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Article

Astrocytic expression of GFAP and serum levels of IL-1β and TNF-α in rats treated with different pain relievers

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Pro-inflammatory cytokines and glial cells, especially microglial cells, have been implicated in persistent pain sensitization. Less is known about the role of astrocytes in pain regulation. This study aimed to observe the expression of the astrocytic biomarker glial fibrillary acidic protein (GFAP) and the serum levels of interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) after short-term administration of central pain relievers in rats not submitted to noxious stimuli. Male Wistar rats were divided into five groups, receiving for nine days- (1) amitriptyline (Amt-10 mg/kg/day, by gavage); (2) gabapentin (Gb-60 mg/kg/day, by gavage; (3) methadone (Me-4.5 mg/kg/day, intraperitoneal route [IP]); (4) morphine (Mo-10 mg/kg/day, IP); or (5) 0.9% saline solution, IP. Brain samples were collected for immunohistochemical study of GFAP expression in the mesencephalon and nucleus accumbens (NAc). The area of GFAP-positive cells was calculated using MetaMorph® software and serum levels of IL-1β and TNF-α were measured by enzyme-linked immunosorbent assay. Serum TNF-α levels were decreased in the groups treated with Mo, Me and Gb, but not in the Amt-treated group. IL-1β decreased only in rats treated with Me. The astrocytic expression of GFAP was decreased in the brainstem with all drugs, while it was increased in the NAc with Amt, Me and Mo.

Uniterms: Neuropathic pain/treatment. Astrocytes/pain regulation. Analgesics/tests/treatment. Cytokines. Glial Fibrillary Acidic Protein/study

INTRODUCTION

Although pain is traditionally considered to be neuronally mediated, recent research shows an important role of glial cells in persistent pain sensitization. There is abundant evidence that certain pro-inflammatory cytokines such as interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) are involved in the process of pathological pain (Watkins, Milligan, Maier, 2003; Hutchinson *et al*., 2007; Ji, Berta, Nedergaard, 2013) and many pain relievers are known to diminish central and peripheral cytokine production (De Waal, Van Der Laan, Van Loveren, 1998; Kenis, Maes, 2002; Hutchinson, Somogyi, 2004; Vallejo, De Leon-Casasola, Ramsun, 2004; Sacerdote, 2006; Bao *et al*., 2014). Moreover, blood-borne cytokines have been shown to cross the blood-brain barrier, despite their large size and low lipid solubility, by using bidirectional and saturable transport systems (Banks, Kastin, Broadwell, 1995), thus permitting the existence of cross-talk between the immune and neuroendocrine systems in the so-called neuroimmune-endocrine axis.

Pain modulation exists in the form of a descending modulatory circuit with inputs that arise in multiple sites, including the hypothalamus, the amygdala and the rostral anterior cingulate cortex, feeding to the midbrain periaqueductal gray matter (PAG) and with outputs from the PAG to the medulla oblongata. Neurons within the nucleus raphe magnus and nucleus reticularis gigantocellularis, which are included within the rostral ventromedial medulla, have been shown to project to the spinal dorsal horns to, directly or indirectly, enhance or diminish nociceptive traffic, thus changing the experience

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of pain (Ossipov, Dussor, Porreca, 2010). These "top-down" modulatory pathways are opioid-sensitive and the actions of, for example, exogenous or endogenous opiates, cannabinoids, serotonin/norepinephrine reuptake blockers, and nonsteroidal anti-inflammatory drugs, mimic, in part, the actions of opiates.

Although microglial cells have been widely implicated in the development of chronic pain, less is known about the role of astrocytes in its regulation. Due to the complexity of the central circuitry for pain modulation, the precise mechanisms by which distinct pain relievers act remain unknown, as well as the effects of such drugs on central nervous system (CNS) cells - neurons and glial cells, especially astrocytes.

Pain modulators include a wide variety of drugs, with distinct mechanisms of action, such as tricyclic antidepressants (TCAs) that act primarily as serotonin/norepinephrine reuptake inhibitors (e.g., amitriptyline - Amt), opioid agonists (e.g., morphine – Mo - and methadone - Me) and GABAergic medications that modulate the activity of chloride channels (e.g., gabapentin - Gb) (Lynch, Watson, 2006).

Many different types of signalling molecules are able to trigger and/or regulate astrocyte function and can be released by all cell types of the CNS tissue, including neurons, microglia, oligodendrocyte lineage cells, pericytes, endothelia and other astrocytes, as well as by invasive inflammatory/immune cells. These molecular signals include (a) large polypeptide growth factors and cytokines; (b) mediators of innate immunity such as lipopolysaccharide and other Toll-like receptor ligands; (c) neurotransmitters such as glutamate, dopamine and norepinephrine; (d) purines (e.g., adenosine triphosphate); (e) reactive oxygen species; (f) products associated with systemic metabolic activity (e.g., ammonium) and (g) regulators of cell proliferation, such as endothelin (Sofroniew, 2009). Astrogliosis may be induced by molecular signals that stimulate astrocyte activity and/or affect neurotransmitter release (Sofroniew, Vinters, 2010).

In such context, the aim of this study was to determine, after the administration of short-term therapeutic doses of Amt, Gb, Me or Mo in rats not submitted to any noxious stimuli, the astrocyte response in the mesencephalon and nucleus accumbens (NAc), through the expression of the astrocytic biomarker GFAP (glial fibrillary acidic protein), as well as the possible changes in the serum levels of IL-1β and TNF-α by such drugs.

MATERIAL AND METHODS

The animal procedures were performed in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources and Brazilian Institutional Ethics Committee guidelines, Universidade Paulista (protocol no. 235/14, CEUA/ICS/UNIP, 16/04/2014). The experiments were performed in accordance with good laboratory practice protocols and all efforts were made to minimize the suffering of the animals.

Male Wistar rats were divided into five groups of six individuals, receiving for nine days - (1) 0.9% saline solution by the intraperitoneal route (IP); (2) Mo (10 mg/kg/day, IP); (3) Me (4.5 mg/kg/day, IP); (4) Gb (60 mg/kg/day, by gavage); or (5) Amt (10 mg/kg/day, by gavage). On the tenth day, the rats were anaesthetized with xylazine combined with ketamine (10 mg/kg and 90 mg/kg, respectively, IP) and tiopenthal (30 mg/kg, IP) for thoracotomy, and blood was collected by cardiac puncture. The samples were centrifuged in 5 mL conical tubes at 1500g for 15 minutes and serum was stored at -80° C. The rats were immediately subjected to intracardiac perfusion with buffered 10% formaldehyde solution. Their brains were then removed and kept for at least 48 hours in the same fixative for immunohistochemical study of GFAP expression in the mesencephalon and NAc. Coronal sections were made to collect the NAc and the mesencephalon and the tissue was embedded in paraffin for processing according to conventional histological procedures. The NAc area was chosen due to its role in processing rewarding and/or reinforcing stimuli, and the mesencephalon (PAG), for its participation in the pain desensitization pathway. The expression of the astrocyte marker GFAP was analyzed using immunohistochemical staining in sections of the mesencephalon and NAc from all groups. Coronal sections were mounted on silanized slides and submitted to GFAP immunostaining using the avidin-biotin peroxidase complex method, according to Bondan *et al*. (2013). Briefly, the sections were deparaffinized in xylene and rehydrated in a crescent-graded series of ethanol solutions. Antigen retrieval was achieved by transferring the slides to 10 mM sodium citrate buffer (pH 6.0) at 95° C for 20 minutes. Endogenous peroxidase was blocked by 3% hydrogen peroxide for 10 minutes at room temperature. Two washes with Tris/HCl buffer pH 6.0 (Wash buffer 10x, S3006, Dako, Glostrup, Danmark) were performed between incubations. Polyclonal rabbit anti-GFAP immunoglobulin (Z0334, Dako), at a dilution of 1:1000, was used as the primary antibody, for 16 hours, followed by the application of a biotinylated secondary antibody (Dako Universal LSAB™ 2 System - HRP, K0690), according to the manufacturer`s instructions. Immunoreactivity was visualized by incubating the sections in a solution containing 0.1% diaminobenzidine

(DAB, K3467, Dako). Sections were then counterstained by Harris´ modified haematoxylin solution, dehydrated and mounted in Entellan (Merck, Germany). Negative controls for immunostaining (sections lacking primary antibody application) were also prepared.

Ten photomicrographs of each mesencephalon section and four of each NAc section were randomly taken with the same microscope using a 40x objective. The area of astrocyte processes, marked in brown, was automatically calculated in pixels using MetaMorph® software that was calibrated with digital colour filters that regulated red, green, and blue areas, in such a manner that only positive cells were included and background staining was excluded from the measurement.

Serum levels of IL-1βb and TNF–α were determined in triplicate using an enzyme-linked immunosorbent assay (ready-to-use sandwich ELISA) according to the manufacturer's instructions (rat IL-1β Platinum ELISA kit, BMS630, and rat TNF-α Platinum ELISA kit, BMS622, eBioscience, San Diego, California, USA).

For statistical analyses, homoscedasticity was verified using Bartlett's test. Normality was verified using the Kolmogorov-Smirnov test. Data were analyzed by one-way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test. Statistical significance was set at $p < 0.05$.

RESULTS

No mortality was found in rats treated with the doses and drugs used in this experiment.

The serum levels (in pg/dL) of TNF-α and IL-1β from the experimental groups are presented, respectively, in Figures 1 and 2. It was observed that, on the tenth day of the experiment, TNF-α decreased compared to control rats (968.31±88.66 pg/dL) in the groups receiving Gb (484.53±70.28 pg/dL), Mo (388.53±17.37 pg/dL) and Me (451.87±22.47 pg/dL), but not in the Amt-group (867.87±61.57 pg/dL). Highly significant differences ($p < 0.001$) were seen between groups Amt vs. Gb, Amt vs. Mo and Amt vs. Me. A significant difference ($p < 0.05$) was found between Gb vs. Mo groups.

As for IL-1β, serum levels decreased only in the group treated with Me (261.56±41.79 pg/dL) compared to control rats (404.52±95 pg/dL, $p < 0.05$) and no significant differences were seen between groups that received the drugs for pain relief.

The expression of the astrocytic marker GFAP (represented in total pixel counts) in the mesencephalon and in the NAc can be seen, respectively, in Figures 3 and 4. In the mesencephalon, GFAP immunoreactivity decreased ($p < 0.001$) in the groups treated with Amt (24,031.48±20,235.34 pixels), Gb (31,033.12±15,923.32 pixels), Me (14,713.75±13,744.54 pixels) and Mo (11,004.82±6,593.14 pixels) in relation to controls (42,243±14,916.46 pixels). Significant differences were found between Mo vs. Amt ($p < 0.001$) , Mo vs. Gb ($p < 0.001$), Me vs. Amt ($p < 0.01$), Me vs. Gb ($p < 0.001$) and Gb vs. Amt ($p < 0.05$), but not in Mo vs. Me. As for the NAc, GFAP increased ($p < 0.001$) in the groups treated with Amt (20,382.75±14,830.65 pixels), Me (24,075.04±21,023.05 pixels) and Mo (37,232.63±21,888.26 pixels), compared

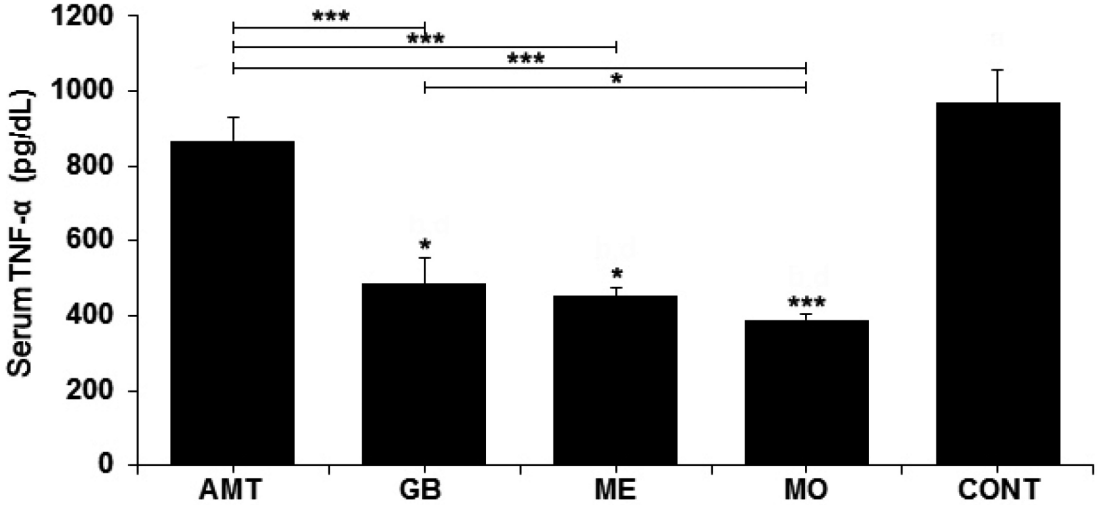

FIGURE 1 - Serum concentrations (represented as mean ± standard deviation) of TNF-α (pg/dL) in the different experimental groups (n=6 per group). AMT - amitriptyline; GB - gabapentin; ME - methadone; MO - morphine; CONT - control group. Data were analyzed by one-way ANOVA followed by Tukey-Kramer test. Asterisks show significant differences for comparisons between drug group vs. control group. Bars show differences between drug groups. *$p < 0.05$; ***$p < 0.001$.

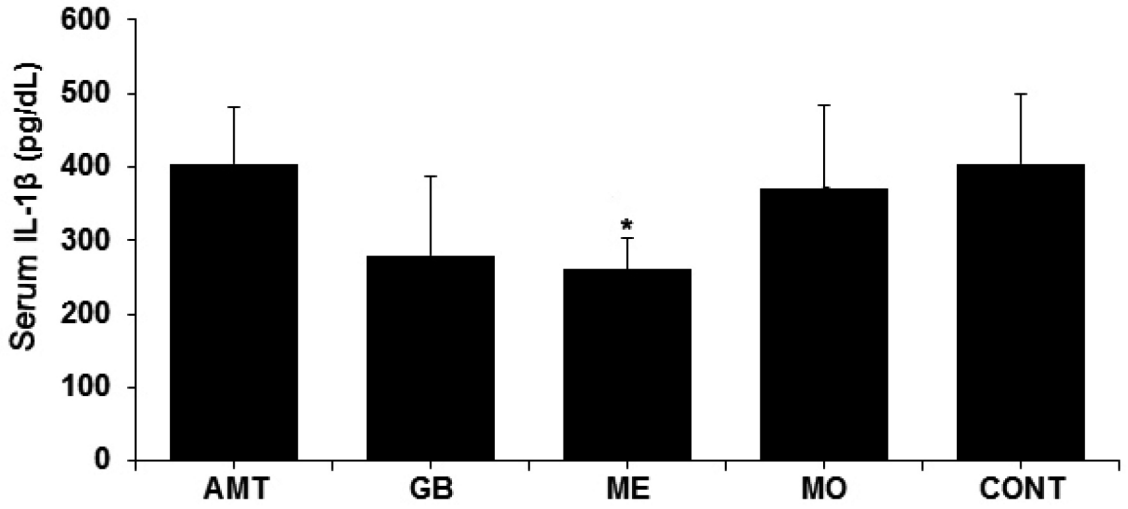

FIGURE 2 - Serum concentrations (represented as mean ± standard deviation) of IL-1β (pg/dL) in the different experimental groups (n=6 per group). AMT - amitriptyline; GB - gabapentin; ME - methadone; MO - morphine; CONT - control group. Data were analyzed by one-way ANOVA followed by Tukey-Kramer test. Asterisk shows significant difference for comparison between drug group vs. control group. *p < 0.05. No differences were found between AMT, GB, ME and MO groups.

to controls (5,602.0±4,797.88 pixels), but not in the Gb-group (3,827.58±3,579.64 pixels). Significant differences were found between Mo vs. Amt ($p < 0.01$), Mo vs. Gb ($p < 0.001$), Mo vs. Me ($p < 0.05$), Me vs. Gb ($p < 0.001$), Me vs. Gb ($p < 0.001$) and Gb vs. Amt ($p < 0.001$), but not in Me vs. Amt.

Astrocytic GFAP immunoreactivity in the mesencephalon and NAc of the different experimental groups are, respectively, presented in Figures 5 and 6.

DISCUSSION

Activation of glial cells and neuro-glial interactions are emerging as key mechanisms underlying chronic pain (Watkins, Milligan, Maier, 2003). Accumulating evidence has implicated three types of glial cells in the development and maintenance of chronic pain, namely the microglia and astrocytes of the CNS and the satellite glial cells of the dorsal root and trigeminal ganglia (Ji, Berta, Nedergaard, 2013).

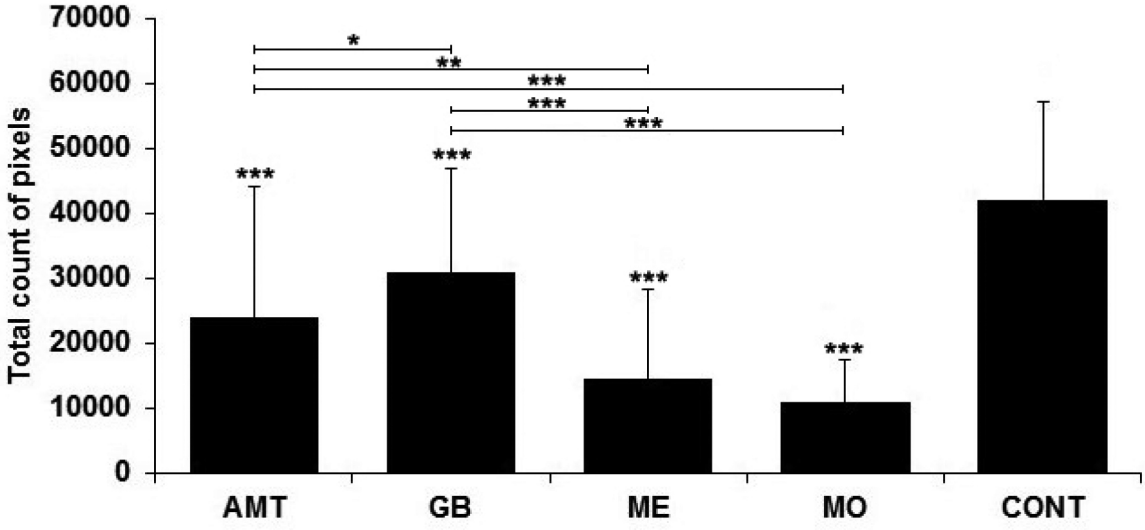

FIGURE 3 - Total count in pixels of GFAP immunoreactive astrocytes (represented as mean ± standard deviation) in the mesencephalon from the different experimental groups (n=6 per group). AMT - amitriptyline; GB - gabapentin; ME - methadone; MO - morphine; CONT - control group. Data were analyzed by one-way ANOVA followed by Tukey-Kramer test. Asterisks show significant differences for comparisons between drug group vs. control group. Bars show differences between drug groups. *p < 0.05; **p < 0.01; ***p < 0.001.

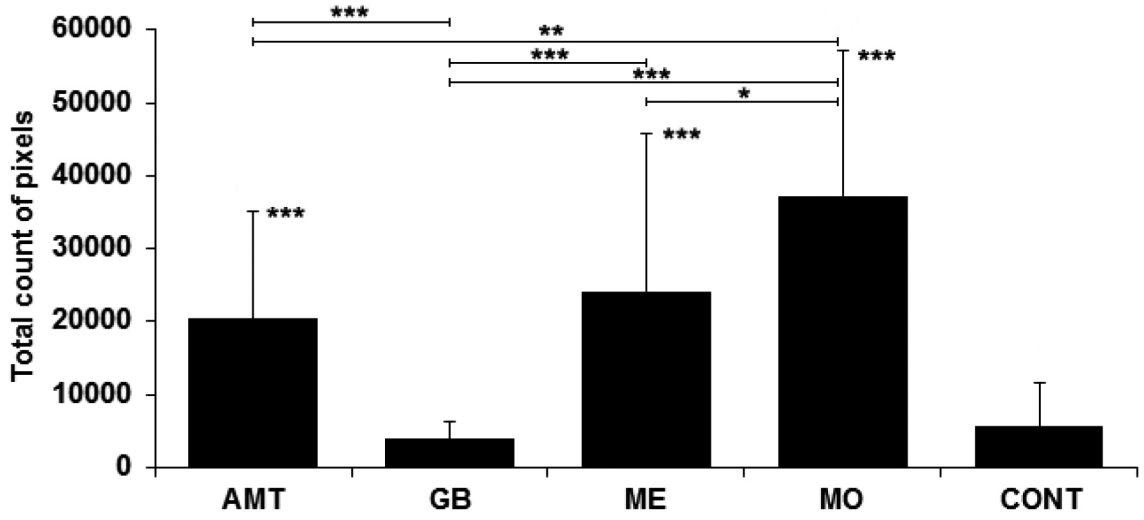

FIGURE 4 - Total count in pixels of GFAP immunoreactive astrocytes (represented as mean ± standard deviation) in the NAc from the different experimental groups (n=6 per group). AMT - amitriptyline; GB - gabapentin; ME - methadone; MO - morphine; CONT - control group. Data were analyzed by one-way ANOVA followed by Tukey-Kramer test. Asterisks show significant differences for comparisons between drug group vs. control group. Bars show differences between drug groups. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

A number of events, including CNS injury, infection and pain states, lead to hypertrophy of both microglia and astroglia, with a concomitant increased production of a variety of proinflammatory cytokines and other potentially pain-enhancing substances. Increased numbers of such cells and expression of markers such as OX-42 (for microglia) and GFAP (for astrocytes) can be used to identify glial cell activation (McMahon, Cafferty, Marchand, 2005). Microglia appear to be more important in the initial phases of neuropathic pain, while astrocytes are more relevant for later phases (Raghavendra, Tanga, DeLeo, 2004). Although glial activation has been extensively studied in the spinal cord (Scholz, Woolf, 2007), less is known about the microglial and astrocyte responses in brain areas when a noxious stimulus is applied to the periphery.

Current therapeutic strategies for neuropathic pain aim to reduce the excitability of neurons in the peripheral nervous system (PNS) or the CNS by modulating the activity of ion channels (Gb, pregabalin, carbamazepine, lidocaine and capsaicin) or by mimicking and enhancing endogenous inhibitory mechanisms (TCAs, duloxetine and opioids). Preclinical studies have explored other routes for neuropathic pain relief by modulating immune and glial responses. For example, global inhibitors of glial metabolism, such as fluorocitrate, propentofylline, minocycline and teriflunomide, reduce cytokine release and attenuate pain-responsive behaviour in several animal models of neuropathic pain (Scholz, Woolf, 2007).

When astrocytes and microglia are not in their activated state, they do not appear to be important regulators of pain transmission; however, when glia become activated, pain is affected. Peripheral immune-activated signalling to the CNS induces sickness responses, including enhanced pain responsivity, fever, increase in sleep, decreases in food and water intake, and generalized suppression of behaviour (Watkins *et al*., 2007).

Opioids such as Mo and Me are known to have profound suppressive effects on the immune system (Van der Laan *et al*., 1995; De Waal, Van der Laan, Van Lovenren, 1998; Hutchinson, Somogyi, 2004). Three distinct opioid receptor classes have been identified and cloned, and are designated μ-, κ- and δ, and all of them are widely distributed in the CNS and PNS. The expression of opioid receptors on cells of the immune system was first implicated by the ability of opioids to alter immune functions (Rittner, Machelska, Stein, 2005). Different opioids have been reported to exert immunosuppressive and modulatory effects on a variety of immune cells, including T and B cells, macrophages and natural killer (NK) cells. Moreover, they have been shown to diminish the levels of inflammatory cytokines, including IL-2, interferon gamma (IFN-γ), and TNF-α, while enhancing the levels of anti-inflammatory cytokines like IL-4 during immune/inflammatory processes (Suo, Weber, 1998; Sacerdote, 2006; Kafami *et al*., 2013). Methadone (Me), for instance, a potent μ-opioid receptor agonist, has been shown to diminish neuroinflammation and severity of symptoms in experimental autoimmune encephalomyelitis (Kafami *et al*., 2013). On the other hand, Monibi *et al.* (2015) did not find any alteration in leucocyte TNF-α, IL-

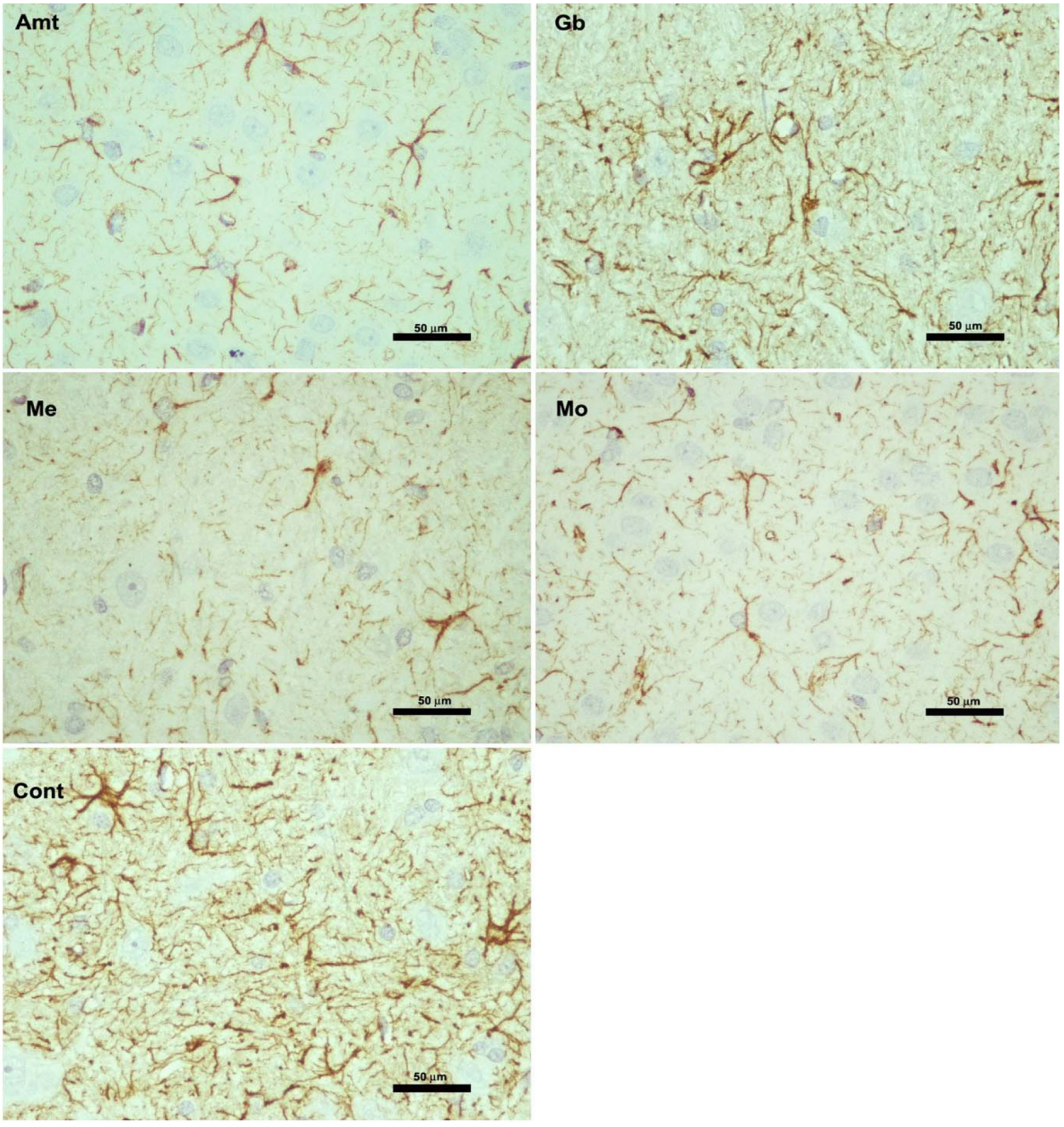

FIGURE 5 - Astrocytic immunostaining for GFAP in the ventral surface of the mesencephalon of the different experimental groups. Amt - amitriptyline; Gb - gabapentin; Me - methadone; Mo - morphine; Cont - control group. Bar = 50 µm.

6, IL-10 production, on early leucocyte apoptosis and on neutrophil phagocytic function after a 24-hour infusion of morphine (Mo) or buprenorphine in healthy dogs.

Our results show that healthy rats treated for nine days with the opiate agonists Mo and Me presented decreased serum levels of TNF-α, although only Me decreased IL-1β. Gb-treated rats also exhibited decreased serum levels of TNF-α. The rats used in this investigation were apparently healthy and were not submitted to any noxious stimuli, inflammation or damage to the PNS/CNS or at peripheral sites; despite this, acute opioid administration is known to have inhibitory effects on humoral and cellular peripheral immune responses including antibody production, NK cell activity, cytokine expression, and phagocytic activity. Opiates behave like cytokines, modulating the immune response by interacting with their receptors in the CNS and in the periphery (Vallejo, De Leon-Casarola, Ramsun, 2004).

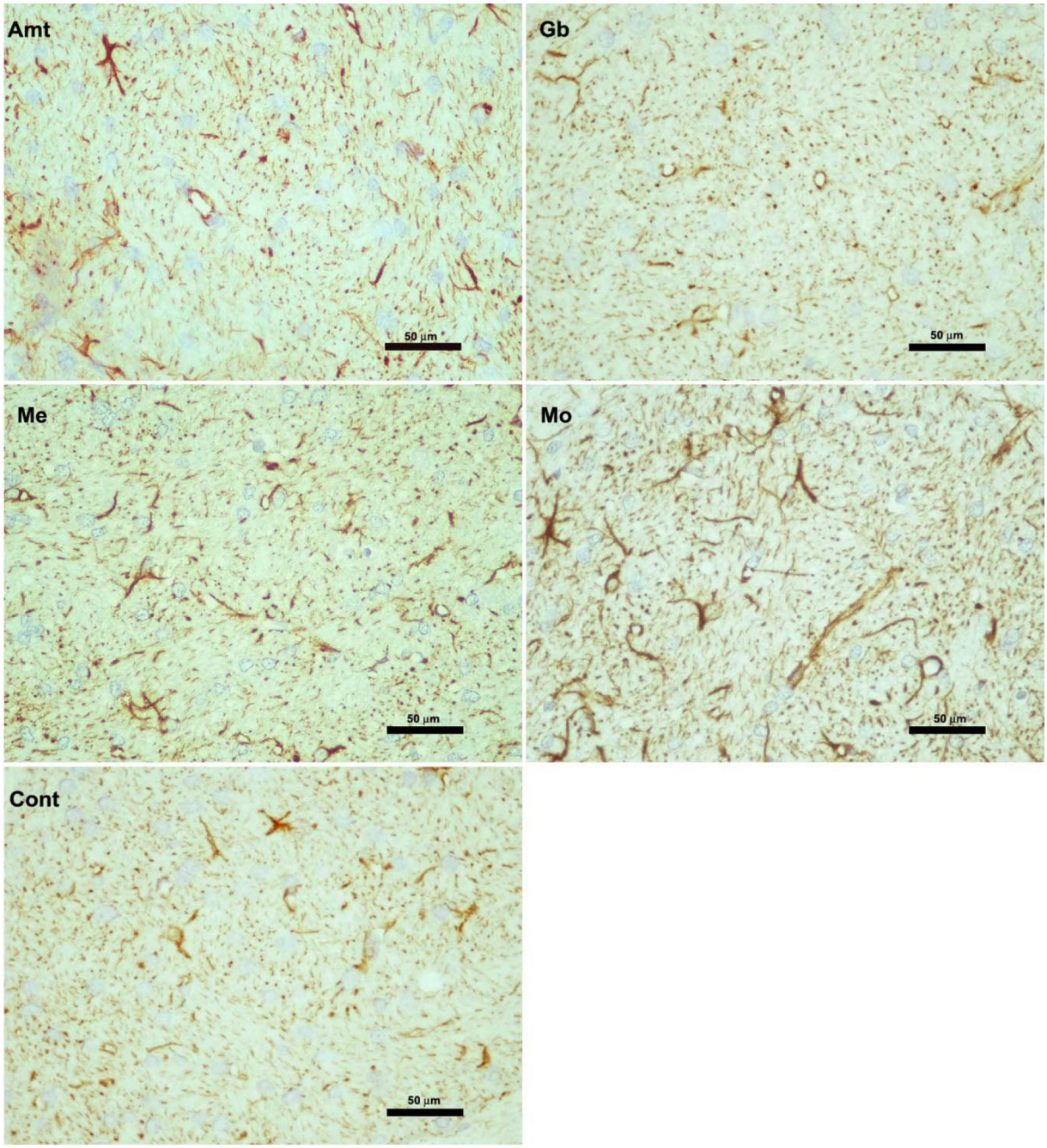

FIGURE 6 - Astrocytic immunostaining for GFAP in the core region of the NAc of the different experimental groups. Amt - amitriptyline; Gb - gabapentin; Me - methadone; Mo - morphine; Cont - control group. Bar = 50 µm.

The PAG of the mesencephalon has been reported to be one of the primary neural foci for the action of centrally administered Mo in eliciting the suppression of splenic NK cell activity. This immunosuppression has been hypothesized to be due to the activation of the hypothalamic-pituitary-adrenal axis or of the sympathetic nervous system or both. Although PAG is involved in the regulation of many physiological functions including pain, fear and anxiety, autonomic regulation, vocalization, aggressive and reproductive behavior, the relationship between PAG and immunity remains obscure (Suo, Weber, 1998; Vallejo, De Leon-Casarola, Ramsun, 2004).

GABAergic medications, such as Gb, appear to reduce the reinforcing effects of cocaine by attenuating cocaine-induced dopamine release in the NAc and corpus striatum (Gerasimov *et al*., 2001; González *et al.*, 2007). Gabapentin (Gb) also enhances the antinociceptive effect

of Mo and attenuates Mo tolerance probably through up-regulation of the anti-inflammatory cytokine IL-10 and inhibition of proinflammatory cytokines in the rat spinal cord (Lee *et al*., 2013; Bao *et al*., 2014); effects that may explain the decreased TNF-α levels in the Gb-treated rats from our investigation. Rossi and colleagues (2013) demonstrated that Gb also reduced reactive gliosis and dendritic loss caused by glutamate excitotoxicity after pilocarpine-induced status epilecticus.

Administration of Amt in association with Mo not only has preserved the antinociceptive effect of Mo, but also attenuated astrocyte activation in the rat spinal cord dorsal horn (Huang *et al*., 2012). It also up-regulated glutamate transporters in Mo-tolerant rats, attenuating Mo tolerance, and suppressed neuroinflammation in this site by inhibiting pro-inflammatory cytokine (TNF-α, IL-1β, IL-6) expression (Tai *et al*., 2006). In vitro studies with human whole blood have also reported that TCAs (e.g. imipramine and clomipramine) and selective serotonin reuptake inhibitors (e.g. citalopram and sertraline) were able to inhibit the production of the proinflammatory cytokines IL-1, IL-2, IL-6, TNF-α, IFN-γ, while stimulating the negative immunoregulatory cytokine IL-10 (Kenis, Maes, 2002; Schiepers, Wichers, Maes, 2005). Our results showed that Amt did not affect TNF-α and IL-1β levels in serum. Although there are many conflicting results in the literature, the lack of effects of antidepressants on cytokine release in humans was also found by Anisman *et al.* (1999) and Rothermundt *et al.* (2001), in relation to IL-1β production, and by Landmann *et al.* (1997) for TNF-α.

Glial fibrillary acidic protein (GFAP) is the main intermediate filament protein in mature astrocytes, and up-regulation of its expression is one of the main characteristics of the astrocyte reaction commonly observed after CNS injury (Middeldorp, Hol, 2011). The exact function of GFAP remains obscure, despite the huge number of studies using it as a marker for astrocytes; however, it has been suggested that GFAP helps astrocytes to maintain mechanical strength, as well their shape (Middeldorp, Hol, 2011). TNF-α, a pluripotent cytokine that is reportedly mitogenic to astrocytes, is associated with the overexpression of GFAP, probably via activation of the mitogen activated protein kinase (MAPK) termed Erk2 (extracellular signal-regulated protein kinase) (Zhang *et al.*, 2000). Interleukin 1 beta (IL-1β) is also identified as a potent inducer of several members of the TNF superfamily, as well as chemokines, growth factors, extracellular matrix proteins and matrix metalloproteinases, although repressing genes of the transforming growth factor (TGF) family and cytoskeleton proteins (John *et al*., 2005). Human fetal astrocyte cultures showed, after hours of stimulation with IL-1β, a morphological conversion from flat, polygonal cells to small, contracted, highly branched cells, displaying intense GFAP and vimentin immunoreactivity in the perikarya and processes.

The periaqueductal gray (PAG) matter of the midbrain plays a key role in the central analgesic system (for review, see Ossipov, Dussor, Porreca, 2010). In our study, GFAP immunoreactivity in the mesencephalon tended to diminish in rats treated with Amt, Gb, Me and Mo compared to the control group. Lazriev *et al.* (2001) showed that high doses of Mo appeared to decrease the total length of astrocyte processes and branching of individual astrocytes in the caudate nucleus. However, it induced hyperplasia and elongation of astrocytic processes in the NAc and lateral septal nucleus.

In contrast, Song and Zhao (2001) demonstrated that chronic Mo administration caused glial activation in the spinal cord and brain, and co-administration of Mo with a glial activation inhibitor resulted in maintenance of analgesic efficacy and a corresponding reduction of glial activation. They linked for the first time opioid-induced glial activation with the development of tolerance. A dose of 50 mg/kg/day (a dose five times greater than the dose used in our investigation) by the IP route was used for nine days to induce systemic tolerance and on the tenth day its analgesic effect disappeared as detected by behavioral testing. In these systemically Mo-tolerant rats, GFAP immunostaining was increased significantly in the spinal cord, posterior cingulate cortex and hippocampus, but not in the thalamus and this increase was attributed primarily to hypertrophy of astrocytes rather than their proliferation or migration since counts of astroglial cells demonstrated no significant difference compared with the control group. It is important to notice that, in contrast with the investigation performed by Song and Zhao (2001) with Mo, our investigation has used (for all drugs) doses that are equivalent to the therapeutic ones and no noxious stimulus was applied to produce pain.

The most characterized change in brain neurochemistry caused by drugs of abuse is an increase in extracellular dopamine levels in the NAc of the basal forebrain, and the findings of many studies suggest that this plays a pivotal role in the rewarding and positive reinforcing effects of μ-agonists, like Mo (Olds, 1982; Pontieri, Tanda, Di Chiara, 1995) and Me (Di Chiara, Imperato, 1988). Di Matteo *et al.* (2000) showed that administration of Amt also increased dopamine release in the rat NAc, suggesting the possible involvement of serotonin $5HT_{2C}$ receptors. Peng *et al*. (2008), in turn,

failed to demonstrate any alteration of the dopamine level within the NAc using Gb or even after cocaine self-administration, cocaine-triggered relapse and cocaine-enhanced NAc dopamine in rats. Our results suggest that drugs which, according to previous studies, exhibit the capacity of increasing dopamine concentrations in the NAc, such as Amt, Me and Mo, also induced an increased GFAP immunoreactivity in this region, probably by enhancing the astrocyte activity of clearing excess dopamine. It is recognized that this neurotransmitter may be actively and specifically removed from the extracellular space by astrocytes and neurons through (1) dopamine transporters and, afterwards, either recycled into vesicles or (2) metabolized by the glial/neuronal enzymes monoamino oxidase B (MAO B) and catechol-O-methyl transferase (COMT) (Karakaya, Kipp, Beyer, 2007). Excess dopamine can cause auto-oxidation to ortho-quinone and consequently oxidative damage and schizophrenia-like symptoms (Whitehead *et al*., 2001).

CONCLUSION

Serum TNF-α levels decreased in the groups that received short-term doses of Mo, Me and Gb, but not in the Amt-treated group. On the other hand, IL-1β has decreased only in rats treated with Me. As for GFAP expression, all drugs decreased astrocyte immunostaining in the mesencephalon, although Amt, Me and Mo caused increased GFAP reactivity in the NAc. Thus, it can be assumed that the pain relievers Gb, Me and Mo (even with the therapeutic doses that were employed in this study) impaired IL-1β and/or TNF-α release. On the other hand, all drugs seemed to modify astrocytic expression of GFAP, but the effect varied according to the region observed (Amt, Gb, Me and Mo decreased this expression in the mesencephalon, while Amt, Me and Mo increased it in the NAc, probably due to the augmented activity of astrocytes in removing the excess of dopamine).

It is important to notice that the pain relievers used in this investigation affected astrocyte behavior and cytokine release in healthy rats that were not submitted to painful conditions, even though it was not possible to determine which cells had the cytokine production impaired. Astrocytes appeared to phenotypically react to distinct classes of pain modulators, probably influencing neurons and the entire CNS microenvironment in physiological conditions. Further studies must be performed in order to make clear the effects of these pain relievers on astrocytes from other CNS regions, both in the absence and in the presence of noxious stimuli.

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