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# Investigation of Hemodynamic Receptors of the Internal Carotid Artery Segments

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## Abstract

**Objectives:** Internal carotid artery (ICA), the main artery of the brain, passes through the cavernous sinus (CS) which forms one of these venous pools. During this transition, while there is arterial blood in the lumen of ICA, its outer surface is in contact with venous blood from the brain. Herein, we aimed to detect the receptor differences of ICA in this highly specialized anatomical region of the skull base.

**Methods:** We performed the study on 10 human cadavers and searched CGRPR, TRP12, ASIC3 and ACTHR receptors via immunostaining using laser scanning confocal microscopy.

**Results:** We determined TRP12 receptor positive in the tunica media and tunica adventitia layers of the cavernous segment of ICA. We did not detect similar positivity in the cervical part of the ICA. In the receptor scan we made in terms of CGRPR, while we detected positivity in the tunica media layer of the cavernous segment, we found positivity in the tunica intima layer of the cervical segment of the ICA. We did not detect any positivity for ASIC3 and ACTHR receptors in both parts of the ICA.

**Conclusions:** As a result, we observed various differences in receptors between ICA segments. While the outer surface of the ICA in the cervical region did not show any receptor positivity, we detected TRP12 receptor positivity along the tissue contour of vessel in the CS. We assume that it may provide a new perspective on pathologies of the CS/ICA and preservation of brain hemodynamics for clinicians.

**Keywords:** Internal carotid artery, Cavernous sinus, Brain hemodynamics

## 1 Introduction

Cerebral arteries appear to have a balanced autonomic reflex when evaluated within the cardiovascular system [1]. As an anatomical view, the internal carotid artery (ICA) has a specific structure with its surroundings. Along with a distance it passes through the temporal bone, there is also a section within the cavernous sinus (CS) which is one of the venous sinuses of the head [2]. This artery makes right angles as it enters and exits the CS. In addition, an artery passes through a venous pool

in this dural sinus. While carotid sheath is located around the vessel in neck and temporal region, it does not exist in the cavernous segment [3]. Besides, the artery is lined with an endothelial layer whose function is not clearly known in the CS [4, 5]. With the consideration of special morphology, this is a unique feature [6].

Arterial blood supply and venous drainage of the brain are anatomically different from each other [7]. Small venules within the brain tissue primarily form venous plexuses and open into larger cerebral veins located in the subarachnoid space. These veins open into the sinus durae matris structures located between the periosteal and meningeal layers of the dura mater. Venous drainage of the brain is provided by these dural sinuses [6]. The artery carrying blood to the brain passes through the CS

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filled with venous blood from the brain. In other words, there is blood flowing to the head in the lumen of the vessel, while there is blood coming from the head on the outside of it in this particular anatomical region. Also; the connective sheath around the artery before entering the skull base is no longer present in this sinus. Instead, it is surrounded by the endothelial cell layer. Consequently, also the artery wall morphologies vary between the segments of ICA [4, 5].

ICA joins the structure of the *circulus arteriosus cerebri* also known as Willis polygon which is the main structure that provides blood supply to the brain by entering the cranium. The brain is an organ with dense blood supply and 70% of the arterial blood it uses is met by ICA [8]. ICA is the essential artery of the brain that provides blood supply to the eye and the auxiliary organs of the eye, the anterior part of the forehead and the nasal cavity [6]. ICA goes upwards in front of the *processus transversus* of the first three cervical vertebrae by following a vertical course and reaches the base of the skull. After entering the *canalis caroticus* located in the *os temporale*, it changes direction towards the anteromedial area by making an angle of 90°. As soon as it leaves the canal in the temporal area, it changes direction upwards in the upper part of the *foramen lacerum* and comes to the *fossa cranii media*. In this area, ICA passes through the CS structure located between the laminae of the *dura mater* [9].

The CS structure was first described in humans by J. B. Winslow; several studies are carried out from eighteenth century to present day [10]. Important cranial nerves pass through the lateral wall of CS as well as through it with ICA one of them. *N. oculomotorius*, *n. trochlearis*, *n. maxillaris* and *n. ophthalmicus* are described as being embedded in the lateral wall of the sinus, while ICA with its sympathetic plexus and *n. abducens* are located in the cavity of the sinus. Most of the small arteries that supply blood to important structures in the *fossa cranii media* region, originated from this segment of the artery [11]. Anatomically; if ICA and CS are evaluated together; while there is blood flowing towards the head in the lumen of the artery, there is blood coming from the head on the outside surface of the artery in this specialized region. In addition; the ICA reaches the base of the skull by making right angles to pass through the CS. In addition to this, there is no longer any sheath structure around the ICA before it enters the skull base. Instead, it is surrounded by endothelial cell layer in the CS [4, 5]. Whether this anatomical course, which may pose a hemodynamic risk, has a possible evolutionary advantage or not, constitutes the main basis of our study.

TRPs (transient receptor potential) are ion channels with high  $\text{Ca}^{2+}$  permeability that respond to stimuli

such as osmotic changes, temperature and proton concentration, which are found in the cell surface membrane as well as in the endoplasmic reticulum [12]. They cause the entry of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  into the cell, causing membrane depolarization with their activation. In this case, the intracellular secondary messenger contributes to triggering the signal transmission. In this case, the intracellular secondary messengers contributes to triggering the signal transmission. This biochemical pathway has important roles in sensory perception in vision, hearing, taste and somatosensory systems [13].

CGRP (Calcitonin gene related peptide) is a neuropeptide that consists of 37 amino acids and has strong vasodilator effect. It is produced in sensory neurons in the central nervous system (CNS) and peripheral tissues. The most important production center is the nucleus of trigeminal nerve in CNS [14]. It has important roles in pain pathways and trigeminovascular reflex [15].

ASICs (acid-sensing ion channels) have a structure that detects the pH level in the extracellular environment and by allowing free  $\text{H}^+$  passage, they are involved in acid-dependent signal transmission. As well it is known to be found in renal epithelial cells and neurons [16]. ASICs subunits can sense extracellular acidification during physiological and pathological processes such as pain perception, inflammation, ischemia and skin contact [17].

The pituitary is of primary importance in the endocrine system and secretes many regulatory hormones from the adenohypophysis. ACTH is one of these hormones and stimulates secretion of glucocorticoids which are important in the regulation of glucose, protein and lipid metabolism [18]. Taken together, it is an important component of the HPA (hypothalamic–pituitary–adrenal) axis. Hormones regulated through this axis are responsible for the control of the sympathetic system. Melanocortin receptors (MC-R) are defined as 5 subunits; MC1-R, MC2-R, MC3-R, MC4-R and MC5-R, respectively. Melanocortin receptors family belong to the seven-transmembrane domain proteins that are coupled to G-proteins and signaled through intracellular cyclic adenosine monophosphate [19]. The melanocortin receptor2 (MC2R) is a receptor subtype specific to the ACTH hormone, also known as the adrenocorticotrophic hormone receptor 'ACTHR'. ACTH hormone is the only agonist ligand for MC2R 'ACTH receptor' [20].

In this context; the aim is to determine the morphological differences of the part of the ICA in the CS, according to the cervical part of the same artery. For this purpose we evaluated potential receptors (TRP12, CGRPR, ASIC3 and ACTHR) differences in the cavernous segment and compare them with the cervical segment of the ICA.

## 2 Materials and Methods

### 2.1 Ethics Committee Approval and Study Materials

Ethical approval for this study was obtained from the Kocaeli University Non-Invasive Clinical Research Ethics Committee (Project number 2019/25). The study was conducted on 10 adult human cadavers, four females and six males (median age 62.4 years). Written consent was obtained from them and/or their families for examine in this study.

### 2.2 Dissection and Tissue Removal

The dissection stages of the study were carried out in Dissection Laboratory in Department of Anatomy. To reach the skull base, the calvarium of cadavers were removed and the meninges were separated from brain tissue. For this aim, the linea horizontalis supraorbitalis line was determined and SCALP incision was made in the cadavers. Sinus cavernosus area was determined on the basis cranii revealed. The lateral walls of CS consisting of the dura mater laminae were removed. Processus clinoideus anteriors were trimmed and the pars cavernosa of ICA was cut out in its widest form. Neck region dissection was performed to remove the pars cervicalis of the arteria carotis interna. The process is carried out as previously described [21].

### 2.3 Histopathological Preparation

The arteries were fixed in 10% buffered formaldehyde for a week. The tissues were dehydrated through upgrading levels of ethanol solutions and cleared in xylene then embedded in paraffin. Afterwards, slides of 4  $\mu$ m thickness were taken for each sample. Samples kept at 4 °C were taken into an oven heated up to 60 °C and 1.5 h incubation was provided for the deparaffinization of tissue sections. After the samples were cooled to room temperature, they were taken into three freshly prepared xylene cuvettes and kept separately in for 10 min. Samples taken from xylene, were kept in 100%, 90%, 80%, 70% alcohol solutions for 5 min and washed in PBS. Then, the 'antigen retrieval' step was applied. 2.94 g trisodium citrate was dissolved in 1 L of sterile distilled water and 120  $\mu$ L of Tween 20 was added. The samples were heated in trisodium citrate solution for 10 min. Slides removed from the solution were incubated in 3% H<sub>2</sub>O<sub>2</sub> PBS buffered solution for 10 min at room temperature and washed again with PBS.

### 2.4 Immunostaining and Laser Scanning Confocal Microscopy

Laser scanning confocal microscopy (DMI8 Confocal Microscope, Leica, Germany) method was used. CGRPR/CRLR Polyclonal (bs-1860R), TRP12

Polyclonal (bs-6425R), ASIC3 Polyclonal (PA5-61898), ACTHR Polyclonal (bs-11408R) were used as the primary antibodies. Goat Anti-Rabbit IgG (ab97075) and Goat Anti-Mouse IgG (ab6944) was also used as secondary antibodies in the study. To blocking tissues, 3:200 PBS solution was prepared as 200  $\mu$ L per sample. Tween20 was added to the solution at a ratio of 1: 1000 for nuclear staining. The prepared block serum was kept at +4 °C until use. 200  $\mu$ L of 1:50 primary antibody solution was prepared and distributed over the samples for staining. Slides were incubated in a hot water bath for 2 h and washed with PBS. Secondary antibody solution was prepared at a ratio of 200  $\mu$ L and 1:100 for each sample in darkroom. Slides were incubated in a hot water bath for 1 h and then washed with distilled water. Cell nuclear stain DAPI (4',6-Diamidino-2-phenylindole dihydrochloride) was added just before confocal imaging. Alexoflor was set for the secondary antibodies and DAPI in the image processing phase.

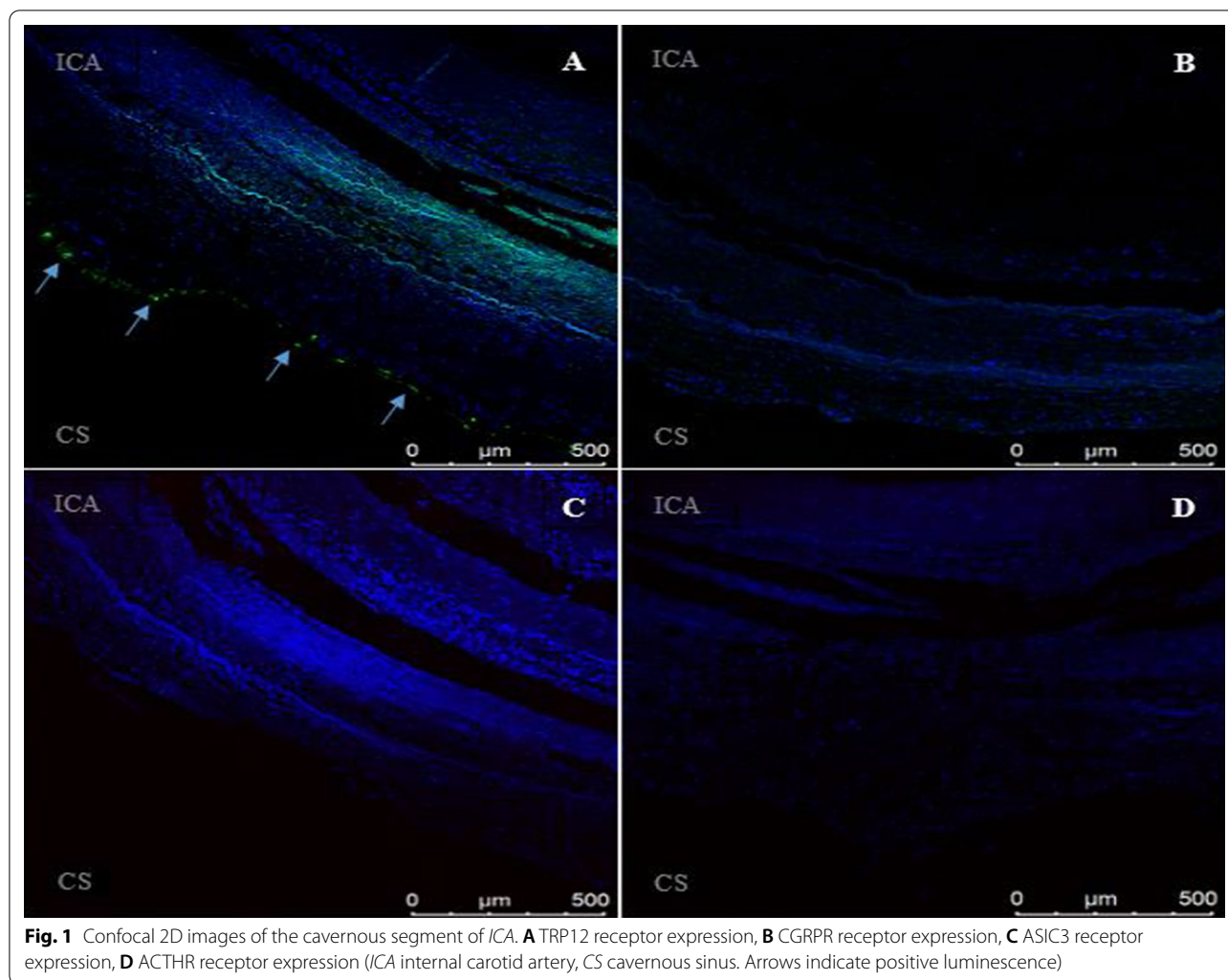
## 3 Results

Herein we examined the expressions of the identified receptors (TRP12, CGRPR, ASIC3 and ACTHR) were categorically in three layers of artery; tunica intima, media and adventitia. The proximal part of the same artery 'pars cervicalis' was used as control group. Microscopy images of the 'pars cavernosa' are given in Fig. 1.

We observed TRP12 cationic ion channel expression was detected along the tissue contour in the outer surface of the artery in cavernous segment of ICA. Besides that as determined by confocal imaging, it was observed that TRP12 was also expressed in the tunica media layer in a disorganized way (Fig. 2). However, TRP12 expression was not detected in the tunica intima layer of cavernous segment of ICA (Fig. 2).

When the expression of CGRPR was examined in the cavernous segment of ICA, it was determined that the receptor was expressed only in tunica media layer and it was not expressed on the outer and inner surfaces of the artery (Fig. 2). No luminescence indicating the presence of CGRPR was detected on the outer surface of the ICA, facing the cavernous sinus. Furthermore, ASIC3 and ACTHR receptor expressions were not detected in all three layers of the cavernous segment of the artery.

Pars cervicalis which segment the part of ICA before entering the cavernous sinus; when examined in terms of related receptors, only positive luminescence related to CGRPR was found. As seen in Fig. 3, the luminescence indicating CGRPR expression are seen in the tunica intima of the artery. Nonetheless, no positivity was detected for other receptors in the cervical segment (Figs. 3, and 4).

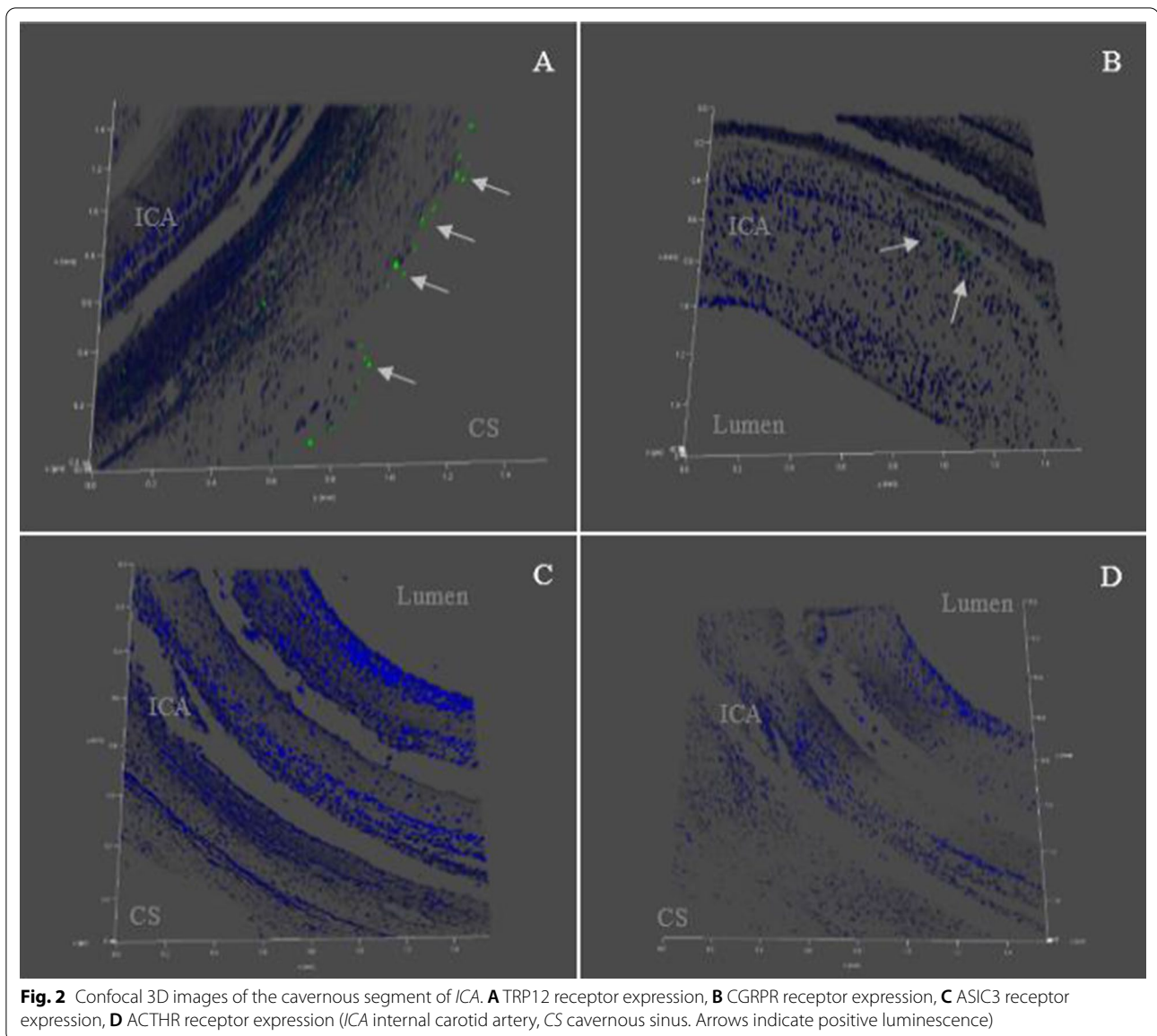


#### 4 Discussion

Neurons are the basic cells of the central nervous system that forms human cognitive activities. As is known neurons and glial cells, which are the main cells of the brain tissue, have very high metabolic activity. Among them, neurons are highly sensitive to energy production and highly depend on glucose and oxygen for energy supply [22]. The fact that the brain tissue which constitutes 2% of the body weight receives approximately 20% (280 mL/min) of the blood pumped from the heart, is an indication that brain blood supply is vital for obtaining oxygen and glucose [8]. Therefore, maintenance of brain hemodynamics is essential for keeping neuron survival with synaptic activities, and energy metabolism as well [23]. There are anatomical and physiological mechanisms that control blood flow. Several reflex mechanisms adjust blood pressure and flow of the target tissue [24]. When considering this information, deteriorating homeostasis via tissue nutrition

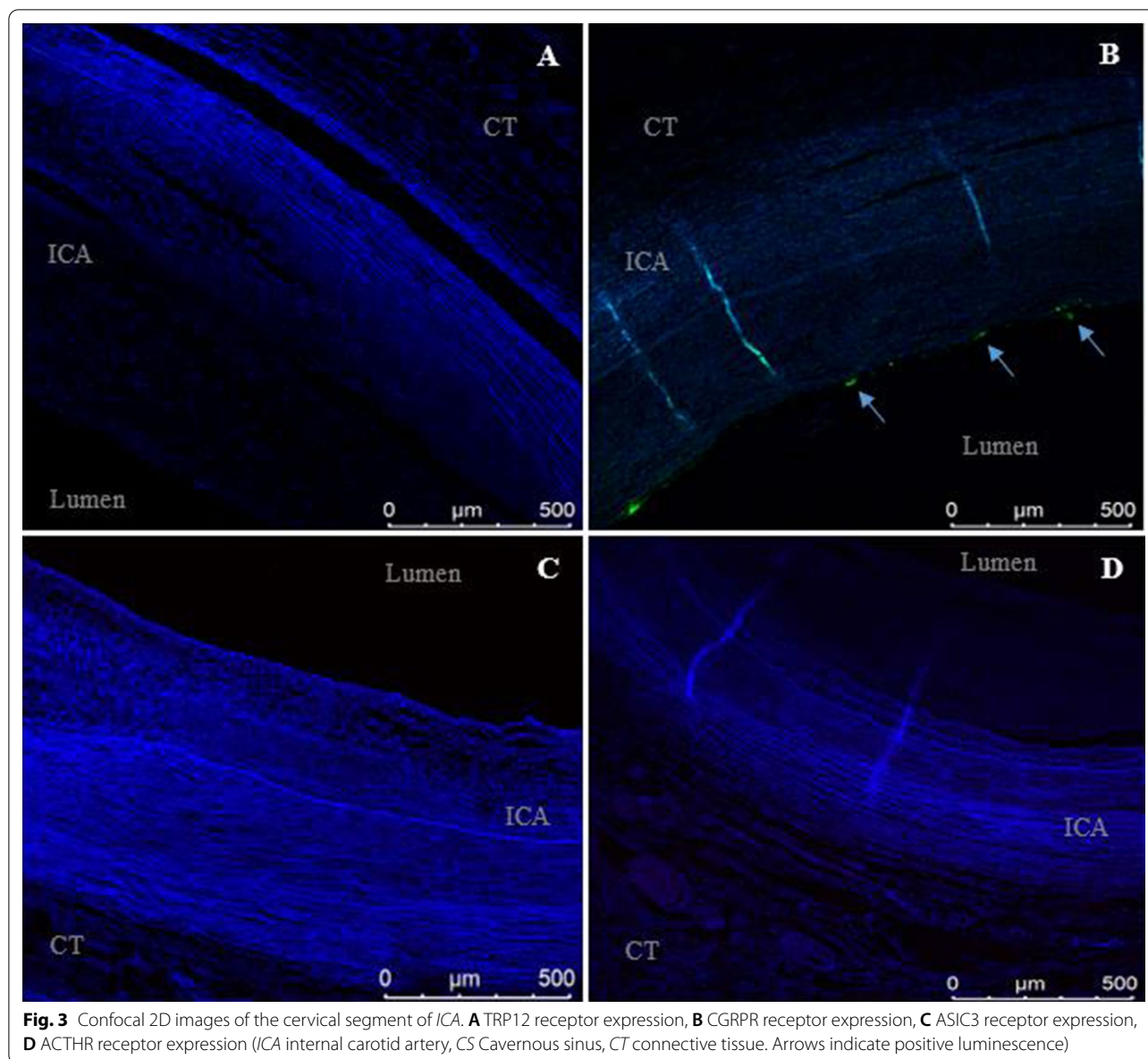
problems, and hypertension may cause significant risks in terms of public health [25].

Numerous ionotropic channels adjust arterial vascular tone by affecting smooth muscle cells [26]. Voltage-gated ion channels may also adjust cell membrane ion passage with difference in ion permeability via extracellular stimuli. This allows for smooth muscle contractions or relaxation. These channels are frequently investigated in current hypertension and tissue perfusion studies [16–18]. TRPs are ion channels with high  $\text{Ca}^{2+}$  permeability that respond to stimuli such as osmotic changes, temperature and proton concentration, which are found in the cell membrane [12]. TRP channels are found in mammals and are divided into six main groups: TRPV (vanilloid), TRPC (canonical), TRPM (melastatin), TRPP (polycystin), TRPA (ankyrin) and TRPML (mucolipin) [27]. Among them, TRP12 (TRPV4) is a member of the TRP ion channel family, it can be activated by a mechanical effect or changes



in extracellular osmolarity [28]. Dural afferent nerves are sensitive to mechanical stimuli such as tension in the dura mater and to extracellular osmolarity changes. Moreover, these neurons have also been shown previously to express mechanically and osmotically sensitive TRP12 channels [13]. Since dural afferents are sensitive to mechanical stimuli and can be stimulated by extracellular osmolarity changes, many studies emphasize that TRP12 ion channel may be associated with migraine in the processes that contribute to headache [29]. It has been shown that the TRP12-mRNA is expressed in the ganglion trigeminale; this nucleus provides the innervation of dura mater which also forms the roof of CS [30, 31]. Also; it has been shown

that TRP channels are expressed in subgroup neurons of dural afferent nerves expressing CGRP [29]. TRP channels are particularly important in the perception of irritant stimuli as a sense of pain in migraine pathophysiology. Intracellular ion exchanges caused by the activation of TRP channels facilitate the excitability of nociceptive afferent nerves and cause pain perception [24, 25]. In this context; due to its perception of dural afferent impulses, active role in the release of CGRP and important functions in vascular tone changes; we thought that researching the expression of the TRP12 (TRPV4) differences between segments of ICA could contribute to the study. We detected luminescence that indicating the TRP12 expression on the outer surface of

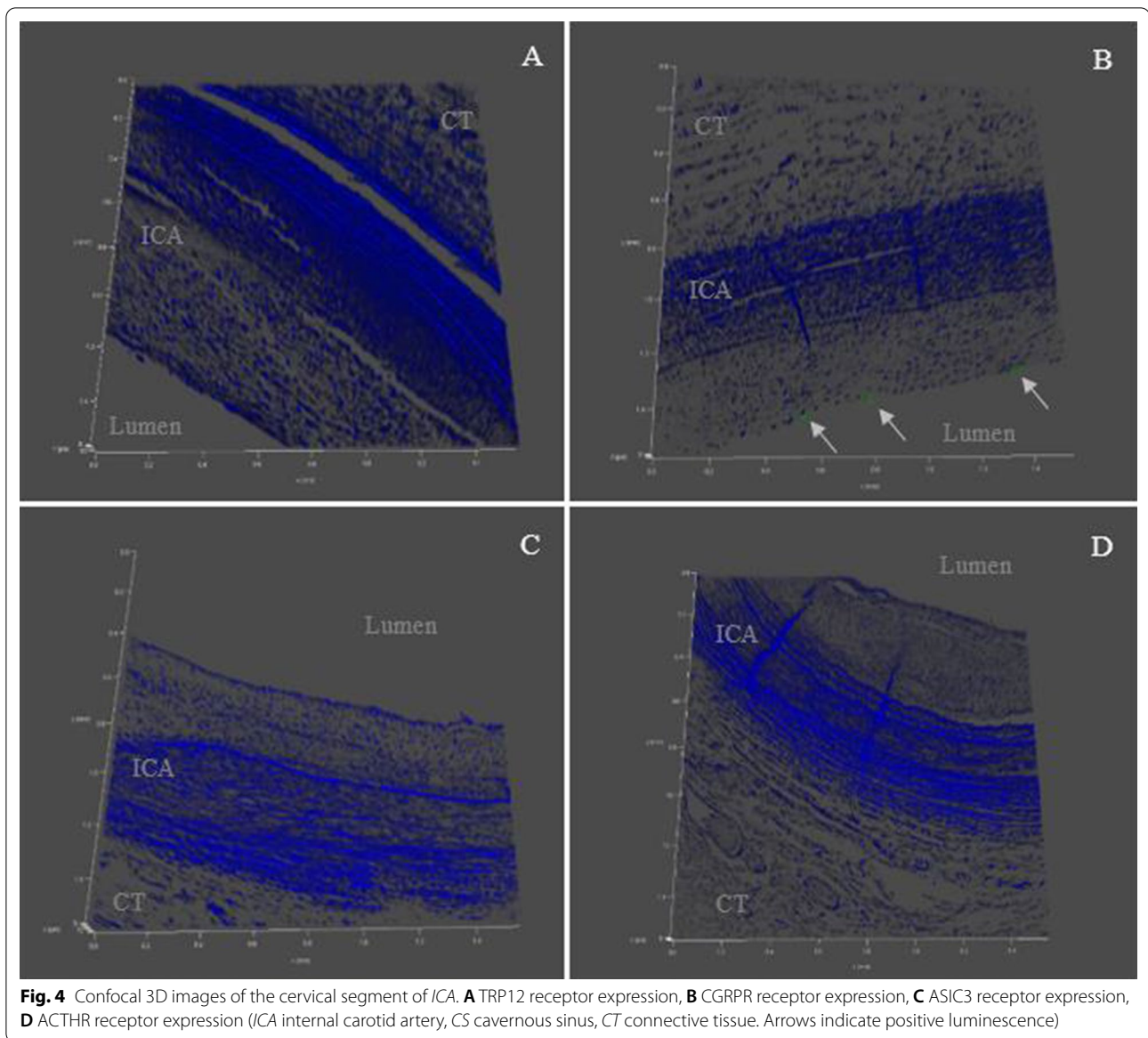


the ICA, facing the CS. Nonetheless, a similar positive luminescence was not observed on the outer surface of the cervical segment.

CGRP (calcitonin gene related peptide) has an important role in pain pathways and trigeminovascular reflex [15]. Therefore; CGRP antagonists are used in the treatment of migraine disease which is known that cerebrovascular changes play a role in the etiopathology [32]. In a morphological study conducted considering similar biological mechanisms, it has been shown that expression of the CGRP is also in the lung tissue. Moreover; it has been reported that it is released from perivascular nerve endings and is effective in the permeability of pericytes.

Hereby, it has been stated that CGRP related pharmaceuticals can be a possible treatment method against pulmonary hypertension [33]. According to all accounts in our study, CGRPR expression was determined in both segments of cavernous and cervical of ICA. CGRPR, affecting the brain hemodynamics, was found to be positive in the tunica media layer of the pars cavernosa and the tunica intima layer of the pars cervicalis.

ASIC3 provides optimal signal transduction at the level closest to blood pH [34]. Glucose and oxygen sensitive brain cells change the extracellular pH thanks to the metabolites is produced during energy recovery. In the light of these studies, it is stated that ASIC3 plays



an active role in synaptic disruptions and neuron damage after brain injury [17]. In another study, by drawing attention to the oxygenase activity caused by ASIC3 activation, the effect of biomolecules with inhibitory effect on the receptor on cerebral blood flow was investigated [35]. As stated priorly; when there is arterial blood in the lumen of ICA, there is venous blood in the lacunae within the CS. Within this context, we thought that determining receptor changes to detect possible pH differences may contribute to our study. In terms of ASIC3 receptor, no luminescence was detected indicating positivity in both parts of the ICA.

HPA (hypothalamic–pituitary–adrenal) axis constitutes the biological equivalent of the “fight or flight”

paradigm [36]. This biological pathway has many effects, primarily on cardiovascular circulation [18]. Considering the complex relationship of the cardiovascular system and sympathetic discharge, its effect on vascular tone works towards generating high blood pressure [20]. Additionally, from the standpoint of anatomical, there are pituitary veins among the veins that drain into the CS [37]. As the receptor of hormone that is at center of the sympathetic system and regulates it, we conducted the investigation of ACTHR in the vascular wall of ICA segments. In our study, no luminescence belonging to ACTHR (MC2R) was found in the vessel walls of both segments of ICA.

The main function of the dural sinuses structures is to provide venous drainage of the brain and cranial structures. Considering the special anatomical structures of the cavernous sinus and ICA, an opinion has also been proposed for the relations and functions of these structures [38]. Krzymowski et al. (2014) used radiolabeled dopamine in their animal experiment and showed that this neuropeptide is retrograde transfer from the cavernous sinus to the ICA. It has been suggested that this is part of a universal physiological regulatory system. In a similar study of the same research group, physiological transition of neuropeptides from the cavernous sinus to the ICA via retrograde transfer was demonstrated [39].

Arteries basically consist of three histological layers: tunica intima, media and adventitia. Tunica intima forms the innermost layer of the artery lumen including the endothelial layer [40]. Tunica intima has a different morphology from other layers and contains a wide variety of receptors [26]. The main functions of the receptors are to perceive and transmit cellular messages. In this context, continuous information is transferred about the content of blood flowing from the lumen. This biological process plays a role in the modulation of vascular tone [25]. On the other hand, tunica adventitia layer, which consists of connective tissue, surrounds the outermost layer of the blood vessel. The task of this layer is to protect the vein and limit its flexibility [40]. The presence of receptors in the tunica intima and media layers where there are intense metabolic activities is a natural biological requirement. On the contrary, the presence of vasomodulatory receptors on the outer surfaces of vessels with dense connective tissue and not in contact with blood is unexpected [41]. Depending on the special anatomical structure of the CS; the outer surface of ICA is in contact with venous blood from the brain. Also the outermost surface of the artery is surrounded by the endothelial layer just inside this venous pool [4, 5]. TRP12 was present on the outermost surface of ICA in the CS while it was not detected in the part of the artery that outside of the CS. In this context, the hypothesis is maturing that venous blood in the CS may have an effect on the modulation of the blood flow to the brain.

## 5 Conclusion

Within the scope of the data we obtained in our study, TRP12 (TRPV4) receptor was detected in tunica media and tunica adventitia layers of cavernous segment. However, it was not detected in the cervical segment of ICA. It was also observed that the cavernous segment in tunica adventitia was on the outer surface of the artery. The CGRPR was detected in the tunica media layer of cavernous segment, and in tunica intima layers in cervical

segment of ICA. In addition, in both segments of ICA for ASIC3 and ACTHR receptors were not detected.

ICA makes right angles to pass through the cavernous sinus and it undergoes morphological differences while passing through this narrow anatomical compartment at the base of the skull. During this anatomical course, we think that the presence of receptor differences between the segments of the same artery and also the presence of a vasomodulatory ion channel receptor such as TRP12 on the outer surface of the artery in contact with venous blood may contribute to the literature on brain hemodynamics. These new findings should be confirmed by future studies. We assume that our findings will provide a new vision for scientists working on the subject.

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### Author contributions

MDY, TC and BB conducted the dissection. MDY and SHR conducted the histological preparation stage. MDY and AO performed immunostaining and AO used laser scanning confocal microscopy. MDY, AO and YY contributed in data processing. All authors read, critically evaluated, and approved the final version of the manuscript.

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### Declarations

#### Conflict of interest

The authors declare they have no conflicts of interest.

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