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Acute mechanical sensitization of peripheral nociceptors by aldosterone through non-genomic activation of membrane bound mineralocorticoid receptors in naive rats

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ABSTRACT

Recently, there is increasing interest in the role of peripheral mineralocorticoid receptors (MR) to modulate pain, but their localization in neurons and glia of the periphery and their distinct involvement in pain control remains elusive. In naive Wistar rats our double immunofluorescence confocal microscopy of the spinal cord, dorsal root ganglia, sciatic nerve and innervated skin revealed that MR predominantly colocalized with calcitonin-gene-related peptide (CGRP)- and trkA-immunoreactive (IR) nociceptive neurons and only marginally with myelinated trkB-IR mechanoreceptive and trkC-IR proprioceptive neurons underscoring a pivotal role for MR in the modulation of pain. MR could not be detected in Schwann cells, satellite cells, and astrocytes and only scarcely in spinal microglia cells excluding a relevant functional role of glia-derived MR at least in naïve rats. Intrathecal (i.t.) and intraplantar (i.pl.) application of increasing doses of the MR selective agonist aldosterone acutely increased nociceptive behavior which was reversible by a MR selective antagonist and most likely due to non-genomic effects. This was further substantiated by the first identification of membrane bound MR specific binding sites in sensory neurons of dorsal root ganglia and spinal cord. Therefore, a crucial role of MR on nociceptive neurons but not on glia cells and their impact on nociceptive behavior most likely due to immediate non-genomic effects has to be considered under normal but more so under pathological conditions in future studies.

1. Introduction

Mineralocorticoid receptors and their endogenous ligand aldosterone are best known for their control of the water and electrolyte balance in the kidney and their involvement in volume and blood pressure regulation (Te Riet et al., 2015). In addition, aldosterone has also been reported to promote inflammation, fibrosis, and remodeling in the heart and vasculature (Ferrario and Schiffrin, 2015; Young and Rickard, 2012). Interestingly in older studies, a combination of aldosterone and its antagonist spironolactone revealed an immunosuppressive effect in allogenic skin grafts (Baethmann et al., 1971), multiple sclerosis (Mertin et al., 1972), and progressive systemic sclerosis (Altmeyer et al., 1985). All these effects occur by the classical pathway at which aldosterone easily diffuses the cellular membrane, binds to its cytoplasmic MR and – upon dissociation of chaperons and formation of MR dimers - is translocated to the nucleus resulting in the enhanced or inhibited expression of several genes (Te Riet et al., 2015). The effects of aldosterone via intracellular MR are usually characterized by a 45-min up to several hours lag period (Funder, 2005). In contrast to these genomic effects more rapid non-genomic effects have also been demonstrated particularly in neurons of the nervous system (Groeneweg et al., 2012). Corticosteroids for example rapidly alter neuronal excitability throughout the brain and as a consequence regulate adaptive behavior and memory (Groeneweg et al., 2012).

Recently, increasing interest has focused on the role of MR in different conditions of pain. In a model of chronic compression of

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the L5 lumbar dorsal root ganglion the twice daily intrathecal injection of the MR antagonist spironolactone over three days resulted in a significant reduction of mechanical allodynia (Gu et al., 2011; Sun et al., 2012). In another model of zymosan-induced local inflammation of the L5 dorsal root ganglion, the combined local application of zymosan with the MR antagonist eplerenone reduced for an extended period of time the mechanical hypersensitivity (Dong et al., 2012). In the same study MR were shown in dorsal root ganglia to colocalize with the pan-neuronal cell marker NeuN and eplerenone treatment apparently decreased the number of activated satellite glia cells. Since these effects occurred with some delay and lasted several days, they were most likely due to genomic effects of the MR. Up to now, evidence for the exact subpopulations of DRG neurons and glia cells that express MR and for putative short lasting non-genomic effects resulting from neuronal membrane bound MR is lacking.

Therefore, we systematically investigated in naive rats i) the presence of MR specific mRNA and receptor protein in spinal cord, dorsal root ganglia and innervating sciatic nerve compared to kidney; ii) the localization of MR in neurons, astrocytes and microglia of the spinal cord, iii) the localization of MR to myelinated, unmyelinated, nociceptive, mechanoreceptive and proprioceptive neurons in dorsal root ganglia; iv) the characterization of MR-ir nerve terminals in the subepidermal and epidermal layer of the skin; v) the changes in nociceptive behavior following the local i.pl. or i.th. administration of a MR selective agonist with and without antagonist; vi) the evidence for membrane bound MR by saturation binding with the radiolabeled MR selective ligand [^{3}H] aldosterone.

2. Methods

2.1. Reagents

The following drugs were used: aldosterone, canreonate-K (Sigma-Aldrich, St. Louis, MO, USA); doses were calculated - where applicable - in terms of the free base. Canreonate-K was dissolved in NaCl 0.9%, aldosterone was dissolved in a vehicle composed of 10% ethanol and 90% normal saline, as described previously (Gravez et al., 2013). Routes and volumes of drug administration were i.t. 20 μL and i.pl. 100 μL. Intrathecal injections were performed under inhalational anesthesia with the rat in the elevated lumbar position. Intraplantar injections were given under inhalational anesthesia into the subcutaneous tissue of the glabrous skin directly proximal to the callosities of the toes. The drug or its solvent were injected into the intrathecal L3-L4 interspace (spontaneous tail movement being a positive indication for correct i.t. positioning) with a 30-gauge needle connected to a 50 μL syringe. In accordance with previous studies (Myers and Van Meerveld, 2009; Khan and Bakshi, 2009), separate groups of animals for each dose and injection technique received i.pl. or i.t. administrations of different doses of: aldosterone (i.pl. 25–100 μg or i.t. 4–40 μg) with and without canreonate-K (150–500 μg). Control animals received vehicle treatment. Experiments were performed in a blinded way to the drugs and doses applied.

2.2. Animals

Experiments were conducted in male Wistar rats (200–250 g) (breeding facility, Charité-Universitätsmedizin Berlin, Germany) after approval by the local animal care committee and in accordance with the European Directive on the protection of animals used for scientific purposes (2010/63/EU).

2.3. Characterization of antibodies

The species, sources, dilutions, and immunogens of the primary antibodies used in this study are summarized in Table 1.

2.4. Tissue preparation

Rats were deeply anesthetized with isoflurane and the subcutaneous paw tissue, sciatic nerve, dorsal root ganglia, spinal cord, and kidney were removed from adult rats for subsequent qRT-PCR and western blot experiments.

2.5. RT-PCR

The total RNA was prepared from kidney, DRG and spinal cord of rats with the commercially available Kit Qiazol Lysis Reagent, (Qiagen, Hilden, Germany) according to the manufacturer's protocol. 500 ng total RNA, measured by Nanodrop (Peqlab) was applied for transcription of cDNA using Omniscript RT Kit (Qiagen, Hilden, Germany) as followed: 0.5 mM dNTP, 1 μM Random Primer, 10 units RNase Inhibitor and 4 units Omnisript reverse Transcriptase. Samples were incubated at 42 °C for 1 h and cDNA were stored

Table 1
Characterization of primary antibodies used.

Antigen	Immunogen	Manufacturer, species, type, catalogue number	Dilution used
MR	a 142-amino-acid peptide sequence from the unique DNA-binding domain of the rat MR gene	a gift from M. Kawata (Kyoto Prefectural University of Medicine, Japan), rabbit polyclonal, # Ito et al., 2000	1:2000
MR	amino acids 1–300 of the human MR that is recommended for the detection of mouse, rat and human MR	Santa Cruz Biotechnology (USA), rabbit polyclonal, # sc-11412, Kapoor et al., 2008	1:1000
Calcitonin-Gene Related Peptide	synthetic entire calcitonin gene-related peptide	Peninsula Laboratories (CA, USA), guinea pig polyclonal, # T-5027, Mousa et al., 2013	1:1000
trkA	extracellular domain Ala33-Pro418 of rat trkA	R&D Systems (USA), goat polyclonal, # AF1056, Matsumoto et al., 2012	1:1000
NF200	carboxy terminal tail segment of dephosphorylated NF200	Sigma-Aldrich (USA), mouse monoclonal # N0142/N52, Kestell et al., 2015	1:1000
trkB	extracellular domain Cys32-Thr429 of recombinant mouse trkB	R&D Systems (USA), goat polyclonal, # AF1494, Matsumoto et al., 2012	1:1000
trkC	extracellular domain Cys32-Thr429 of recombinant mouse trkC	R&D Systems (USA), goat polyclonal, # AF1404, Matsumoto et al., 2012	1:1000
GFAP	clone G-A-5	Sigma-Aldrich (USA), mouse monoclonal # G3893, Liu and Chien, 2012	1:1000
CD11b	clone OX-42	AbD Serotec (Germany), mouse monoclonal # MCA275R, Robinson et al., 1986	1:1000