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Alarin in cranial autonomic ganglia of human and rat

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ABSTRACT

Extrinsic and intrinsic sources of the autonomic nervous system contribute to choroidal innervation, thus being responsible for the control of choroidal blood flow, aqueous humor production or intraocular pressure. Neuropeptides are involved in this autonomic control, and amongst those, alarin has been recently introduced. While alarin is present in intrinsic choroidal neurons, it is not clear if these are the only source of neuronal alarin in the choroid. Therefore, we here screened for the presence of alarin in human cranial autonomic ganglia, and also in rat, a species lacking intrinsic choroidal innervation.

Cranial autonomic ganglia (i.e., ciliary, CIL; pterygopalatine, PPG; superior cervical, SCG; trigeminal ganglion, TRI) of human and rat were prepared for immunohistochemistry against murine and human alarin, respectively. Additionally, double staining experiments for alarin and choline acetyltransferase (ChAT), tyrosine hydroxilase (TH), substance P (SP) were performed in human and rat ganglia for unequivocal identification of ganglia. For documentation, confocal laser scanning microscopy was used, while quantitative RT-PCR was applied to confirm immunohistochemical data and to detect alarin mRNA expression.

In humans, alarin-like immunoreactivity (alarin-LI) was detected in intrinsic neurons and nerve fibers of the choroidal stroma, but was lacking in CIL, PPG, SCG and TRI. In rat, alarin-LI was detected in only a minority of cranial autonomic ganglia (CIL: 3.5%; PPG: 0.4%; SCG: 1.9%; TRI: 1%). qRT-PCR confirmed the low expression level of alarin mRNA in rat ganglia.

Since alarin-LI was absent in human cranial autonomic ganglia, and only present in few neurons of rat cranial autonomic ganglia, we consider it of low impact in extrinsic ocular innervation in those species. Nevertheless, it seems important for intrinsic choroidal innervation in humans, where it could serve as intrinsic choroidal marker.

1. Introduction.

The autonomic nervous systems plays an important role in control of proper eye functions such as ocular blood flow, intraocular pressure, or production of aqueous humor. For that, various sources are responsible, deriving from the parasympathetic and sympathetic nervous system, and further also primary afferent (sensory) nerve fibers contribute to this autonomic control (Burnstock and Sillito, 2000). These autonomic pathways use several relay stations, the cranial autonomic ganglia, in which preganglionic fibers of the brainstem connect to neurons that further project into the eye with their postganglionic nerve fibers. These ganglia are the parasympathetic pterygopalatine ganglion (PPG) and ciliary ganglion (CIL), and the sympathetic superior cervical ganglion (SGG). Primary afferent nerve endings take the opposite way, coursing from within the eye to the pseudounipolar nerve cells residing in the trigeminal ganglion (TRI), and further into the brainstem (Neuhuber and Schrödl, 2011).

The situation in the posterior uvea is different, since here an

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additional source of innervation exists, the so called intrinsic choroidal neurons (Nickla and Wallman, 2010). These nerve cells form a dense plexus in the choroid of man and some bird species, and it is highly assumed that this intrinsic innervation contributes to a local network responsible for the local control of choroidal blood flow and choroidal thickness (Schrödl, 2009). Interestingly, this intrinsic system is also integrated in parasympathetic, sympathetic and primary afferent control, as revealed by morphological studies (May et al., 2004; Stübinger et al., 2010). For this local control, various neurotransmitters and neuropeptides are known, such as e.g., neuronal nitric oxide synthase (nNOS) or vasoactive intestinal polypeptide (VIP), galanin (GAL) or calretinin (Troger et al., 2007), however the exact mechanisms and interaction of these various intrinsic and extrinsic sources are unknown yet.

Recently, the neuroregulatory peptide alarin (Eberhard et al., 2012; Santic, 2006; Van Der Kolk et al., 2010) was detected in various tissues of human, mouse and rat eye (Schrodl et al., 2013). As a splice variant of the galanin-like peptide (GALP) mRNA lacking exon3, it misses the galanin-receptor binding domain and possibly using different receptors for its action. Since alarin has been shown to have vasoconstrictive activity in murine skin (Santic et al., 2007), it has been assumed that, besides other possible mechanisms, an alarin mediated vasomodulatory effect is also present in the eye. In this respect, rat would represent a favorable animal model regarding ocular blood flow experiments since these are technically possible (Strohmaier et al., 2010, 2013) compared to smaller sized mouse eyes. While alarin-immunoreactivity was also detected in human intrinsic choroidal neurons (Schrodl et al., 2013) it is not clear if alarin-immunoreactivity in the choroid derives from intrinsic origin only (i.e., intrinsic choroidal neurons) or also from extrinsic sources (i.e., PPG, CIL, SCG, TRI). This, however, is important to properly interpret interactions of the various sources.

Therefore, we screened for the presence of alarin in human cranial autonomic ganglia. Additionally, we monitored the presence of alarin in the rat, a common animal model lacking intrinsic choroidal innervation (Flügel et al., 1994) to get answers about possible “extrinsic-only” sources of alarin, and we have confirmed immunhistochemical results by applying molecular biological methods (i.e., qRT-PCR).

2. Methods

2.1. Tissue preparation

Cranial autonomic ganglia of rat ($n = 3$, adults, of both sex) and human ($n = 4$, 75–84 years of age, of both sex, post mortem time 8–12 h), i.e. ciliary ganglion (CIL), pterygopalatine ganglion (PPG), trigeminal ganglion (TRI) and superior cervical ganglion (SCG), and human choroids were obtained. Experiments were performed in accordance with the Helsinki declaration of 1975 (revised 1983) and with approval of the Salzburg State Ethics Research Committee. The study did not extend to examination of individual case records and the anonymity of the patients has been ensured. Human tissue samples (unfixed tissue) were obtained from the cornea bank of the Department of Ophthalmology and Optometry, Salzburg, Austria, from the Department of Legal Medicine, Salzburg, Austria, during routine autopsy, or from the body donor program of the Department of Anatomy I, University Erlangen-Nuremberg, Germany. Animal tissue was obtained in accordance with the guidelines of the Association for Research in Vision and Ophthalmology (ARVO) regarding the use of animals in ophthalmic and visual research.

Cranial ganglia as well as choroids were dissected free and fixed by immersion in phosphate buffered saline (PBS) containing 4% paraformaldehyde (PFA; 2 h at room temperature, RT). They were rinsed in PBS (24–48 h) and transferred into PBS containing 15%

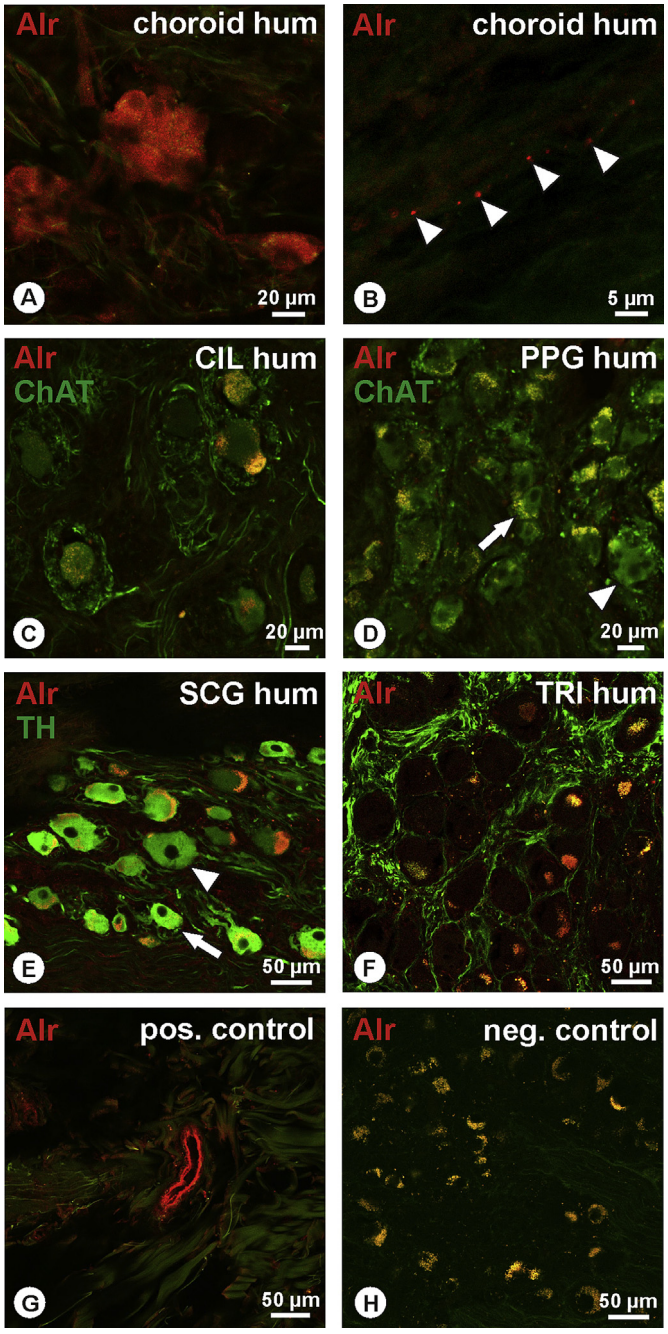

Fig. 1. Human tissue A) alr-LI (red) was detected in somata and processes of intrinsic choroidal neurons (yellow dots in neurons represent lipofuscin granules). B) alr-LI (red) was detected in nerve fibers forming boutons (arrowheads) within the choroidal stroma. C) Neurons and nerve fibers in the human ciliary ganglion (CIL) display immunoreactivity for ChAT (green) while alr-LI (red) was absent in this ganglion (yellow dots in neurons represent lipofuscin granules). D) Neurons and nerve fibers in the human pterygopalatine ganglion (PPG) display immunoreactivity for ChAT (green) while alr-LI (red) was absent in this ganglion, irrespective of neuron size (large neurons = arrowhead vs. small neurons arrow; yellow dots in neurons represent lipofuscin granules). E) Neurons and nerve fibers in the human superior cervical ganglion (SCG) display immunoreactivity for ChAT (green) while alr-LI (red) was absent in this ganglion, irrespective of neuron size (large neurons = arrowhead vs. small neurons arrow; yellow dots in neurons represent lipofuscin granules). F) Alr-LI (red) was not detectable in the human trigeminal ganglion (TRI; green: autofluorescence; yellow dots in neurons represent lipofuscin granules). G, H) In all specimens investigated, alr-LI (red, G) was detectable in the wall of blood vessels, serving as positive control, while omission of the primary antibody served as negative control (H; superior cervical ganglion; yellow dots in neurons represent lipofuscin granules). All micrographs in Fig. 1 represent confocal images in single optical section mode.