MA (4)		14 days MA (14)	
		ipsi	contra	ipsi	contra	ipsi	contra
mGluR1							
Pons							
Median raphe (MnR)	1.1±0.1	1.0±0.2	1.0±0.2	1.0±0.2	1.0±0.2	0.9±0.2	0.9±0.2
Dorsal raphe (DR)	1.1±0.1	0.8±0.1	0.9±0.2	1.0±0.1	1.4±0.2	0.9±0.1	1.0±0.2
Mesencephalon							
Periaq. grey, dorsal (PAGD)	1.1±0.1	0.9±0.2	0.9±0.2	1.1±0.2	1.4±0.2	1.0±0.1	1.0±0.1
Periaq. grey, ventrolateral (PAGVL)	1.1±0.1	0.7±0.1	0.9±0.1	0.9±0.1	1.2±0.2	0.8±0.1	0.9±0.2
Sup. Colliculus, deep layers (dSC)	1.3±0.1	0.8±0.2	1.1±0.2	1.2±0.1	1.5±0.3	1.2±0.1	1.2±0.2
mGluR3							
Pons							
Median raphe (MnR)	1.5±0.3	1.4±0.2	1.4±0.2	1.5±0.2	1.5±0.2	1.2±0.1	1.2±0.1
Dorsal raphe (DR)	1.1±0.1	1.2±0.2	1.3±0.2	1.1±0.3	0.9±0.2	1.2±0.2	1.1±0.2
Mesencephalon							
Periaq. grey, dorsal (PAGD)	1.3±0.1	1.5±0.1	1.6±0.2	1.7±0.7	1.4±0.2	1.4±0.2	1.3±0.2
Periaq. grey, ventrolateral (PAGVL)	1.4±0.1	1.6±0.1	1.9±0.3	1.7±0.4	1.5±0.2	1.3±0.2	1.1±0.2
Sup. Colliculus, deep layers (dSC)	1.4±0.1	2.1±0.3	2.5±0.4	2.0±0.8	2.0±0.6	1.7±0.1	1.7±0.2
mGluR4							
Pons							
Median raphe (MnR)	1.4±0.1	1.3±0.2	1.3±0.2	1.2±0.3	1.2±0.3	1.9±0.1	1.9±0.1
Dorsal raphe (DR)	1.2±0.1	1.4±0.2	1.5±0.2	1.1±0.3	1.1±0.2	1.7±0.2	1.6±0.1
Mesencephalon							
Periaq. grey, dorsal (PAGD)	1.5±0.1	1.4±0.2	1.5±0.2	1.3±0.3	1.2±0.3	1.7±0.2	1.8±0.2
Periaq. grey, ventrolateral (PAGVL)	1.6±0.1	1.7±0.3	1.5±0.2	1.7±0.4	1.4±0.2	1.7±0.2	1.8±0.2
Sup. Colliculus, deep layers (dSC)	1.2±0.1	1.5±0.1	1.5±0.3	1.5±0.2	1.2±0.2	**1.8±0.1***	2.0±0.2
mGluR7							
Pons							
Median raphe (MnR)	1.0±0.1	0.8±0.1	0.8±0.1	0.8±0.2	0.8±0.2	0.6±0.1	0.6±0.1
Dorsal raphe (DR)	1.2±0.1	1.0±0.1	0.9±0.1	1.4±0.5	0.8±0.1	1.0±0.2	0.8±0.2
Mesencephalon							
Periaq. grey, dorsal (PAGD)	1.1±0.1	1.0±0.1	1.0±0.1	0.9±0.2	0.8±0.2	0.8±0.2	0.7±0.1
Periaq. grey, ventrolateral (PAGVL)	1.2±0.1	1.0±0.1	1.0±0.2	0.9±0.1	0.8±0.2	0.8±0.2	0.9±0.2
Sup. Colliculus, deep layers (dSC)	1.2±0.1	0.9±0.1	0.8±0.1	0.9±0.2	0.7±0.2	0.8±0.1	0.9±0.1

Grain density values in bold with an * depicts significant differences with the control group.

were similar to control values (Fig. 1). At 2 days MA, statistically significant decreases were detected bilaterally in the VPL (−22 and −33% for the ipsi- and contralateral sides, respectively, Table 1 and Fig. 3) and contralaterally in the VPM (21% decrease, Table 1). Comparable changes were also detected contralaterally in the Po and Pf, although not reaching statistical significance (Table 1). In the 4 days MA group, significant reductions were found bilaterally in the VPL (−31 and −38% for the ipsi- and contralateral sides, respectively, Table 1 and Fig. 3) and contralaterally in the VPM (38% reduction). The contralateral decrease in the Po and the bilateral reduction in the Pf were not statistically significant. In the 14 days MA animals, only the ipsilateral VPL displayed significant decreases (−22%), whereas the remainder of the thalamic regions investigated were in the range of control animals. No significant changes were detected in the submedius nucleus.

3.1.3. mGluR7

The global time course pattern of thalamic mGluR7 mRNA expression showed decreases of similar magnitude in the 2, 4 and 14 days MA rats in comparison to controls (reductions of 31, 36 and 26%, respectively; Fig. 1). At 2 days MA, the grain density values for the mGluR7 transcript were significantly decreased bilaterally in the VPM (−40% for both the ipsi- and contralateral sides, Table 1) and in the contralateral Po (40% reduction). The VPL displayed similar reductions, which, however, were not statistically significant (Table 1). At 4 days MA rats

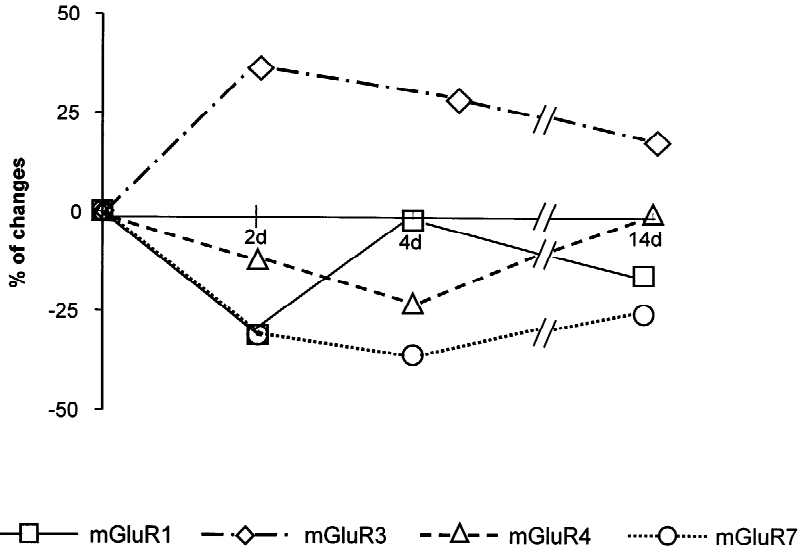

Fig. 1. General time course pattern of mGluR1, 3, 4 and 7 mRNA expression in the thalamus during monoarthritis, expressed as percentage of changes in relation to saline-injected controls. Grain density values of the ipsi- and contralateral sides in the thalamic regions analysed were pooled and averaged for each time point investigated. A biphasic time course pattern was observed for the mGluR1 transcript with higher decreases at 2 days (2d), return to control values at 4 days (4d) and decreases again at 14 days of MA (14d). Decreases of mGluR4 mRNA signal were maximum at 4 days recovering to control values at 14 days while the mGluR7 transcript showed decreases of similar magnitude at the three time points. In contrast, mGluR3 mRNA expression was always increased with maximum changes at 2 days.

significant reductions were verified in the contralateral VPL and Po, (−45 and −35%, respectively), as well as in both sides of the VPM (−40 and −50%). The reductions observed in 14 days MA rats were similar to those found at 2 days. Thus, the signal for mGluR7 mRNA was diminished by 35% in the contralateral Po and by 35% and 40%, respectively, in the ipsi- and contralateral sides of the VPM. No changes were observed in the Sub at any time point (Table 1). In the Pf, no specific mGluR7 mRNA expression was detected in control or MA animals.

3.1.4. mGluR3

Expression of mGluR3 mRNA in the thalamus of normal animals is almost entirely restricted to the Rt [58] and therefore the quantification of that mGluR subtype mRNA was limited to this nucleus. Contrarily to the other mGluR analysed, the signal for the mGluR3 transcript was generally increased during monoarthritis (Figs. 1 and 4). Broad maximum increases were seen at 2 days (+40%), but the grain density values at 4 and 14 days of MA were still 31 and 22% above controls, respectively (Fig. 1). Increases were mainly located in the most rostral part of the nucleus both ipsi- and contralaterally. In this region, increments were more prominent ipsilaterally at 2 days (124% increase) but they were also high in the contralateral side for the same group of animals as well as bilaterally in the 4 and 14 days MA (between 76 and 94% increases). Increases ranging from 25 to 42% were also observed in the other rostro-caudal levels of the Rt, but these changes were not statistically significant.

3.2. Brainstem

3.2.1. mGluR1, 3, 4 and 7

The grain density of labelling for the mGluR1, 3 4 and 7 subtypes mRNA was obtained in five brainstem nuclei located in the pons and mesencephalon of control and MA rats at 2, 4 and 14 days after CFA injection (Table 2). Both in the pons, where the MnR and DR were analysed, and in the mesencephalon (PAGD, PAGVL and dSC), the mRNA expression of the mGluR subtypes studied was weak or in the background range in control animals. In MA rats, apart from an incidental increase of mGluR4 mRNA expression in the ipsilateral dSC at 14 days, no further changes were observed (Table 2).

4. Discussion

Inflammatory nociceptive lesions of peripheral tissues induce several changes in the somatosensory system that lead to hyperalgesia and allodynia. Peripheral mechanisms comprise the activation of inflammatory mediators, and sensitisation of nociceptors [3,14,21,22,53,85]. At the spinal cord level, central sensitisation is represented by an enhanced excitability of spinal cord neurones, which acquire new low-threshold inputs and/or increase their responsiveness to high-threshold inputs, and by an enlargement of their receptive fields [14,41,43,49]. Also supraspinally, e.g. in the thalamus, the neuronal activity or the responsiveness to sensory stimuli is profoundly altered

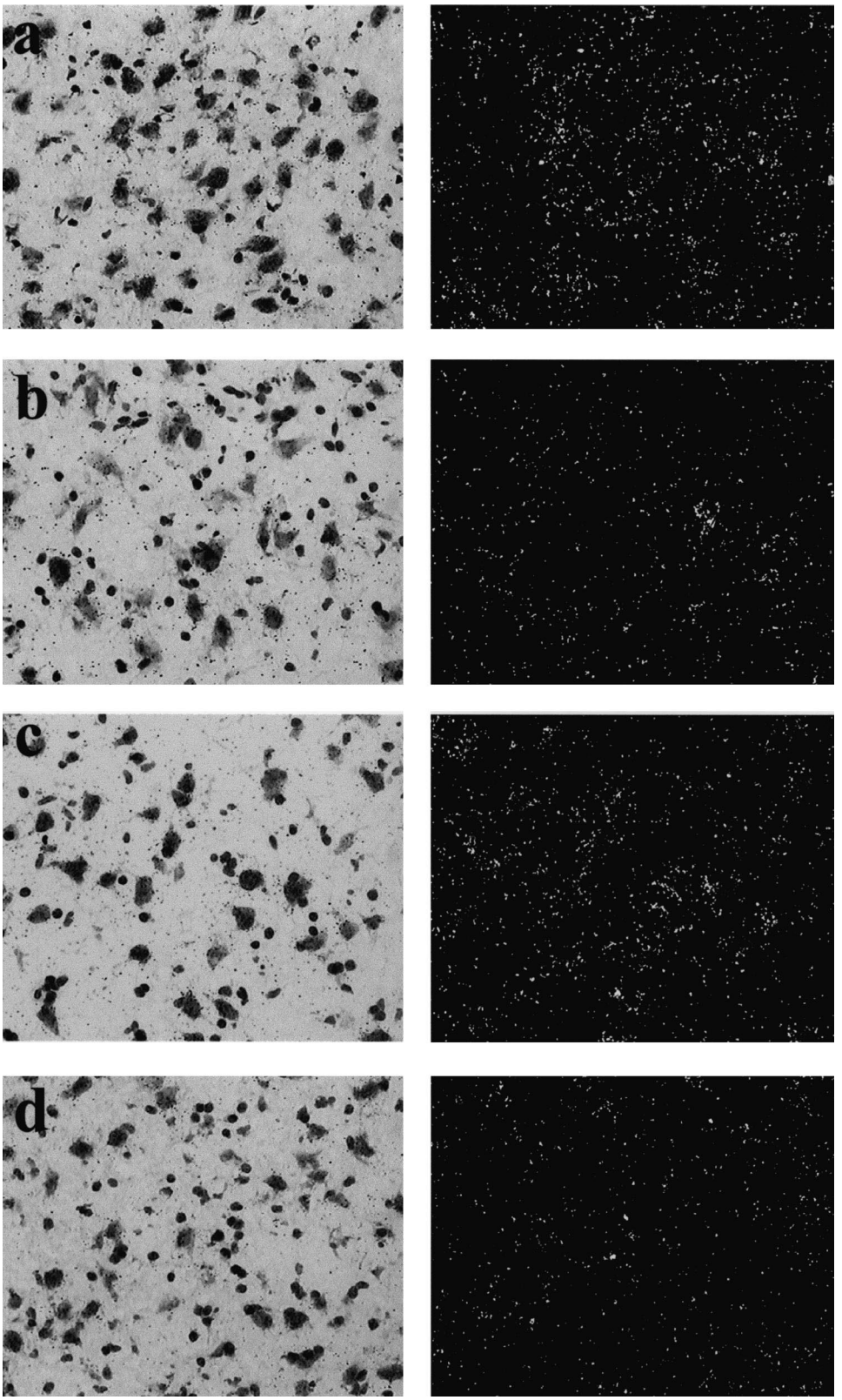

Fig. 2. High power bright field (left column) and corresponding dark field (right column) digitalised images of emulsion-dipped sections illustrating mGluR1 mRNA expression in the VPM of controls (a) and of MA rats at 2 (b), 4 (c) and 14 days (d) of the disease. The signal for mGluR1 mRNA shows a biphasic time course pattern, decreasing at 2 days of MA, returning to control levels at 4 days and decreasing again at 14 days.

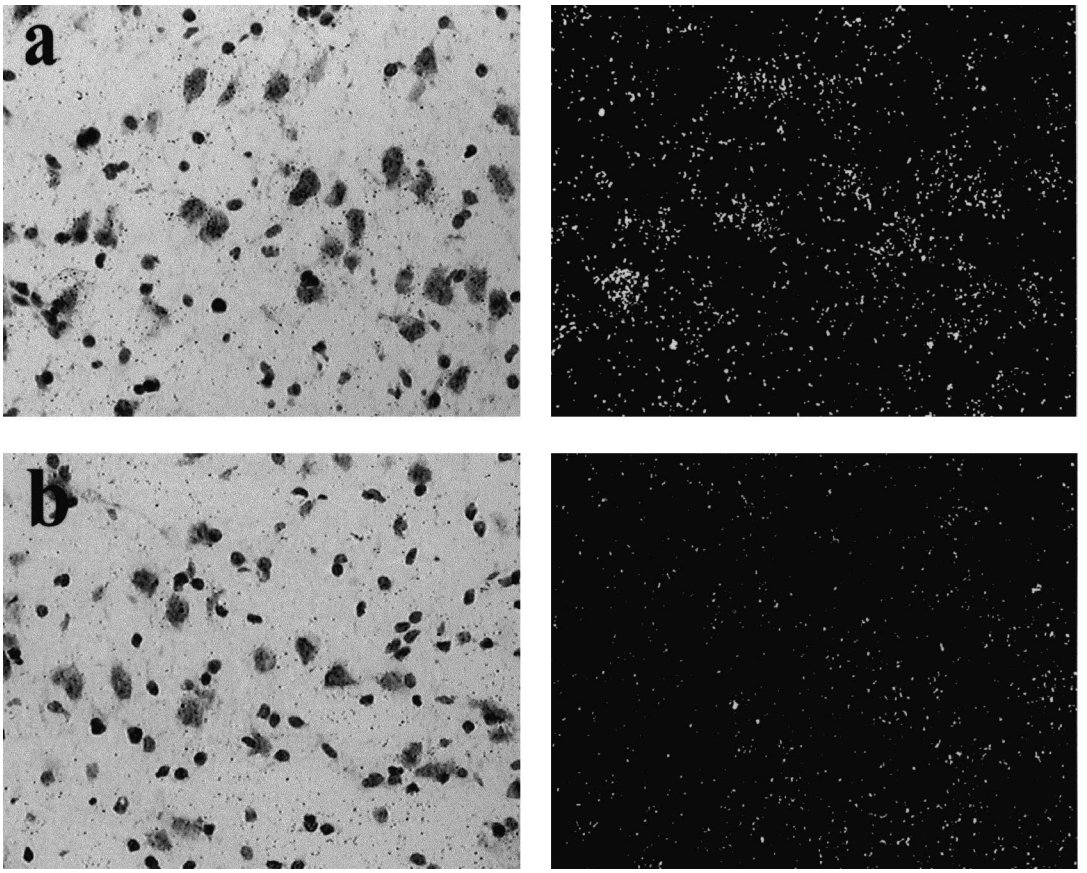

Fig. 3. High power bright field (left column) and corresponding dark field (right column) digitalised images of emulsion-dipped sections illustrating mGluR4 mRNA expression in the VPL of controls (a) and of MA rats at 4 days (b) of the disease. Maximum decreases of mGluR4 mRNA labeling were observed at this timepoint.

during chronic nociception, particularly in the VB [30,32,57].

A large variety of mechanisms contribute to this neuroplastic reaction to prolonged nociceptive input, which include changes in the expression of transmitters, receptors, ion channels and second messengers systems, that have been extensively studied mainly at the spinal cord level [3,10–13,15,19,22,29,34,53,69]. In the present study, supraspinal changes in the mRNA expression levels of metabotropic glutamate receptor subtypes were observed in thalamic nuclei of monoarthritic rats, while no alterations could be detected in the brainstem nuclei investigated. In the thalamus, mGluR1, 4 and 7 transcripts were down regulated in the VB (VPL and VPM), in the Po, and in some cases in the Pf, whereas mGluR3 mRNA was up regulated in the Rt. These alterations displayed specific time course patterns during the evolution of the monoarthritis, studied at 2, 4 and 14 days of the disease. The molecular mechanisms subserving these changes in mGluR gene expression are still unknown. In the dorsal root ganglions (DRGs) and spinal dorsal horn, the generation of inflammatory nociception leads to an alteration in the expression of several effector molecules which seems to be under the regulation of second-messenger system cascades activating a whole range of transcription factors. From these transcription factors, the cAMP responsive element-binding protein (CREB) appears to be very important in neuronal systems [98]. It is thus possible, that also in the thalamus, the mGluR mRNA expression is under the regulation of similar mechanisms. Whatever the mechanisms involved, mGluR changes found in MA animals in the present study are most likely a direct consequence of the enhanced nociceptive input, and not part of a generalised reaction to the disease. In support of this assumption, no changes could be found in the Sub or in brainstem nuclei, contrarily to what would be expected in a systemic response. Moreover, changes in mGluR mRNA expression are not likely to result from an inespecific activation of the thalamus by, for instance, an increased brainstem tonic activity. In fact, no changes of mGluR mRNA expression could be found in thalamic nuclei such as the Sub, which receives projections from the dorsal raphe nucleus as well as from the ventrolateral and medial parts of the periaqueductal grey [47]. In addition, the Pf, where no changes in mGluR mRNA expression were found except at 2 days MA for the mGluR1 subtype, is also reciprocally connected with most of the nuclei in the raphe system [46]. Incidentally, both the submedius and parafascicular nuclei,

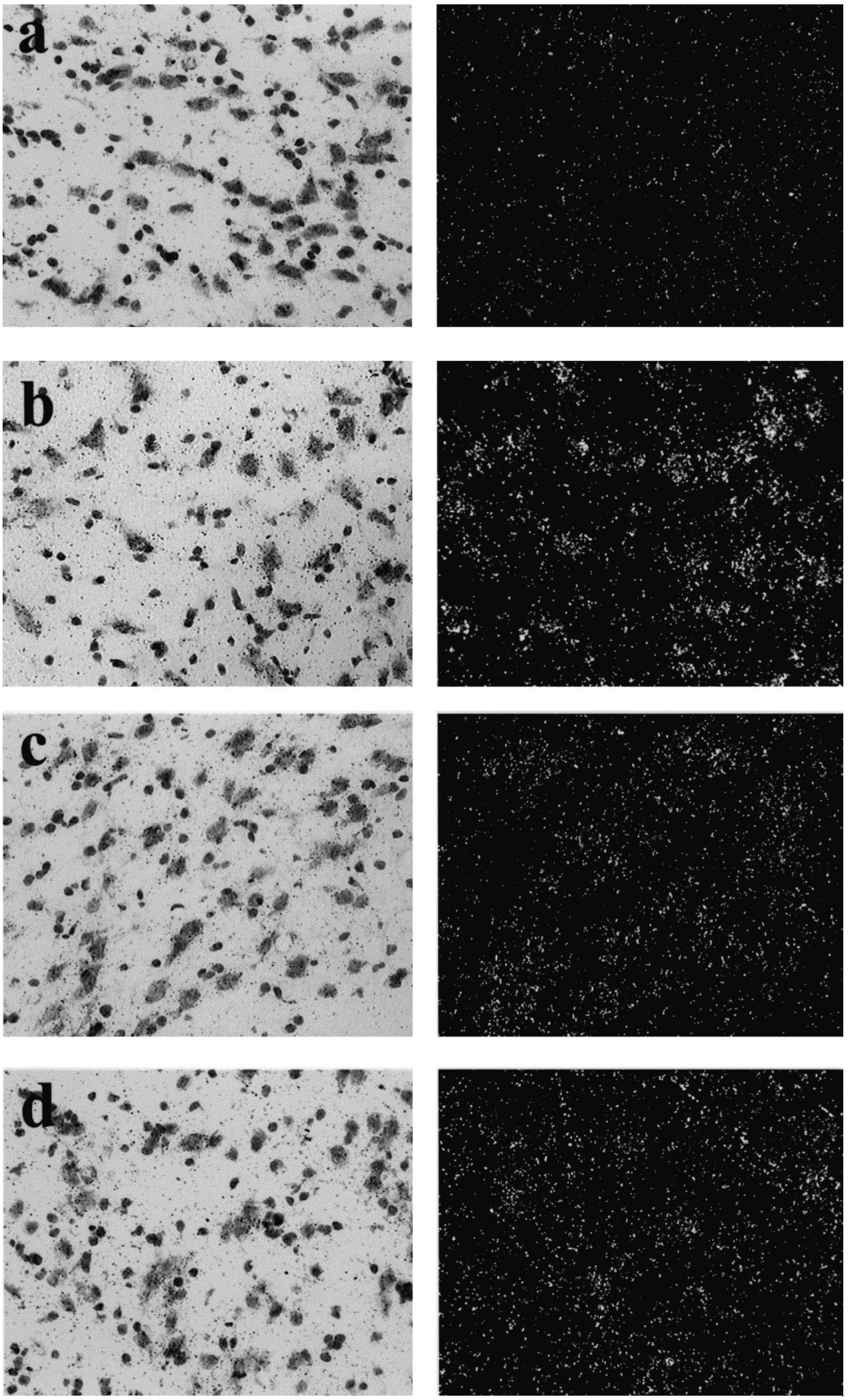

Fig. 4. High power bright field (left column) and corresponding dark field (right column) digitalised images of emulsion-dipped sections illustrating mGluR3 mRNA expression in the Rt of controls (a) and of MA rats at 2 (b), 4 (c) and 14 days (d) of the disease. The expression of mGluR3 mRNA is increased maximally at 2 days, but at 4 and 14 days it is still augmented.

although being related with pain mechanisms, have been ascribed to the motivational-affective aspects of nociception [4,20,52,70], in contrast with the VB and Po which are involved in the sensory-discriminative components of nociception [4,32,71] and where changes of mGluR mRNA expression were now detected.

Previous studies in normal rats have described the presence of various mGluR subtype mRNAs in thalamic nuclei of normal rats [37,58,63,66,86,92], most of which relaying the activity from the major sensory and motor systems to specific areas of the somatosensory and motor cortices. The same mGluR subtypes have been found expressed in DRGs and in the superficial layers of the spinal dorsal horn, which are also part of the nociceptive pathways [37,39,40,63,64]. Together, these anatomical studies point to an important role of mGluR in the nociceptive system. Recent work has corroborated their importance in the modulation of noxious stimuli, namely at the spinal cord level, by participating in the generation of inflammation-evoked hyperexcitability [60], in the production of acute mechanical hyperalgesia [51] or in the facilitation of formalin-induced nociception [26]. In the thalamus, several electrophysiological studies point to an important involvement of mGluR in the mediation of sensory inputs [25,79–82]. However, little is yet known regarding dynamic changes of neurochemical systems, particularly the mGluR, in response to a prolonged nociceptive input.

4.1. Reduction in thalamic mGluR1, 4 and 7 mRNA expression in monoarthritic animals

The decreased expression of mGluR1, 4 and 7 mRNA in MA rats occurred bilaterally in most cases, but was more pronounced in the VPL, VPM and Po contralateral to the affected paw. This agrees with the well known role of the ventrobasal complex nuclei and Po in the sensory-discriminative processing of noxious input [4,32,71], and with the bilateral activation of thalamic neurones in MA rats, as revealed by 2-DG mapping [57].

At the spinal cord level, previous in vivo studies suggested an involvement of group I (mGluR1 and 5) in nociceptive processing, particularly in prolonged pain states [27,59,60,101,102]. Intrathecally applied anti-mGluR1 and mGluR5 antibodies showed antinociceptive activity in neuropathic pain models [27], as well as in group I mGluR-agonist induced persistent pain. Moreover, selective antisense deletion of mGluR1 expression in the spinal cord dorsal horn abolished behavioural and electrophysiological nociceptive responses, without affecting responses to innocuous stimuli in multireceptive spinal neurones [100]. These findings extended earlier studies proposing an important role of the mGluR1 subtype in spinal processing of persistent nociceptive input [101,102]. At the thalamic level, iGluR as well as mGluR are involved in sensory transmission [79], and cortically-elicited excitatory postsynaptic potentials are reduced by group I mGluR antagonists [25]. The higher abundance of the mGluR1 subtype, in the thalamus [58], as compared to mGluR5, and the participation of the former in the electrophysiological responses of the ventrobasal complex neurones to noxious stimuli [77,81] suggests that mGluR1 rather than mGluR5 is highly implicated in the thalamic processing of nociceptive input. Several studies have shown that mGluR1 is located postsynaptically at the periphery of dendrites of thalamic relay neurones which receive corticothalamic input [45,79,94]. Accordingly, it has been proposed that activation of group I mGluR induces synaptic excitation in relay neurones, and that the responses of these cells to noxious stimuli may depend on the recruitment of corticothalamic transmission ([79] and references therein). In this view, it may be hypothesised that the present decreases in mGluR1 mRNA expression observed in MA rats reflect a compensatory mechanism, counteracting the increases in noxious-induced recruitment of corticothalamic transmission. Due to this decrease, relay cells would be less activated leading to a reduction in thalamo-cortical activation, overall compensating an enhanced excitability in thalamo-cortico-thalamic circuits.

Expression of mGluR4 and mGluR7 mRNA was also reduced in the ventrobasal complex nuclei and in the Po of MA rats, but with a differential time course. The expression of these group III mGluR subtypes in numerous sensory thalamic nuclei, spinal cord dorsal horn and DRGs has been well documented [37,39,40,58,63,64]. This points to their participation in the nociceptive processing, although studies in clear support of this assumption are still lacking. Agonists acting at group III mGluR (and others acting at group II) reduced sensory-evoked GABA-mediated inhibition in the VB, which was abolished by selective antagonists [78,80]. Group III (and group II) mGluR in the VB have been suggested to be localised presynaptically in terminals of GABAergic neurones projecting from the Rt, and the activation of these presynaptic mGluR would prevent the release of GABA [78–80]. It may be speculated that the present reductions in mGluR4 and mGluR7 mRNA expression found in MA animals would decrease the inhibitory action of those receptors on GABA release, resulting in an increase in GABAergic inhibitory activity in the VB. In this way, the augmented neuronal activity observed in this region in MA animals, as revealed by enhanced metabolic activity [57], would be counteracted.

Altogether, the down-regulation of mGluR1, 4 and 7 mRNA expression in thalamic sensory relay cells of MA rats possibly contributes to reduce the excitability of the thalamo-cortico-thalamic circuit driven by the ongoing nociceptive input arising from inflammation of the affected joint. In order to accomplish this function, these mGluR subtypes probably interact with iGluR [25,79], as has already been suggested for the spinal cord [6,36,51].

Although the Sub and the Pf have also been implicated

in pain processing [4,20,52,70,74], mGluR1, 4 or 7 mRNA expression was not significantly altered in those nuclei in MA animals, besides an incidental decrease in mGluR1 mRNA expression in the Pf in 2 day MA rats. Thus, it is possible that these mGluR subtypes in those particular nuclei are not substantially involved in modulating chronic hyperexcitability. Incidentally, it should be recalled that both the Sub and Pf, contrarily to the VB and Po nuclei, have been implicated in the motivational-affective aspects of nociception [4,20,52,70].

4.2. Increased mGluR3 mRNA expression in the Rt of monoarthritic animals

In contrast to the decreased expression of mGluR1, 4 and 7 mRNA in the VB and Po, mGluR3 mRNA expression was bilaterally augmented in the Rt of MA rats. A bilateral up-regulation of mGluR3 mRNA expression in the spinal cord has recently been shown in young rats bearing unilateral UV-induced peripheral inflammation of the hind paw [7]. Interestingly, intrathecally applied group II selective agonists produced mixed effects (facilitatory or inhibitory) in noxious-evoked dorsal horn neuronal responses in normal animals [89], while only inhibitory effects were observed in rats bearing a carrageenan-induced inflammation of the paw [89]. This suggests that peripheral inflammation may also induce changes of group II mGluR mechanisms in spinal nociceptive processing.

The Rt surrounds dorsolaterally the other thalamic nuclei and separates them from the cerebral cortex. It plays a primordial role in gating and modulating the corticothalamic and thalamocortical inputs by receiving collaterals from the fibres connecting the two regions [48,73,91]. A high number of GABAergic (inhibitory) neurones occur in the Rt, which send their axons into the other thalamic nuclei, including the VB and Po relay nuclei [73]. A recent study using current-clamp recordings of Rt neurones showed that a selective group II agonist caused a small amplitude membrane hyperpolarization, and this inhibitory action was due to the activation of postsynaptic receptors in the Rt neurones, as it persisted in the presence of TTX [17]. These results raise the possibility that corticothalamic and thalamocortical glutamatergic inputs inhibit Rt cells through the activation of postsynaptic mGluR3. In this case, the present increased mGluR3 expression in the Rt of MA animals would contribute to a disinhibition of VB and Po relay cells due to the enhanced inhibition of Rt neurones. In the VB, pharmacological and electrophysiological studies suggest a presynaptic localisation of group II mGluR, including mGluR3, on terminals of GABAergic neurones projecting from the Rt, causing a reduction of sensory-evoked GABAergic afferent inhibition possibly by preventing GABA release [78–80,82]. In this sense, the present overexpression of the mGluR3 transcript in presumptive GABAergic Rt neurones could result in an enhancement of its inhibitory action on GABA release from the presynaptic terminals in the VB and Po, thereby disinhibiting relay cells in these nuclei through a presynaptic mechanism. On the other hand, a recent electron microscopic study revealed that few GABAergic terminals were immunoreactive for mGluR2/3 in the VB, but rather prominent labelling was found in astrocytic processes surrounding synaptic terminals [55]. These authors proposed that, when a strong stimulation is applied, mGluR2/3 activation in astrocytes surrounding GABAergic terminals in the VB may up-regulate the uptake of GABA (mediated by specific transporters) and consequently reduce its diffusion in the extracellular space, thus reducing the inhibitory effects of the neurotransmitter, as reported [78,80,82]. Alternatively, it has been proposed that mGluR in astrocytes may regulate the release of a specific modulator (non-excitatory amino acid) which influences GABAergic inhibition in the VB [82].

It has been known that expression of mGluR3 is not restricted to neurones but also occurs in glial cells [35,55,58,65,92,]. This is also the case in the Rt [58], and since in the present study a differential quantitative analysis for neuronal or glial cells was not performed separately, it is not possible to estimate the relative contribution of each cell type for the up-regulation observed in the Rt of MA animals. Qualitative analysis, however, indicated that mGluR3 mRNA increased both in neurones and glial cells. Although the physiological role for an up-regulation in glial mGluR3 transcript is unknown, there is some evidence that glia participates in the control of neuronal activity and synaptic neurotransmission (astrocyte-to-astrocyte and astrocyte-to-neuron/neuron-to-astrocyte signalling) by increasing their internal Ca^{2+} concentration and releasing neurotransmitters in response to various stimuli [1]. Additionally, it has been shown that activation of mGluR (and iGluR) in astrocytes, induces a number of immediate-early genes that modulate gene expression [90].

4.3. Time course of thalamic mGluRs mRNA expression changes during monoarthritis

The changes in the various mGluR subtype mRNAs expression in the thalamic nuclei analysed were not homogeneous throughout the development of monoarthritis. Interestingly, in previous experiments employing the 2-DG method to map alterations in neuronal activity in the CNS of monoarthritic rats, a non-linear temporal profile was observed [57,84]. In the thalamus, similar to other supraspinal regions, elevated neuronal metabolic activity was observed at 2 and 14 days of MA, while in the 4 day MA rats the values were in the range of normal controls [57]. Possibly, at 4 days, increased activity in inhibitory systems, acting mainly at spinal level, counterbalance the ongoing noxious input arising from the inflamed joint [57,84]. In the present study, decreases in mGluR1 mRNA expression were found at 2 and 14 days, while at 4 days the mRNA levels were comparable to controls, thus showing a biphasic temporal profile which agrees very

well with the neuronal activity changes in the 2-DG studies. This strongly suggests that in thalamic nociceptive processing mGluR1 expression is highly sensitive to changes in neuronal activity. Such alterations may, though, also directly contribute to the modulation of neuronal activity.

The time course of the reduction of mGluR4 and mGluR7 mRNA expression was different from mGluR1. Reduced expression was found at 2 days, more pronounced decreases were observed at 4 days, while in the 14 day MA animals the mGluR7 transcript levels were reduced but mGluR4 mRNA was in the control range. Since the two receptor subtypes belong to the same mGluR group (group III) it is possible that they share the same regulatory mechanisms. In contrast to mGluR1, the time course of mGluR4 and mGluR7 mRNA down-regulation did not correlate so closely with the metabolic activity changes found in the 2-DG experiments.

The thalamic mRNA expression of mGluR3 was up regulated in MA rats, with maximum values at 2 days of inflammation and slightly lower values at 4 and 14 days. Therefore, no correlation could also be found between the non-linear time-profile of the 2-DG changes and the alterations of mGluR3 mRNA expression. A time-dependence for the increase of mGluR3 mRNA was also detected in the rat spinal cords during UV-induced peripheral inflammation of the hind paw [7].

Time-dependent plastic changes have been reported for various other systems in DRGs and at the spinal level in experimental models of CFA-induced inflammation [10,19,29,34]. Accordingly, Goff et al. [29] have shown increases in the immunolabelling for the μ-opioid (MOR) and neurokinin-1 (NK1) receptors in the spinal cord of CFA-treated rats, that were highest at 4 days of inflammation for NK1 and at 7 days for MOR, gradually decreasing thereafter with time. These changes were paralleled by alterations in behavioural parameters in the case of NK1, or were slightly delayed concerning the MOR. In another study, a non-linear pattern for changes in the mRNA levels of various peptides was found in the DRGs of rats with a developing monoarthritis [19]. These elaborate changes of multiple neuroactive substances or their receptors most likely reflect the complexity of modifications in the transducing and modulating systems involved in the transition from ‘phase 1’ to ‘phase 2’ pain [14,98].

4.4. No changes of mGluR1, 3, 4 and 7 mRNA expression in the brainstem during monoarthritis

In the brainstem nuclei analysed the basal expression of mGluR1, 3, 4 and 7 mRNA was very weak or nil. Although the grain density levels were in general slightly lower than previously reported [37,63,65,86], the pattern of expression was in accordance with previous studies where low levels of these subtypes mRNA were also found in most of the nuclei that were analysed in the present study. In contrast to the thalamic nuclei, the levels of the various mGluR subtypes mRNA were not changed in brainstem nuclei of MA rats at the time points investigated. In the previous 2-DG studies assessing the metabolic activity changes in the CNS in MA rats [57], increased neuronal metabolic activity with a non-linear time course pattern was found in these brainstem nuclei. The present findings, though, indicate that in those nuclei the altered neuronal activity does not modulate the mRNA expression of the mGluR subtypes analysed. Theoretically, it cannot be excluded that, besides changes at the mRNA expression level, mGluR receptors might be regulated at the post-transcriptional or translational level. To our knowledge only very few studies have focussed on the involvement of brainstem mGluR in nociception. One study reported that microinjections of group I mGluR agonists in the PAG in mice increased the latency of the nociceptive reaction, while group II or group III mGluR agonists caused the opposite effect [44].

In conclusion, the present study showed that neurochemical changes during ongoing persistent nociception are not restricted to the spinal cord level, where the majority of the studies regarding central sensitisation, neuronal plasticity and chronic nociception have been performed in the past, but also take place in supraspinal sites involved in sensory transmission such as the thalamus. In rats bearing an experimental hind paw monoarthritis for up to 2 weeks, a differential and time-dependent modulation of various mGluR subtypes mRNA expression was observed in several thalamic nuclei. Assuming that these mRNA changes result in alterations of the respective protein levels, the down-regulations of mGluR1, mGluR4 and mGluR7 in thalamic nuclei specifically involved in the transmission of nociceptive information to the cortex, as well as the up-regulation of mGluR3 in the Rt, a thalamic structure that importantly modulates the activity of cortico-thalamo-cortical loops, suggest that these changes may reflect adaptive (inhibitory) mechanisms in response to the known increase in neuronal activity in the thalamus during ongoing nociceptive input. In the brainstem, where little is yet known regarding mGluR mechanisms involved in nociceptive processing, and where mGluR appear to be less prominent, no such alterations in mGluR mRNA expression were detected at the time points investigated. An increasing understanding of supraspinal neurochemical changes induced by ongoing peripheral noxious stimulation will highlight the importance of supraspinal modulatory mechanisms in pain chronification.

Acknowledgements

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