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Anandamide enhances expression of heat shock proteins Hsp70 and Hsp25 in rat lungs

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ABSTRACT

Anandamide (AEA), an endogenous cannabinoid and vanilloid receptor ligand, possesses anti-inflammatory properties. Transport of AEA through cytoplasm is facilitated by heat shock protein (HSP) Hsp70, which enhances the rate of cellular AEA uptake, possibly via direct interactions. In lungs, increased HSP expression is an endogenous, protective mechanism against acute lung inflammation. We hypothesised that AEA could enhance the expression of cytoprotective Hsp70 and Hsp25. Anaesthetised rats were injected intravenously with 1 mg/kg AEA or saline. Lungs were removed 2 and 24 h after injection for evaluation of HSP expression. Hsp70 and Hsp25 expression in lungs was evaluated by immunohistochemistry. The relative levels of these HSPs in lung sections were determined through optical density measurements and western blotting of lung homogenates. Western blot and immunohistochemistry analyses indicated that expression of both proteins was significantly higher in AEA-injected animals than in control animals 2 and 24 h after treatment. AEA administration enhanced Hsp70 and Hsp25 expression in lungs. Therefore, AEA-HSP interactions could be involved in mechanisms protecting against lung inflammation, indicating a possible use of AEA as a treatment for lung inflammation.

1. Introduction

Heat shock proteins (HSPs), also commonly referred to as stress proteins, are a group of highly conserved proteins that are found in every cellular compartment, including the nucleus, cytoplasm, and mitochondria. HSPs are classified according to their molecular weight, e.g., Hsp25 and Hsp70, and are induced in response to thermal stress and a wide variety of non-thermal stressors including endotoxemia/sepsis (Hauser et al., 1996; Ryan et al., 1992), acute lung injury (Hiratsuka et al., 1998; Villar et al., 1993), ischemia-reperfusion injury (Hiratsuka et al., 1998), and pharmacological agents such as nonsteroidal anti-inflammatory drugs (Wheeler and Wong, 2007). Increased expression of HSPs results in cytoprotective responses in several cell types such as alveolar epithelial cells (Wheeler and Wong, 2007).

Anandamide (arachidonyletanolamide, AEA), one of several endogenous cannabinoid (CB_1 and CB_2) receptor ligands (Devane et al., 1992; Matsuda et al., 1990; Munro et al., 1993), acts as an endovanilloid by binding the transient receptor potential (TRP) vanilloid type 1 ($TRPV_1$) (Smart et al., 2000; Starowicz et al., 2007; Zygmunt et al., 1999). AEA may elicit these effects through GPR55, a purported CB_3 receptor (Lauckner et al., 2008). Due to the multitude of receptors that AEA can activate, AEA likely has modulatory roles in the nervous, immune, cardiovascular, respiratory, and reproductive systems (Di Marzo, 2008; Klein, 2005; Kopczyńska, 2007; Piomelli, 2003; Wang et al., 2006).

Recently, Oddi et al. (2009) examined the mechanisms of intracellular transport of AEA. Transport of AEA through the cytoplasm was proposed to be facilitated by Hsp70, which enhanced the rate of the cellular uptake of AEA. The enhanced uptake of AEA may be a result of its direct interaction with Hsp70. Hsp70 has several extracellular functions, some of which involve immunoregulation, cellular homeostasis, and protection. One of the most important Hsp70 family members is BiP (HSPA5), which is a key endoplasmic reticulum-lumenal molecular chaperone involved in the unfolded-protein response (Henderson and Pockley, 2010). BiP blocks antigen presentation and induces leukocyte anti-inflammatory profiles, including IL-10 and TNFRII (Corrigall et al., 2004), somewhat resembling the actions of Hsp27.

Hsp27 is a potential therapeutic molecular chaperone (Henderson and Pockley, 2010). This small ATP-independent protein is a member of the small HSP group and has several important intracellular functions (Kostenko and Moens, 2009). Extracellular recombinant Hsp27 activates human monocytes to a state characterised by the overproduction of IL-10 relative to TNF-alpha, suggesting that Hsp27 has anti-inflammatory properties (De et al., 2000). Hsp25 (rat analogue of Hsp27) can exert protective effects during a variety of

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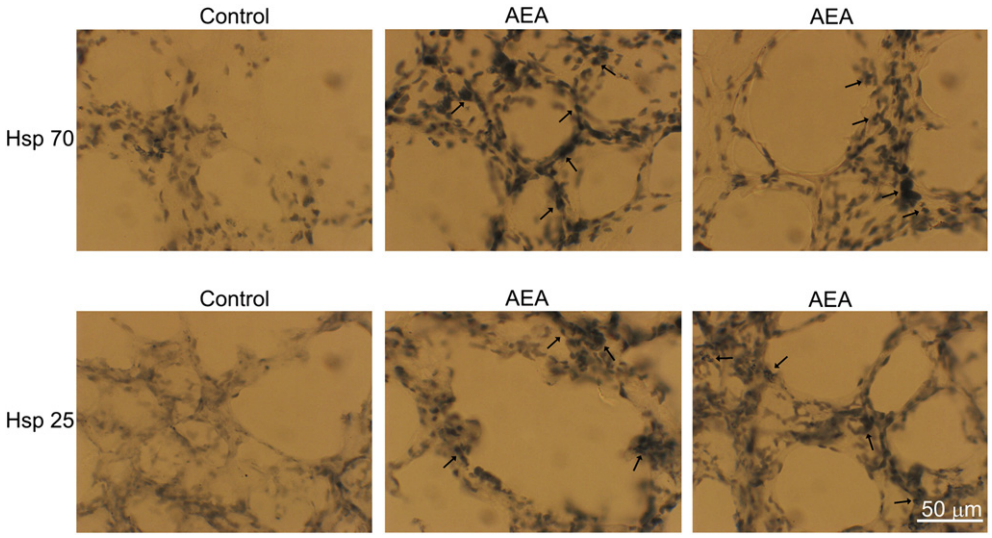

Fig. 1. Hsp70 and Hsp25 immunoreactivity in control and anandamide (AEA)-treated rat lungs. Rats were intravenously injected with 1 mg/kg AEA or saline alone. Lungs were removed, sectioned, and stained for Hsp70 and Hsp25 expression 2 and 24 h after treatment. SG blue was used as the chromogen. Representative images are shown. Arrows demonstrate the increase of Hsp70 and Hsp25 immunoreactivity in lungs 2 (middle panel) and 24 h (right Panel) after AEA injection.

stress situations (Schwarz et al., 2010). HSP25/27 can confer resistance to apoptotic stimuli and may protect the cytoskeleton during stress (Richter-Landsberg and Goldbaum, 2007).

The present experiments were designed to test the hypothesis that AEA, as an anti-inflammatory drug (Klein, 2005; Pacher et al., 2006), can enhance the expression of cytoprotective Hsp70, through interactions with Hsp70 and Hsp25, a protein highly-inducible in lungs following exposure to a variety of stressors (Arrigo, 2001; Hastie et al., 1997; Meredino et al., 2002; Wu et al., 1999).

2. Materials and methods

Ethical approval for the experimental procedures used in this study was obtained from the local animal care committee. All animal procedures were performed in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Twenty-one adult male Sprague Dawley rats (180–200 g body weight) were anaesthetised with an intraperitoneal (i.p.) injection of 10% ketamine (90 mg/kg) (Bioketan, Vetoquinol Biovet) and 2% xylazine (10 mg/kg) (Rometar, Bioveta) in saline solution.

Animals were housed in a temperature- and humidity-controlled environment (20 C and 60–70% relative humidity) with a 12-h/12-h light/dark cycle, and were supplied standard food for laboratory rodents (Ssniff M-Z, ssniff Spezialdiäten GmbH, Soest, Germany) and purified tap water ad libitum.

2.1. Lung histology

AEA ($C_{22}H_{37}NO_2$, Tocris) was injected intravenously (i.v.) into experimental rats at a dose of 1 mg/kg, based on previously published methods (Kopczyńska, 2007). A control group was i.v. injected with the solvent (Kopczyńska, 2007). Rats were re-anaesthetised at 2 and 24 h post-injection.

The lungs of the first group of control and AEA-treated rats were removed after 2 h and the lungs of the second group (controls and AEA-treated) were removed after 24 h. Lungs were fixed in 4% formaldehyde, cryoprotected with a 10–30% solution of saccharose in

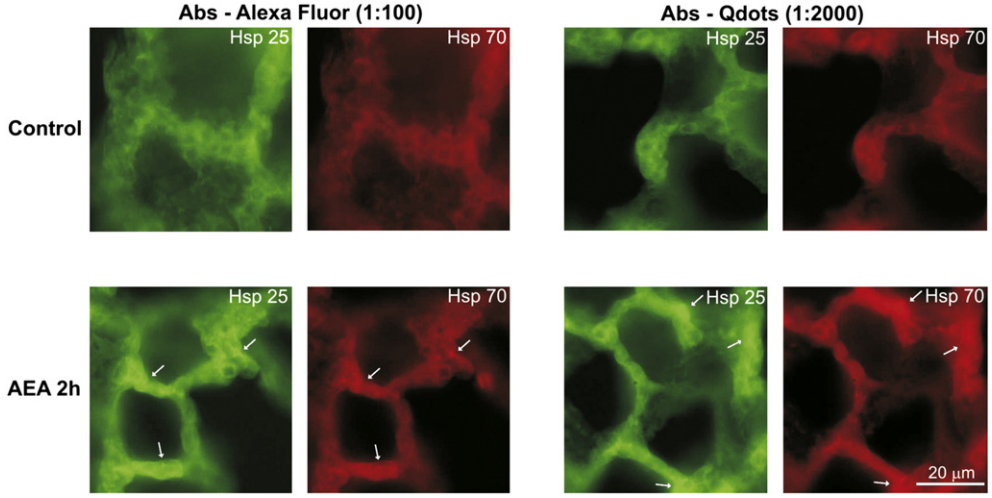

Fig. 2. Immunofluorescent double-staining for Hsp25 (green) and Hsp70 (red) in rat lung sections. Comparison of two immunohistochemical methods using Alexa Fluor (left panel) and Qdots (right panel) as fluorescent dyes. Microphotographs show sections of lung tissues from control and anandamide-treated rats 2 h after drug administration. Note the rise of Hsp70 and Hsp25 immunoreactivity in lungs 2 h after AEA injection (arrows).