Brain Research Bulletin 86 (2011) 326–333

Contents lists available at SciVerse ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Regulation of CCL2/MCP-1 production in astrocytes by desipramine and atomoxetine: Involvement of α2 adrenergic receptors

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ARTICLE INFO

Article history:
Received 20 July 2011
Received in revised form
12 September 2011
Accepted 19 September 2011
Available online 24 September 2011

Keywords:
CCL2
MCP-1
Astrocytes
Noradrenaline
Atomoxetine
Desipramine

ABSTRACT

Having previously observed that noradrenaline activation of β adrenergic receptors induces the synthesis of the chemokine monocyte chemoattractant protein (CCL2/MCP-1) in astrocytes, it is our interest to analyze the mechanisms involved in this process, particularly the possible effect of noradrenaline-modulating drugs.

The treatment of primary rat astrocyte cultures with the noradrenaline transporter inhibitors desipramine or atomoxetine induced the expression and synthesis of CCL2/MCP-1 in these cells. This effect of both drugs in vitro suggests that CCL2/MCP-1 expression could also be modulated by some mechanism independent of the elevation of brain noradrenaline levels. This was confirmed by measuring a reduction in CCL2/MCP-1 production by the treatment with the α2 adrenergic receptor agonist clonidine. Accordingly, the blockade of α2 adrenergic receptors with yohimbine potentiated the production of MCP-1 stimulated by the activation of β receptors.

While the activation of β adrenergic receptors and the subsequent elevation of cAMP levels seem to be the main pathway for noradrenaline to induce CCL2/MCP-1 in astrocytes, our data indicate that the α2 adrenergic receptors also regulate CCL2/MCP-1 expression working as inhibitory mediators.

1. Introduction

The monocyte chemoattractant protein (CCL2/MCP-1) has been well characterized as a key regulator of the attraction of monocytes and other types of cells involved in inflammatory/immune processes [12]. Because of this, it has been linked to the development of inflammation [11,22,51,10] and some studies have shown the possibility to reduce neuronal damage by blocking its action [56,60,32]. However, during the last years, new functions have been attributed to MCP-1 and pointed that there may still be a large number of facts about this chemokine that remain unknown.

The expression of MCP-1 is known to require the translocation of the transcription factors activator protein-1 (AP1) and nuclear factor-kappa B (NFkB) [1,15,33,58]. These factors can be activated by cytokines or other agents of different nature [3,26]. Based on this, the putative modulators of MCP-1 cannot be easily predicted. In fact, our previous finding of astrocytic MCP-1 up-regulation by noradrenaline was rather unexpected considering the anti-inflammatory profile of noradrenaline in brain [41,14] and the pro-inflammatory potential commonly attributed to MCP-1.

Having observed this, it became a priority for us to elucidate the mechanisms activated by noradrenaline that lead to MCP-1 expression. This way we confirmed that the activation of β adrenergic receptors and the subsequent elevation of cAMP is one of the main pathways through which noradrenaline activates MCP-1 promoter and initiates its expression in astrocytes [37]. Our next goal was to analyze if these phenomena translate from the astrocyte culture to the whole brain. This way, we treated rats with a noradrenaline precursor (L-DOPS) and found that the elevation of brain noradrenaline levels results in the augmented expression of MCP-1 in astrocytes [38], the cell type described as the main producers of MCP-1 [1,21]. Based on the neuroprotective actions of MCP-1 previously described by us [37] and others [6,13,49] it became interesting to explore the potential therapeutic use of noradrenaline-elevating drugs, particularly if these drugs can also modulate brain MCP-1. For this purpose we decided to use desipramine, an antidepressant known to elevate brain noradrenaline levels, and observed that its administration to rats induces the expression of MCP-1 in brain cortex [38].

While this effect could be due to the elevation of brain noradrenaline levels, the existence of other noradrenaline-independent mechanisms cannot be discarded, particularly

Abbreviations: CCL2/MCP-1, monocyte chemoattractant protein; PBS, phosphate buffered saline; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; NA, noradrenaline; NAT, noradrenaline transporter.

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doi:10.1016/j.brainresbull.2011.09.014

considering the presence of α and β adrenergic receptors in astrocytes [25,30,42,45].

In order to clarify this possibility, in the current study astrocyte cultures have been incubated with desipramine or atomoxetine (another noradrenaline reuptake inhibitor) observing the induction of MCP-1 in both cases. The main uses of these two drugs are related to their ability to elevate the extracellular noradrenaline concentration, but some other effects can be caused by their interaction with certain cell receptors. In fact, it has been proposed that postsynaptic α2 receptors play a key role in desipramine effects [61]. In order to analyze this possibility, the control of MCP-1 production by α2 modulating drugs was evaluated.

2. Materials and methods

2.1. Reagents

Cell culture reagents and fetal calf serum (FCS) (<10 EU endotoxin per ml) were from GIBCO Life Technologies (Carlsbad, CA, USA). Noradrenaline, desipramine hydrochloride, atomoxetine, yohimbine, clonidine and isoproterenol were from Sigma (St. Louis, MO, USA). Antibody against MCP-1 was from R&D SystemsSerotec (Minneapolis, MN, USA). Secondary antibodies were from Jackson ImmunoResearch (West Grove, PA, USA). Taq polymerase and cDNA synthesis reagents were from Biotools (Madrid, Spain).

2.2. Astrocyte cultures

Rat cortical astrocytes were obtained as described previously [36]. All experimental protocols adhered to the guidelines of the Animal Welfare Committee of the Universidad Complutense (permission SAF2010-21984) in accordance with the European Communities (86/609/EEC) Laws for the Care and Use of Laboratory Animals. Briefly, 1-d-old Wistar rats (Harlan) were used to prepare primary mixed glial cultures; microglia were detached by gentle shaking after 11–13 days in culture, astrocytes were prepared by mild trypsinization of the remaining cells, replated at 1×10^6 cells/ml onto 96 (0.1 ml) or 12 well (1 ml) plates and consisted of >95% astrocytes as determined by staining for GFAP and <5% microglial as determined by staining with the specific marker OX-42.

2.3. mRNA analysis

Total cytoplasmic RNA was prepared from cells using TRIZOL reagent (Invitrogen); aliquots were converted to cDNA using random hexamer primers. Quantitative changes in mRNA levels were estimated by real time PCR (Q-PCR) using the following cycling conditions: 35 cycles of denaturation at 95 °C for 10 s, annealing at 58–61 °C for 15 s depending on the specific set of primers, and extension at 72 °C for 20 s. Reactions were carried out in the presence of SYBR green (1:10,000 dilution of stock solution from Molecular Probes, Eugene, OR, USA), carried out in a 20-μL reaction in a Corbett Rotor-Gene (Corbett Research, Mortlake, NSW, Australia). The primers used for MCP-1 were: forward: 5′-TGC TGT CTC AGC CAG ATG CAG TTA-3′ and reverse: 5′-TAC AGC TTC TTT GGG ACA CCT GCT-3′. The primers used for IL-10 were: forward: 5′-ATA ACT GCA CCC ACT TCC CAG TCA-3′ and reverse: 5′-ACA AGG CTT GGC AAC CCA AGT AAC-3′. The primers used for IL-12p40 were: forward: 5′-ACT CAC ATC TGC TGC TCC ACA AGA-3′ and reverse: 5′-TCC GGA GTA GTT TGG TGC TTC ACA-3′. The primers used for GAPDH were: forward: 5′-TGC ACC ACC AAC TGC TTA GC-3 and reverse: 5′-GGC ATG GAC TGT GGT CAT GAG-3′. Relative mRNA concentrations were calculated from the take-off point of reactions using included software, and GAPDH levels used to normalize data

2.4. MCP-1 measurement

MCP-1 levels in the culture medium were detected using a specific ELISA for rat MCP-1, according to manufacturer's instructions (R&D Systems Inc.). The assay detection limits were of 31.3–2000 pg/ml.

2.5. MCP-1 immunocytochemistry

Astrocytes on glass coverslips were rinsed with cold PBS and fixed with 4% paraformaldehyde for 10 min at RT. Slides were washed for 5 min with PBS and blocked with 5% normal goat serum in PBS at room temperature for 30 min. Primary antibodies were diluted in 1% normal goat serum in PBS. Cells were incubated with primary antibodies (rabbit anti-MCP-1, 1:1000 dilution) at 37 °C for 1 h. After this, the slides were washed three times with PBS and incubated with the correspondent secondary antibody (diluted 1:1000 in PBS with 1% normal goat serum) for 1 h at 37 °C. Slides were washed three times for 5 min with PBS and postfixed in 3.7% formaldehyde in PBS for 20 min. Autofluorescence was quenched with 50 mM NH_4Cl in PBS for 15 min. ProLong Gold antifade reagent with DAPI (Invitrogen) was used as mounting medium.

2.6. Data analysis

All experiments were done at least in triplicate. When more than two experimental groups were present in the same experiment, data were analyzed by one-way ANOVA followed by Newman–Keuls multiple comparison tests and p values <0.05 were considered significant. When two experimental groups were present in the same experiment, data were analyzed by unpaired t-tests, and p values <0.05 were considered significant.

3. Results

3.1. Desipramine and atomoxetine induce MCP-1 expression in astrocyte cultures

Our previous work demonstrated that the in vivo treatment with desipramine elevates the concentration of MCP-1 in rat brain cortex [38]. Since noradrenaline induces MCP-1 in astrocytes [37], this effect of desipramine could be attributed to the elevation of brain noradrenaline levels caused by the inhibition of its reuptake. However, in order to ascertain if other alternative mechanisms are involved, isolated astrocytes were treated with desipramine or with atomoxetine, a structurally different drug also known to inhibit noradrenaline reuptake.

The incubation of primary rat astrocytes with different concentrations of desipramine (Fig. 1a) or atomoxetine (Fig. 2a) for 24 h caused a concentration-dependent elevation of MCP-1 concentration in the culture media.

Desipramine seemed to be more effective since higher concentrations of atomoxetine were necessary to achieve a significant increase in the concentration of MCP-1.

This increase was associated with a higher amount of MCP-1 mRNA, as assessed by quantitative PCR (Figs. 1b and 2b). These observations were further supported by immunocytochemistry studies performed on astrocyte cultures for MCP-1; following the same experimental conditions as described above, we observed an accumulation of MCP-1 in cells treated with desipramine or atomoxetine (Fig. 3).

Since desipramine treatment has been shown to elevate the production of IL-10 in mice plasma [48], we also analyzed the expression of this anti-inflammatory cytokine and of the pro-inflammatory one IL-12 in our experimental conditions. The 24 h incubation of astrocytes with 10 μM desipramine or atomoxetine did not cause significant changes of IL-10 or IL12p40 mRNA levels (Figs. 1c and 2c).

3.2. Activation of α2 adrenergic receptors inhibits MCP-1 production

Since desipramine and atomoxetine also induce MCP-1 production in isolated astrocytes in the absence of noradrenaline or noradrenergic neurons, we can conclude that this effect is independent of their ability to inhibit noradrenaline uptake. Based on this, we searched for different pathways which could participate in this process. Since the α2 type of adrenergic receptors seem to play an important role on the mechanisms of action of desipramine [61], we analyzed if the modulation of its action affects MCP-1 expression. To evaluate this, astrocyte cultures were treated with noradrenaline together with different concentrations of the α2 agonist clonidine. The elevation of MCP-1 caused by noradrenaline was reduced in a concentration dependent manner by clonidine (Fig. 4).

3.3. Yohimbine treatment potentiates MCP-1 induction

The effect of a β adrenergic receptor agonist such as isoproterenol was assessed, and we could observe its concentration-dependent induction of MCP-1 production in astrocyte cultures (Fig. 5a). Having established this, astrocytes were incubated with