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# Inflammation and peripheral 5-HT$_7$ receptors: The role of 5-HT$_7$ receptors in carrageenan induced inflammation in rats

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ABSTRACT

The aim of this study was: (1) to investigate possible role for 5-HT$_7$ receptors in carrageenan induced inflammatory paw oedema in rats; (2) to determine the presence of 5-HT$_7$ receptors in rat paw tissue; (3) to observe the effects of 5-HT$_7$ receptor agonist and antagonist administration on inflammation; and (4) to determine a unique mechanism for inflammatory processes via 5-HT$_7$ receptors. Effects of 5-HT$_7$ receptor agonist, antagonist and indomethacin were investigated in carrageenan induced paw oedema in rats. Blood and tissue samples were collected and evaluated biochemically for serum cytokine levels, tissue oxidant-antioxidant balance and histopathologically for inflammatory cell accumulation. We performed Real Time PCR analyses for tissue 5-HT$_7$ receptor and COX mRNA expressions. The 5-HT$_7$ receptor agonist AS-19 exerted significant anti-inflammatory effect both alone and in combination with indomethacin. Antagonist, SB269970, did not affect inflammation alone but decreased the effects of agonist when co-administered. 5-HT$_7$ mRNA levels were higher in the carrageenan group than healthy control. Carrageenan+indometacin group decreased the mRNA expression of 5-HT$_7$ when compared to carrageenan group. While agonist administration decreased 5-HT$_7$ mRNA expression when compared to carrageenan group. Agonist decreased paw COX expression. Agonist also decreased serum cytokine levels and tissue oxidative stress. In conclusion, this study demonstrated for the first time that 5-HT$_7$ receptors are expressed in rat paw tissue and that this expression responds to inflammatory stimuli. The 5-HT$_7$ receptor may be a promising new therapeutic target for prevention of inflammation and inflammatory disorders and may also provide a new glimpse into inflammation pathophysiology.



## 1. Introduction

Inflammation is part of the beneficial anti-microbial immune defence system (Marchalonis et al., 2002) in which granulocytes act as foot soldiers and are dispatched in large numbers to overcome many challenges to the host organism (Leitch et al., 2009). The leucocytes of the granulocyte lineage, notably neutrophil and eosinophil, are key players in the immune response and inflammation.

The most common pathway targeted in anti-inflammatory therapy is the arachidonic acid pathway. For this reason, glucocorticoids, which inhibit phospholipase enzymes, are used to treat many inflammatory diseases. In contrast, nonsteroidal anti-inflammatory drugs (NSAIDs) work primarily by inhibiting the cyclooxygenase (COX) enzymes 1 and 2 (Odabasoglu et al., 2008; Suleyman et al., 2010), which convert arachidonic acid to inflammatory prostaglandins. Other recent anti-inflammatory approaches have utilized 'biological therapies' to target specific cytokines. Of these, anti-tumour necrosis factor (anti-TNF) therapy is the standout success and considerable evidence now shows that TNF-α is the sought-after essential cytokine in inflammatory disease, although other agents are also showing promise (Feldman et al., 1998; Williams et al., 2007). Consequently, this redundancy in the system almost certainly guarantees that there will always be room for new pharmacological agents that have novel targets within the inflammatory system (Hallett et al., 2008).

Abundant evidence has now been accumulated to suggest that mucosal serotonin (5-HT) modulates the immune response and

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is therefore potentially able to influence intestinal inflammation (Spiller, 2007). Several serotonergic receptors have been characterized in lymphocytes, monocytes, macrophages, and dendritic cells, which suggests a role for 5-HT in immune cell function (Cloez-Tayarani and Changeux, 2007). 5-HT is a classical neurotransmitter and vasoactive amine best known for its role in the regulation of variety of physiologic states and behaviours, including pain, appetite, mood and sleep (Mossner and Lesch, 1998). Despite these major roles for 5-HT in the central nervous system, however, the vast majority is produced in the periphery ( > 90%), primarily by enterochromaffin cells in the gut (Racke et al., 1996).

Consistent with its abundance in the periphery, 5-HT is recognized as an important inflammatory mediator with significant immune-modulatory effects (Gordon and Barnes, 2003; Meredith et al., 2005). Mast cells and platelets both express the 5-HT specific transporter, which enables them to sequester 5-HT from the microenvironment. In turn, 5-HT is released in response to injury and/or inflammatory signals, such as platelet activating factor, complement components and IgE complexes (Gordon and Barnes, 2003; Matsuda et al., 1997). It is well established that various biological effects of 5-HT are mediated through different serotonin receptors and their signal transduction pathways (MaassenVanDenBrink et al., 2008). The 5-$HT_7$ receptor is among the most recently discovered receptors for 5-HT and is also one of the least well characterized (Hedlund, 2009; Hedlund et al., 2004; Hedlund and Sutcliffe, 2004).

In situ hybridization studies, in both rat and guinea pig, have confirmed that 5-$HT_7$ receptor mRNA in brain is found predominantly in thalamus, hypothalamus (including the SCN), cerebral cortex, hippocampus and amygdala (Neumaier et al., 2001; To et al., 1995; Tsou et al., 1994). However, studies regarding the peripheral locations of these receptors are limited. These receptors have been shown in arteries (e.g. pulmonary and coronary arteries and the aorta) (Nilsson et al., 1999; Ullmer et al., 1995) and in T cells, where 5-$HT_7$ receptor stimulation contributes to T cell functions and activation (Leon-Ponte et al., 2007). Soga et al. (2007) showed that 5-HT prevents monocyte apoptosis via 5-$HT_1$ and/or 5-$HT_7$ receptors. Stefulj et al. (2000) showed the presence of 5-$HT_7$ receptor mRNA in both human and rat immune tissues such as thymus, spleen, peripheral lymphocytes and mitogen activated spleen cells . The presence of 5-$HT_7$ receptors in both human (Heidmann et al., 1997) and rat (Shen et al., 1993; Stefulj et al., 2000) immune tissues led us to hypothesize that these receptors contribute to immune responses such as inflammation. However, no study has yet investigated the effects of the 5-$HT_7$ receptor on peripheral inflammation.

The aim of the present study was therefore: (1) to investigate a possible role for 5-$HT_7$ receptors in carrageenan induced inflammatory paw oedema in rats; (2) to determine the presence of 5-$HT_7$ receptors in rat paw tissue; (3) to observe the effects of 5-$HT_7$ receptor agonist and antagonist administration on inflammation; and (4) to determine a unique mechanism for inflammatory processes via 5-$HT_7$ receptors.

## 2. Materials and methods

### 2.1. Animals

The present study used 48 male Wistar rats for all experiments. All animals weighed 200 to 220 g and were obtained from Ataturk University's Experimental Animal Laboratory of Medicinal and Experimental Application and Research Centre. Animal experiments and procedures were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by Ataturk University's local animal care committee. Rats were housed in standard plastic cages on sawdust bedding in an air-conditioned room at 22 °C under lighting controls (14-h-light/10-h-dark cycle). Standard rat chow and tap water were given ad libitum.

### 2.2. Chemicals

AS-19 ((2*S*)-*N*,*N*-dimethyl-5-(1,3,5-trimethylpyrazol-4-yl)-1,2,3,4-tetrahydronaphthalen-2-amine, 5-$HT_7$ receptor agonist) (Cat. No. 1968; TOCRIS Bioscience) and SB 269970 hydrochloride ((2*R*)-1-[(3-hydroxyphenyl)sulfonyl]-2 -(2-(4-methyl-1-piperidinyl)ethyl)pyrrolidine, 5-$HT_7$ receptor antagonist) (Cat. No. 1612; TOCRIS Bioscience) were purchased from TOCRIS. Indomethacin (IND) was obtained from Deva (Istanbul, Turkey). Carrageenan was purchased from Sigma Chemical (Munich, Germany) and all other chemicals for laboratory experimentation were purchased from Sigma Chemical Co and Merck (Germany).

### 2.3. Carrageenan induced paw oedema

A 10 mg/kg dose of 5-$HT_7$ receptor agonist AS-19 (Bosker et al., 2009; Hedlund, 2009) or antagonist SB 269970 (Galici et al., 2008; Monti et al., 2012) was dissolved in 100 mM DMSO for intraperitoneal administration. 25 mg/kg dose of indomethacin was prepared by suspending in the same vehicle, while 1% carrageenan (CAR) solution was prepared with distilled water. Rats were divided into eight groups: 1: healthy, 2: CAR, 3: CAR+IND, 4: CAR+agonist, 5: CAR+antagonist, 6: CAR+agonist+IND, 7: CAR +antagonist+IND, 8: CAR+agonist+antagonist. The rat paw oedema was induced by injection 0.1 ml of 1% carrageenan solution into the plantar surface of the right hind paw in all rats, but not in the healthy control (Group 1) (Houle et al., 2005; Winter et al., 1962). The measurement of basal paw volumes was carried out using a plethysmometer. Briefly, after determination of the basal volume, the animals received drugs. One hour later, 0.1 ml of 1% carrageenan solution was injected into the right hind paw plantar surface and changes in the paw volumes were monitored at 1-h intervals for 5 h. The results were presented as the paw volume variation in relation to the basal values. The anti-inflammatory potency of all treatments was determined relative to (%) the animals that received only 1% carrageenan solution (Group 2).

### 2.4. Biochemical analyses

#### 2.4.1. Serum measurements of cytokine, IL-1β, IL-6 and TNF-α

Sera were separated from blood by allowing it to clot, followed by centrifugation at 2860*g* for 10 min at 4 °C and kept at −86 °C until they were thawed for the assay. The amounts of IL-1β, IL-6 and TNF-α in each sample were determined in duplicate with highly sensitive ELISA kits specifically designed for rats (Invitrogen-KRC0011 (USA), RayBiotech-ELR-IL6.001 (USA), and Invitrogen-KRC3011(USA), respectively) according to the manufacturer's instructions.

#### 2.4.2. Biochemical investigation of paw tissues

After macroscopic analyses, rat tissues were kept at −86 °C. A 100 mg sample of tissue from each rat was perfused with PBS/heparin and then ground with liquid nitrogen and homogenized in an appropriate buffer on ice using an Ultra-Turrax homogenizer. The samples were then centrifuged according to manufacturer's instructions. Biochemical investigations included duplicate assays of superoxide dismutase (SOD) activity, 8-iso-prostaglandin f2α (8-ISO) and glutathione (GSH) levels from each supernatant using highly sensitive ELISA kits specifically designed for rat tissue