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The Ubiquity Nature of Acetylcholine

Carlota Saldanha* and Ana S Silva-Herdade

Instituto de Bioquímica, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Portugal

***Corresponding author:** Carlota Saldanha, , Tel: 351217922600; carlotasaldanha@fm.ul.pt

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Background

Acetylcholine (ACh) was viewed as the "vagusstoff" after Loewi`s experiment with the frog heart [1]. In 1936 Otto Loewi, and Henry Dale were recipients of the Nobel Prize in Physiology and Medicine, by their contribution for the acetylcholine discover as a neurotransmitter [1]. So, ACh was regarded as a neurotransmitter during several decades [2]. After that it was evidenced a widespread expression of the cholinergic system within a variety of non-neuronal tissues. The "Non-Neuronal Cholinergic System" (NNCS) is well established as the extra-neural effects of acetylcholine [3]. Beyond these neural and non neuronal cholinergic systems there is another one named by Tracey et al. as the Cholinergic Anti-Inflammatory Pathway (CAIP) [4]. Afferent and efferent signals transmitted in the vagus nerve are components of a neural circuit that modulates the innate immune response. The CAIP could be considered as a liaison between the cholinergic neural system and NNCS.

The enzymes implicated in the synthesis and responsible for hydrolysis of ACh in different mammalian cells are isoenzymes of Choline-Acetyltransferase (ChAT), and Acetylcholinesterase (AChE) respectively [5]. ACh is synthesised in the nerve cell from choline and acetyl-CoA by a process mediated by choline acetyltransferase and is stored in vesicles in pre-synaptic neurons [2]. Upon arrival of the nerve impulse, a membrane depolarisation is induced and ACh is released to the synaptic cleft. At postsynaptic membrane, ACh binds to its cholinergic receptor, initiating a cascade of actions that are stopped by the enzyme AChE, which hydrolyses ACh to Choline (Ch) and acetic acid [2].

Non-Neuronal Cholinergic System (NNCS)

Circulating acetylcholine can be produced by T-lymphocytes and endothelial cells [6,7].

Lymphocytes express most of the cholinergic elements and upon interaction with the antigen presenting cells or the endothelial cells, T cells synthesize and release acetylcholine [8]. The autocrine ACh action depends on cell membrane receptor and in case of TCR/CD3 receptors for example enhances the expression of both ChAT and M5 mACh receptors [8].

At the vascular wall the ACh produced by the endothelium acts through the auto-paracrine fashion in the intrinsic intima [5]. All the neuronal components, ACh, ChAT and VAChT and AChE above mentioned, are also present in the NNCS [9].

We are the first to perform the biochemical characterization of the AChE in endothelial cell membrane of human umbilical vein. We have identified, with C-terminal anti-AChE, the expression of one molecular form membrane with 70 kDa, (the molecular mass characteristic of the human monomeric form of AChE). When the N-terminal anti-AChE was used two molecular forms with approximately 66 kDa and 77 kDa are expressed at membrane bound level [10]. The molecular form of 70 kDa is also expressed at cytoplasm and nuclear compartments, where the latter also expressed an AChE isoform with approximately 55 kDa [11]. We verified that the nuclear expression is not endothelial cell-specific but is also evidenced in non-neuronal and neuronal cells [11].

The widespread expression of non-neuronal acetylcholine is accompanied by the ubiquitous presence of acetylcholinesterase and nicotinic/muscarinic receptors. Red blood cells account for the blood elements with highest expression of membrane-bound acetylcholinesterase [12]. The enzyme AChE has the particularity to be inhibited by high concentrations of its own substrate, ACh. So, different types of enzyme complexes may be presented namely, active, inactive and less active ones according the amount of ACh existent [13].

Fuji and co-workers quantified the levels of ACh in plasma and blood at normal physiological conditions, and verified differences of its amount among animal species [14].

The circulating ACh induces vasodilation in dependence of integrity of the endothelium via the Nitric Oxide (NO) synthesised and released to smooth muscle [15,16]. Also the NO released from endothelial cells can move to the lumen of the vessels where is scavenged by erythrocyte and free hemoglobin present in the blood circulation [17]. The NO-Heme Hemoglobin adduct (HbFe (II) NO) has been detected during NO inhalation therapy used for pulmonary hypertension relief, but it also occurs when deoxygenated blood enters into a vascular bed in which NO is produced such as the pulmonary circulation [18,19].

Low tissue oxygen tension is perceived by erythrocytes with induced hemoglobin structural allosteric transitions favouring the transfer of its NO bound molecule to band 3 that allow the NO efflux to the tissues in the capillary bed [20,21]. Among the heterotrophic effectors of oxygen binding hemoglobin, NO binds to the thiol group of cysteine β93 at high tissue oxygen tension. At low tissue oxygen tension there is a NO release from either S-nitrosothiol of the S-nitrosated hemoglobin or from the reduction of the anion nitrite to NO in a non exclusive way [22,23]. It is known that the T state of SNO-Hb promotes the transnitrosation by which NO groups are transferred to thiol acceptors biomolecules in RBCs [24]. One of these is the protein band 3 [25], but the exact mechanism by which NO escape from erythrocyte membrane still remain uncertain.

We have verified that in presence of ACh there is an increased of the erythrocyte deformability, of the nitrite and nitrate concentration and the oxygen hemoglobin affinity and a decreased of erythrocyte aggregation [26,27].

The lower erythrocyte deformability expressed in blood samples obtained from hypertensive, hypercholesterolemic and kidney

transplant patients was ameliorate when in presence of ACh as we verified by studies conducted *in vitro* [28].

Non-neuronal acetylcholine appears to be involved in the regulation of elementary cell functions such as cell mitosis, cell-cell interaction, cell automaticity, locomotion, ciliary activity, barrier function, resorption and secretion [29-33]. In the airways, for instance, the great majority of cells express the components of NNCS and it is documented that a substantial increase on ACh levels triggers the release of proinflammatory effectors [34]. In addition, the excitability of airway mast cells can be powerfully inhibited by acetylcholine [35].

The Cholinergic Anti-Inflammatory Pathway (CAIP)

The vagus nerve innervates major organs, including the spleen and the gut, regulates physiological responses to stress, injury and infection. It was observed that the action potential transmitted in sensory nerve fibers to the brain reports the presence of inflammatory stimuli in peripheral issues [14].

The electric stimulation of the vagus nerve enhances the release of acetylcholine from the spleen [36]. Several studies using immumohistochemistry, electron micrsoscopy and neurophysiological techniques [37,38] demonstrate communication between the nerve endings and the T cells, B cells and macrophages that lead to anti-inflammatory signals through the efferent vagus nerve. Tracey et al. based on his work proposes the cholinergic anti-inflammatory pathway [39-41]. It consists in the activation of adrenergic neurons in the spleen that liberate nor-epinephrine near the T cell capable to secrete acetylcholine. This pathway plays a critical role in controlling the inflammatory response through ACh interaction with peripheral α7 subunit–containing nicotinic acetylcholine receptors expressed on macrophages which suppress the synthesis and secretion of inflammatory cytokines. Macrophages act as an interface between the brain and the immune system [4] by the participation of its JaK2/STAT3 signal pathway or via inhibition of the transcription factor NF-kB [42,43]. So, a crosstalk is established between the immune system and the central nervous system that contribute for the reposition of homeostasis in the former.

The activation of afferent vagus nerve by endotoxin or pro-inflammatory cytokines stimulates hypothalamic-pituitary-adrenal anti-inflammatory responses conducted by the efferent vagus nerve [44].

Acetylcholine is an anti-inflammatory molecule that suppresses the production of pro-inflammatory cytokines [3] suggesting that the neuronal activation of the adrenergic system, for example in spleen, release nor-epinephrine from the efferent fibers near T cells which became able to liberate non neuronal acetylcholine. In consequence this ACh interacts with α7 nAChR expressed on cytokine production macrophages. The extraneuronal cholinergic system in lymphocytes is responsible for the levels of acetylcholine in blood circulation [6]. In the case for example of nicotine application it generates complex effects in dependence of the route or local of its administration [45,46].

In vivo Experimental Studies of the Effects of ACh in Inflammatory Response

The study done by Silva-Herdade and Saldanha was conducted *in vivo* on an animal model of Lipopolysaccharide (LPS) induced inflammation aimed to evaluate the effects of ACh on the leukocyte-endothelial cells interactions and to quantify the concentrations of TNF-alpha in blood circulation. Using intravital microscopy the number of rolling and adherent leukocytes in post-capillary venules of Wistar rats was registered after the intravenously administration of LPS alone or with further addition of ACh. Those results evidenced the anti-inflammatory effect of ACh showed by a decrease in TNF-alpha plasma levels and by the decrease of the number of adherent leukocytes [47]. Taking in consideration the non neuronal origin of ACh that we mimesis in these *in vivo* studies we cannot exclude with sure the participation of the cholinergic anti-inflammatory pathway when LPS was administered.

In a previous study the same authors using the same animal model protocol but without induced inflammation showed decrease of the number of rolling and adherent leukocytes while the rolling velocity was reduced without changes in plasma levels of IL-1-beta [48].

We conclude that ACh has an anti inflammatory effect decreasing the concentration of TNF-alpha in plasma and the adhesion of the leukocytes to the endothelial cells that is one of the former steps in the inflammatory innate response. The acute inflammatory state implicated by the surgery show also the anti-inflammatory action of ACh that left unchanged IL-1-beta.

Conclusions

Apart from the former discovers of the neuronal action of acetylcholine much more different cellular locals of synthesis of ACh are evidenced which implicate diverse biological effects conferring its ubiquitous molecular behavior. For instance its ubiquity is different from that of coenzyme Q which moves inside the inner mitochondrial membrane occupying diverse places closer or away from one complex of the electron chain but with the same oxi-reduction function.

Acetylcholine acts in the parasympathetic system, in the neuron muscle junction, in the T-lymphocytes, in endothelial cells, in erythrocytes demonstrate the ability to bind and be recognized by a different membrane cell types. ACh induces in the endothelium the NO synthesis; when within the airway epithelium ACh is involved in the regulation of water and ion transport; acts as anti inflammatory agent when liberate by T-cell activation increasing its concentration in blood circulation. In general terms, the biochemistry point of view that the structure of one biomolecule dictates its physiological function has been enlarged by a multitude of actions and effects resulting from the signal transduction pathway associated with their different kind of receptors or with the same type of receptors in distinct cells.

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