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Forum Minireview

Basic and Clinical Aspects of Non-neuronal Acetylcholine: Overview of Non-neuronal Cholinergic Systems and Their Biological Significance

Koichiro Kawashima[1,*] and Takeshi Fujii[2]

[1]*Department of Pharmacology, Kyoritsu College of Pharmacy, Minato-ku, Tokyo 105-8512, Japan*
[2]*Department of Pharmacology, Faculty of Pharmaceutical Sciences, Doshisha Women's College of Liberal Arts, 97-1 Minamihokodate, Kodo, Kyotanabe, Kyoto 610-0395, Japan*

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Abstract. Acetylcholine (ACh) is a phylogenetically ancient molecule involved in cell-to-cell signaling in almost all life-forms on earth. Cholinergic components, including ACh, choline acetyltransferase, acetylcholinesterase, and muscarinic and nicotinic ACh receptors (mAChRs and nAChRs, respectively) have been identified in numerous non-neuronal cells and tissues, including keratinocytes, cancer cells, immune cells, urinary bladder, airway epithelial cells, vascular endothelial cells, and reproductive organs, among many others. Stimulation of the mAChRs and nAChRs elicits cell-specific functional and biochemical effects. These findings support the notion that non-neuronal cholinergic systems are expressed in certain cells and tissues and are involved in the regulation of their function and that cholinergic dysfunction is related to the pathophysiology of certain diseases. They also provide clues for development of drugs with novel mechanisms of action.

Keywords: acetylcholine, choline acetyltransferase, non-neuronal cholinergic system, muscarinic receptor, nicotinic receptor

1. Introduction

Much progress has been made in our understanding of non-neuronal cholinergic systems since the first symposium on "The Non-neuronal Cholinergic System" was held at the 73rd Annual Meeting of The Japanese Pharmacological Society (March 24, 2000; Yokohama) (1). In the interim, there has been worldwide recognition of the biological significance of non-neuronal cholinergic systems, and the First and the Second International Symposiums on Non-neuronal Acetylcholine were held in San Francisco, CA, USA (2002) and Mainz, Germany (2006), respectively (2, 3). Furthermore, the renowned pharmacology text book "Goodman and Gilman's The Pharmacological Basis of Therapeutics (11th ed, 2006)" now has a new section entitled "Extraneuronal Cholinergic Systems (page 153)," which introduces the idea that acetylcholine (ACh) is present in the vast majority of human cells, including epithelial, mesothelial, and endothelial cells (ECs); circulating cells; and immune cells. With all of that as background, we organized a symposium on the "Basic and Clinical Aspects of Non-neuronal ACh" for the occasion of the 80th Annual Meeting of The Japanese Pharmacological Society (March 15, 2007, Nagoya). The aim of this review is to provide additional insight into the nature of non-neuronal cholinergic systems and their biological and clinical significance.

*Corresponding author. koichiro-jk@piano.ocn.ne.jp
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2. Cholinergic components

2-1. ACh and its synthesizing enzymes

Almost all the life-forms on the earth appear to have the ability to synthesize acetylcholine (ACh). We have detected ACh and ACh-synthesizing activity in bacteria, archaea, and eucarya (4 – 6); and it is evident that ACh is a phylogenetically ancient substance that evolved well before the advent of animals with a nervous system

(7, 8). Nonetheless, most currently available information on ACh was derived from research on the nervous systems of mammalian species, where ACh is synthesized mainly by choline acetyltransferase (ChAT) from choline and acetyl coenzyme A (AcCoA), although in the periphery, the mitochondrial enzyme carnitine acetyltransferase (CarAT) also participates in ACh synthesis (9). This is noteworthy, as the activities of ACh-synthesizing enzymes determined using the radiometric method of Fonnum (10) with [^{3}H]choline do not always correlate well with the observed ACh synthesis in tissues. This can be ascribed, at least in part, to the fact that both ChAT and CarAT are involved in ACh synthesis in the periphery. We found that in lymphocytes the ACh content correlates rather well with ChAT activity but not with either CarAT activity or total ACh synthesis (11). Furthermore, immunological stimulation of lymphocytes selectively stimulates ChAT activity without affecting CarAT activity. This suggests that in lymphocyte ACh is mainly synthesized by ChAT and that CarAT plays only a minor role in ACh synthesis. It is therefore highly advisable to determine both ChAT and CarAT activities using selective inhibitors for the respective enzymes, such as bromoACh (BrACh) and bromoacetylcarnitine (BrACar) (9).

The ACh-synthesizing activity found in bacteria, archaea, fungi, higher plants, and invertebrate animals can show resistance to or varying degrees of susceptibility to BrACh and BrACar (4, 5). Most samples containing relatively high levels of ACh, such as Shiitake mushroom, bamboo shoot, squid, and neresis show ACh-synthesizing activities that were highly sensitive to BrACh, which suggests that a ChAT-like enzyme is responsible for ACh synthesis in samples where substantial amounts of ACh are actively synthesized. However, further studies will be necessary to fully characterize the enzymes involved in ACh synthesis in these organisms.

2-2. ACh receptors

Expression of both muscarinic and nicotinic ACh receptors (mAChRs and nAChRs, respectively) has been detected in numerous non-neuronal cells and organs (3). Most express all five mAChR subtypes ($M_1 - M_5$), although the level of expression of each subtype may vary. In addition, a number of neuron type nAChR subunits (α2, α3, α4, α5, α6, α7, α9, α10, β2, β3, and β4) have been identified in non-neuronal cells and organs, but no muscle type subunits (α1, β1, γ, and δ) have been detected.

2-3. Acetylcholinesterase and butyrylcholinesterase

At the neuromuscular junction, ACh released from nerve endings is cleared within several milliseconds by the action of acetylcholinesterase (AChE), which cleaves ACh into choline and acetic acid. Expression of both AChE and butyrylcholinesterase (BuChE) has been detected in most non-neuronal cells and organs (3); however, the precise roles they play in the regulation of non-neuronal cholinergic activity remain unclear.

2-4. Vesicular ACh transporter

In cholinergic neurons, ACh is transported into the synaptic vesicles via the vesicular ACh transporter (VAChT) and stored there until released by exocytosis. Expression of VAChT has been detected in bronchial epithelium (12), but skin (13), urothelium (14), and T cells (7) do not appear to express VAChT. Instead, ACh appears to be synthesized and released when necessary, without storage. On the other hand, two polyspecific organic cation transporter subtypes, OCT1 and OCT3, are reportedly involved in the release of ACh in the human placenta (15) and in murine bronchial epithelium (16).

2-5. High affinity choline transporter

In cholinergic neurons, choline taken up via the high affinity choline transporter (CHT1) is utilized exclusively for ACh synthesis. OCT1, OCT2, and OCT3 also have the ability to transport choline from the extracellular space into some cells. CHT1 is expressed in airway epithelial cells (17) and T cells (18), while OCT1, OCT2, and OCT3 are expressed in airway epithelial cells (19) and placenta (15).

Although detailed information about the source of choline for ACh synthesis in non-neuronal cells and organs is not yet available, choline taken up via either CHT1 or OCTs is certainly one of candidate substrates for ACh synthesis.

3. Non-neuronal cholinergic systems expressed in mammalian species

In this review, we will briefly discuss the non-neuronal cholinergic systems expressed in the following cells and organs:

- Keratinocytes
- Cancer cells
- Immune cells
- Urinary bladder
- Airway epithelial cells
- Vascular endothelial cells
- Reproductive organs
- Miscellaneous

3-1. Keratinocytes

The non-neuronal cholinergic system in human skin was extensively reviewed recently by Kurzen et al. (20). In addition, the biological and clinical significance of epithelial nAChRs is discussed later in this issue (21). ACh synthesis in keratinocytes is catalyzed by ChAT, while its degradation is catalyzed by AChE (20, 22, 23). Epidermal and gingival keratinocytes express the $M_1 - M_5$ and $M_2 - M_5$ mAChR subtypes, respectively (24 – 26), as well as the α3, α5, α7, α9, and β2 nAChR subunits (3, 23). ACh synthesized by and released from keratinocytes acts on both mAChRs and nAChRs in an autocrine and/or paracrine fashion to modulate basic functions of the skin, including keratinocyte proliferation, differentiation, adhesion and migration, and epidermal barrier formation (20, 27).

A novel endogenous ligand for the nAChR α7 subunit, secreted mammalian Ly-6/urokinase plasminogen-type activator receptor-related protein (SLURP)-1, which is a member of the Ly-6/uPAR protein superfamily isolated from human blood and urine, increases the incidence of apoptosis among cultured human keratinocytes (28, 29). Conversely, SLURP-2, which shares substantial sequence homology with SLURP-1 and has been identified in human epidermal and oral keratinocytes, prevents keratinocyte apoptosis by acting on the α3 subunit (30, 31). That both SLURP-1 and SLURP-2 appear to be expressed in a variety of cells and tissues (32) suggests study of their functions as endogenous nAChR ligands in non-neuronal cholinergic systems is warranted.

3-2. Cancer cells

Various cancer cells express both mAChRs and nAChRs and have the ability to synthesize ACh (33 – 35).

3-2-1. Brest cancer cells

Two different murine mammary adenocarcinoma cell lines, LM2 and LM3, express functional mAChRs (36, 37). Stimulation of these cell lines with carbachol increases their proliferation via M_2 and M_3 mAChR-mediated pathways and facilitates angiogenesis via M_1 and M_2 mAChR-mediated pathways.

3-2-2. Small cell lung cancer cells

Human small cell lung cancer (SCLC) cells have the ability to produce ACh via ChAT and to express a number of mAChR and nAChR subtypes (38). The roles of mAChRs in the regulation of SCLC cell proliferation are described in detail in this issue (39).

Exposing SCLC cells to nicotine increases expression of α7 nAChRs and causes influx of Ca^{2+} and activation of PKC, Raf-1, ERK1/2, and c-myc, which leads to increases in cell proliferation or inhibition of apoptosis (40). Moreover, Schuller (40) has suggested the possibility that in addition to nicotine, its derivative nitrosamine NNK may interact with α7 nAChRs, leading to the development of lung cancer.

3-3. Immune cells

Although Pavlov and Tracey (41) proposed that there is neurological control over inflammatory cell function, lymphocytes, dendritic cells (DCs), and macrophages all clearly express cholinergic components sufficient to constitute their own cholinergic system (7, 42 – 46). A detailed description of the lymphocytic cholinergic system appears later in this issue (47).

Lymphocytes, bone marrow-derived DCs, and peritoneal macrophages all express the five mAChR subtypes ($M_1 - M_5$) and several nAChR subunits (48). Moreover, DCs express ChAT mRNA upon stimulation with lipopolisaccharide. These findings suggest that during immunological reactions, stimulated T cells and DCs have the ability to synthesize and release ACh, which in turn acts in an autocrine and/or paracrine fashion on mAChRs and nAChRs on T cells, DCs, and macrophages to modulate immune function (48).

3-4. Urinary bladder

The expression of a non-neuronal cholinergic system in urinary bladder is reviewed in this issue by Yoshida et al. (49). Briefly, they observed strong ChAT-positive immunostaining in the urothelial and suburothelial regions of the human urinary bladder (50). What is more, they also observed ACh release from urothelium that was facilitated by stretching the bladder. That this release of ACh was tetrodotoxin-insensitive indicates it to be of non-neuronal origin, which suggests that a non-neuronal cholinergic system contributes to the physiology and pathophysiology of human urinary bladder function.

Hanna-Mitchell et al. (14) found that rat urothelial cells express CHT1, ChAT, and CarAT. They also discovered expression of OCT3, which, as mentioned above, is thought to be involved in the release of ACh from non-neuronal cells, but they did not detect VAChT. RT-PCR analysis revealed that human urothelium expresses all five mAChR subtypes in the ranked order $M_2 \gg M_3 = M_5 > M_4 = M_1$ (51). Immunohistochemical analysis demonstrated differing patterns in the distribution of mAChR subtypes: M_1 is restricted to basal cells; M_2 is found almost exclusively in umbrella cells; M_3 and M_4 are homogenously distributed; and M_5 is distributed in decreasing gradient from luminal to basal cells. It may be that the apparent layer-specific distribution of mAChRs serves to stratify cholinergic regulation of urothelial function. Several nAChR α

subunits are expressed in human urothelium in the ranked order $\alpha7 \gg \alpha10 > \alpha9$, as detected by real-time RT-PCR (51). RT-PCR analysis also showed that mouse urothelium expresses the $\alpha2$, $\alpha4$, $\alpha5$, $\alpha6$, $\alpha7$, $\alpha9$, and $\alpha10$ subunits, but not the $\alpha3$ subunit (52).

3-5. Epithelial cells in the airways

Cholinergic components expressed by airway epithelial cells in humans, rats, and mice include ChAT, CHT1, VAChT, and OCT1, as well as various mAChR and nAChR subtypes, demonstrating the presence of a non-neuronal cholinergic system involved in the maintenance of cell-cell-contacts, stimulation of fluid-secretion, and regulation of ciliary beat frequency (8, 16). Lips et al. (12) discovered that the pulmonary non-neuronal cholinergic system is down-regulated during acute allergic airway inflammation, suggesting a contribution to the epithelial shedding and ciliated cell dysfunction seen in such cases. In addition, Wessler et al. (53) investigated the activity of the cholinergic system in cystic fibrosis (CF) patients and found reduced levels of non-neuronal ACh. This could contribute to the deleterious changes in epithelial ion and water movements seen in CF, as ACh stimulates apical Cl^- secretion and inhibits apical Na^+ and water absorption, thereby facilitating mucociliary clearance.

3-6. Reproductive organs

Reproductive organs express cholinergic system components and have the ability to synthesize ACh and express mAChRs or nAChRs.

3-6-1. Ovary cell

Under the control of follicle stimulating hormone (FSH), ACh is produced in granulosa cells (GCs) isolated from human antral follicles and likely also in their in vivo counterparts within the growing follicle (54). mAChRs have been identified in GC membranes and cultured human GCs, where a number of mAChR-mediated actions have been described, including regulation of proliferation and gap junctional communication. ACh may thus act as a local signaling factor within the GC compartment of growing antral follicles (55).

3-6-2. Amniotic membrane

Sakuragawa et al. (56) detected the presence of ChAT immunoreactivity in amniotic epithelial cells and the presence of ACh in the amniotic fluid. In addition, Horikoshi et al. (57) found that amniotic fluid collected after transient ligation of the uterine vessels near the lower and upper ends of the right horn of the pregnant rat contained higher levels of ACh than control samples, suggesting intrauterine hypoxia leads to increases in the level of ACh in amniotic fluid.

3-6-3. Placenta

ACh is expressed in the human placenta, where it acts via nAChR-mediated pathways to regulate nutrient transport, blood flow, and fluid volume in the placental vessels and vascularization during placental development (58). Immunohistochemical analyses revealed the expression of the $\alpha2$, $\alpha3$, $\alpha4$, $\alpha5$, $\alpha7$, $\alpha9$, and $\alpha10$ subunits in different combinations in rat cytotrophoblasts, human and rat syncytiotrophoblasts, Hofbauer cells, human amniotic epithelium, and rat visceral yolk sac epithelium (59). In addition, Wessler et al. (60) showed that ACh is released via OCT1 and OCT3 in human placenta.

3-7. Vascular endothelial cells

It has long been known that vascular endothelial cells (ECs) express mAChRs and that their stimulation by agonists, including exogenously administered ACh, induces vascular smooth muscle relaxation, leading to a reduction in blood pressure mediated by the so called endothelium-derived relaxing factor, which was later revealed to be nitric oxide (NO). Given the absence of postsynaptic parasympathetic innervation of ECs and the presence of high levels of AChE and ChE activity in the blood, the origin of the ACh that should activate the mAChRs expressed by ECs had long been an enigma. However, by using a sensitive and specific radioimmunoassay to measure ACh levels in cultured bovine arterial ECs and primary cultured ECs from porcine cerebral microvessels, as well as in their conditioned media, Kawashima and his colleagues were able to demonstrate for the first time that ECs have the ability to synthesize and release ACh (61 – 63). In addition, they found phorbol myristate 13-acetate (PMA) significantly increased ACh synthesis in primary cultured ECs from porcine cerebral microvessels (63), suggesting a role for PKC in the regulation of ACh synthesis.

Kirkpatrick et al. (64) discovered the presence of ChAT immunoreactivity and activity in ECs from human dermal blood vessels, monolayer cultures of human umbilical vein ECs, and in a human angiosarcoma EC line. They also detected both ACh and VAChT in human ECs.

Using northern blot analysis, Tracey and Peach (65) identified the expression of mRNAs encoding the M_1, M_2, and M_3 mAChR subtypes in freshly isolated bovine ECs, while Elhusseiny et al. (66) found consistent expression of M_2 and M_5 mAChRs and occasional expression of M_1 mAChRs in human brain microvessel ECs. M_1 mAChRs have also been detected in the

endothelium of human pulmonary veins (67).

ECs also express several nAChR subunits, including the $\alpha 3$, $\alpha 5$, $\beta 2$, and $\beta 4$ subunits in primary cultures of ECs from human aorta (68) and the $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 7$, $\beta 2$, and $\beta 4$ subunits in rat microvascular ECs (69). $\alpha 7$ nAChRs have been shown to mediate EC proliferation, survival, migration, and tube formation in vitro and to mediate angiogenesis in vivo. It also has been suggested that $\alpha 7$ nAChR signaling is related to pathological angiogenesis, playing a significant role in tumor growth and the progression of atherosclerosis (70).

3-8. Miscellaneous

3-8-1. Fibroblasts

Human fibroblasts and myofibroblasts isolated from lung tissues express the M_1, M_2, and M_3 mAChR subtypes (71). ACh-stimulated proliferation of these cells is inhibited by tiotropium, an mAChR antagonist, suggesting that cholinergic signaling mediated by mAChRs could contribute to the remodeling processes seen in chronic airway disease.

3-8-2. Tendon

Prompted by the knowledge that stimulation of AChRs affects processes such as pain sensation, collagen production, angiogenesis, and cell proliferation and outgrowth, like that seen in tendinosis, Danielson et al. (72) investigated the expression of cholinergic components in human tendon cells (tenocytes). They found strong expression of ChAT and VAChT in tenocytes, as well as the mRNAs for M_2 mAChRs and ChAT, suggesting that excessive local production of ACh might be related to the pain sensation and the pathophysiology underlying the development of tendinosis.

3-8-3. Embryonic stem cells

It has been reported that developing tissues express cholinergic markers like AChE, BChE, and AChRs (73, 74) and that their expression is highly regulated developmentally. Paraoanu et al. (75) used RT-PCR, histochemistry, and measurements of enzyme activity to study the expression of cholinergic components in a murine embryonic stem cell line. As expected, they found that embryonic stem cells express ACh, all five mAChR subtypes ($M_1 - M_5$), ChAT, AChE, and BuChE. Inhibition of BuChE caused a reduction in proliferation, suggesting that locally produced ACh functions as an intercellular signal acting via mAChRs to modulate stem cell proliferation.

3-8-4. Skeletal muscle

Tuček (9) showed that some ACh synthesis in denervated extensor digitorum longus muscles of the rat is catalyzed by CarAT, but the physiological function of ACh synthesized by CarAT is not known. Later, Hamann et al. (76) found that proliferating myoblasts contained a small amount of ACh-like compound and that this compound was synthesized by BrACh-sensitive ChAT in all myogenic cells. The ACh-like compound was spontaneously released from both myoblasts and myotubes in a partially Ca^{2+}-dependent manner. It was therefore suggested that human myogenic cells synthesize and release an ACh-like compound to promote the fusion process that occurs in muscle during growth and regeneration.

4. Conclusion

Studies have shown that non-neuronal cholinergic systems are expressed in numerous tissues and cells and are involved in the regulation of their function. Moreover, abnormalities of these non-neuronal cholinergic systems appear to be related to the pathology of certain diseases, and the evidence suggests that non-neuronal cholinergic systems are potentially useful therapeutic targets.

References

1 Kawashima K, Nomura Y. The non-neuronal cholinergic system: Preface. Jpn J Pharmacol. 2001;85:1.
2 Grando SA, Kawashima K, Wessler I. Editorial: The non-neuronal cholinergic system in humans. Life Sci. 2003;72:2009–2012.
3 Grando SA, Kawashima K, Kirkpatrick CJ, Wessler I. Introduction: Recent progress in understanding the non-neuronal cholinergic system in humans. Life Sci. 2007;80:2181–2185.
4 Horiuchi Y, Kimura R, Kato N, Fujii T, Seki M, Endo T, et al. Evolutional study on acetylcholine expression. Life Sci. 2003; 72:1745–1756.
5 Yamada T, Fujii T, Kanai T, Amo T, Imanaka T, Nishimasu H, et al. Expression of acetylcholine (ACh) and ACh-synthesizing activity in archaea. Life Sci. 2005;77:1935–1944.
6 Kawashima K, Misawa H, Moriwaki Y, Fujii YX, Fujii T, Horiuchi Y, et al. Ubiquitous expression of acetylcholine and its biological functions in life forms without nervous systems. Life Sci. 2007;80:2206–2209.
7 Kawashima K, Fujii T. Extraneuronal cholinergic system in lymphocytes. Pharmacol Ther. 2000;86:29–48.
8 Wessler I, Kirkpatrick CJ, Racké K. Non-neuronal acetylcholine, a locally acting molecule widely distributed in biological system: expression and function in humans. Pharmacol Ther. 1998;77:59–79.
9 Tuček S. The synthesis of acetylcholine in skeletal muscles of the rat. J Physiol. 1982;322:53–69.
10 Fonnum F. A rapid radiochemical method for the determination of choline acetyltransferase. J Neurochem. 1975;24:407–409.
11 Fujii T, Tajima S, Yamada S, Watanabe Y, Sato KZ, Matsui M,

et al. Constitutive expression of mRNA for the same choline acetyltransferase as that in the nervous system, an acetylcholine-synthesizing enzyme, in human leukemic T-cell lines. Neurosci Lett. 1999;259:71–74.
12 Lips KS, Lührmann A, Tschernig T, Stoeger T, Alessandrini F, Grau V, et al. Down-regulation of the non-neuronal acetylcholine synthesis and release machinery in acute allergic airway inflammation of rat and mouse. Life Sci. 2007;80:2263–2269.
13 Elwary SM, Chavan B, Schallreuter KU. The vesicular acetylcholine transporter is present in melanocytes and keratinocytes in the human epidermis. J Invest Dermatol. 2006;126:1879–1884.
14 Hanna-Mitchell AT, Beckel JM, Barbadora S, Kanai AJ, de Groat WC, Birder LA. Non-neuronal acetylcholine and urinary bladder urothelium. Life Sci. 2007;80:2298–2302.
15 Wessler I, Roth E, Deutsch C, Brockerhoff P, Bittinger F, Kirkpatrick CJ, et al. Release of non-neuronal acetylcholine from the isolated human placenta is mediated by organic cation transporters. Br J Pharmacol. 2001;134:951–956.
16 Kummer W, Wiegand S, Akinci S, Wessler I, Schinkel AH, Wess J, et al. Role of acetylcholine and polyspecific cation transporters in serotonin-induced bronchoconstriction in the mouse. Respir Res. 2006;7:65–76.
17 Pfeil U, Lips KS, Eberling L, Grau V, Haberberger RV, Kummer W. Expression of the high-affinity choline transporter, CHT1, in the rat trachea. Am J Respir Cell Mol Biol. 2003;28:473–477.
18 Fujii T, Okuda T, Haga T, Kawashima K. Detection of the high-affinity choline transporter in the MOLT-3 human leukemic T-cell line. Life Sci. 2003;72: 2131–2134.
19 Lips KS, Volk C, Schmitt BM, Pfeil U, Arndt P, Miska D, et al. Polyspecific cation transporters mediate luminal release of acetylcholine from bronchial epithelium. Am J Respir Cell Mol Biol. 2005;33:79–88.
20 Kurzen H, Wessler I, Kirkpatrick CJ, Kawashima K, Grando SA. The non-neuronal cholinergic system of human skin. Horm Metab Res. 2007;39:125–135.
21 Grando SA. Basic and clinical aspects of non-neuronal acetylcholine: Biological and clinical significance of non-canonical ligands of epithelial nicotinic acetylcholine receptors. J Pharmacol Sci. 2008;106:174–179.
22 Grando SA, Kist DA, Qi M, Dahl MV. Human keratinocytes synthesize, secrete, and degrade acetylcholine. J Invest Dermatol. 1993;101:32–36.
23 Nguyen VT, Hall LL, Gallacher G, Ndoye A, Jolkovsky DL, Webber RJ, et al. Choline acetyltransferase, acetylcholinesterase, and nicotinic acetylcholine receptors of human gingival and esophageal epithelia. J Dent Res. 2000;79:939–949.
24 Arredondo J, Hall LL, Ndoye A, Chernyavsky AI, Jolkovsky DL, Grando SA. Muscarinic acetylcholine receptors regulating cell cycle progression are expressed in human gingival keratinocytes. J Periodontal Res. 2003;38:79–89.
25 Ndoye A, Buchli R, Greenberg B, Nguyen VT, Zia S, Rodriguez JG, et al. Identification and mapping of keratinocyte muscarinic acetylcholine receptor subtypes in human epidermis. J Invest Dermatol. 1998;111:410–416.
26 Elwary SMA, Hasse S, Schallreuter KU. m2 Muscarinic acetylcholine receptor (mAchR) subtype is present in human epidermal keratinocytes in situ and in vitro. J Invest Dermatol. 2004;123:1206–1207.
27 Grando SA, Pittelkow MR, Schallreuter KU. Adrenergic and cholinergic control in the biology of epidermis: physiological and clinical significance. J Invest Dermatol. 2006;126:1948–1965.
28 Adermann K, Wattler F, Wattler S, Heine G, Meyer M, Forssmann W-G, et al. Structural and phylogenetic characterization of human SLURP-1, the first secreted mammalian member of the Ly-6/uPAR protein superfamily. Protein Sci. 1999;8:810–819.
29 Arredondo J, Chernyavsky AI, Webber RJ, Grando SA. Biological effects of SLURP-1 on human keratinocytes. J Invest Dermatol. 2005;125:1236–1241.
30 Tsuji H, Okamoto K, Matsuzaka Y, Iizuka H, Tamiya G, Inoko H. SLURP-2, a novel member of the human Ly-6 superfamily that is up-regulated in psoriasis vulgaris. Genomics. 2003;81: 26–33.
31 Arredondo J, Chernyavsky AI, Jolkovsky DL, Webber RJ, Grando SA. SLURP-2: A novel cholinergic signaling peptide in human mucocutaneous epithelium. J Cell Physiol. 2006; 208:238–245.
32 Moriwaki Y, Yoshikawa K, Fukuda H, Fujii YX, Misawa H, Kawashima K. Immune system expression of SLURP-1 and SLURP-2, two endogenous nicotinic acetylcholine receptor ligands. Life Sci. 2007;80:2365–2368.
33 Gutkind JS, Novotny EA, Brann MR, Robbins KC. Muscarinic acetylcholine receptor subtypes as agonist-dependent oncogenes. Proc Natl Acad Sci U S A. 1991;88:4703–4707.
34 Cunningham JM, Lennon VA, Lambert EH, Scheithauer B. Acetylcholine receptors in small cell carcinomas. J Neurochem. 1985;45:159–167.
35 Song P, Sekhon HS, Jia Y, Keller JA, Blusztajn JK, Mark GP, et al. Acetylcholine is synthesized by and acts as an autocrine growth factor for small cell lung carcinoma. Cancer Res. 2003;63:214–221.
36 Español AJ, Sales ME. Different muscarinic receptors are involved in the proliferation of murine mammary adenocarcinoma cell lines. Int J Mol Med. 2004;13:311–317.
37 Español AJ, de la Torre E, Fiszman GL, Sales ME. Role of non-neuronal cholinergic system in breast cancer progression. Life Sci 2007;80:2281–2285.
38 Song P, Sekhon HS, Jia Y, Keller JA, Blusztajn JK, Mark GP, et al. Acetylcholine is synthesized by and acts as an autocrine growth factor for small cell lung carcinoma. Cancer Res. 2003;63:214–221.
39 Song P, Spindel ER. Basic and clinical aspects of non-neuronal acetylcholine: Expression of non-neuronal acetylcholine in lung cancer provides a new target for cancer therapy. J Pharmacol Sci. 2008:106:180–185.
40 Schuller HM. Nitrosamines as nicotinic receptor ligands. Life Sci. 2007;80:2274–2280.
41 Pavlov VA, Tracey KJ. The cholinergic anti-inflammatory pathway. Brain Behav Immun. 2005;19:493–499.
42 Kawashima K, Fujii T, Watanabe Y, Misawa H. Acetylcholine synthesis and muscarinic receptor subtype mRNA expression in T-lymphocytes. Life Sci. 1998;62:1701–1705.
43 Fujii T, Kawashima K. An independent non-neuronal cholinergic system in lymphocytes. Jpn J Pharmacol. 2001;85:11–15.
44 Kawashima K, Fujii T. The lymphocytic cholinergic system and its biological function. Life Sci. 2003;72:2101–2110.
45 Kawashima K, Fujii T. Minireview: The lymphocytic cholinergic system and its contribution to the regulation of immune

activity. Life Sci. 2003;74:675–696.
46 Kawashima K, Fujii T. Expression of non-neuronal acetylcholine in lymphocytes and its contribution to the regulation of immune function. Front Biosci. 2004;9:2063–2085.
47 Fujii T, Takada-Takatori Y, Kawashima K. Basic and clinical aspects of non-neuronal acetylcholine: Expression of an independent, non-neuronal cholinergic system in lymphocytes and its clinical significance in immunotherapy. J Pharmacol Sci. 2008;106:186–192.
48 Kawashima K, Yoshikawa K, Fujii YX, Moriwaki Y, Misawa H. Expression and function of genes encoding cholinergic components in murine immune cells. Life Sci. 2007;80:2314–2319.
49 Yoshida M, Masunaga K, Satoji Y, Maeda Y, Nagata T, Inadome A. Basic and clinical aspects of non-neuronal acetylcholine: Expression of non-neuronal acetylcholine in urothelium and its clinical significance. J Pharmacol Sci. 2008;106:193–198.
50 Yoshida M, Inadome A, Maeda Y, Satoji Y, Masunaga K. Non-neuronal cholinergic system in human bladder urothelium. Urology. 2006;67:425–430.
51 Bschleipfer T, Schukowski K, Weidner W, Grando SA, Schwantes U, Kummer W, et al. Expression and distribution of cholinergic receptors in the human urothelium. Life Sci. 2007;80:2303–2307.
52 Zarghooni S, Wunsch J, Bodenbenner M, Brüggmann D, Grando SA, Schwantes U, et al. Expression of muscarinic and nicotinic acetylcholine receptors in the mouse urothelium. Life Sci. 2007;80:2308–2313.
53 Wessler I, Bittinger F, Kamin W, Zepp F, Meyer E, Schad A, et al. Dysfunction of the non-neuronal cholinergic system in the airways and blood cells of patients with cystic fibrosis. Life Sci. 2007;80:2253–2258.
54 Kunz L, Roggors C, Mayerhofer A. Ovarian acetylcholine and ovarian KCNQ channels: insights into cellular regulatory systems of steroidogenic granulosa cells. Life Sci. 2007;80:2195–2198.
55 Mayerhofer A, Kunz L, Krieger A, Proskocil B, Spindel E, Amsterdam A, et al. FSH regulates acetylcholine production by ovarian granulosa cells. Reprod Biol Endocrinol. 2006;4:37–43.
56 Sakuragawa N, Elwan MA, Uchida S, Fujii T, Kawashima K. Non-neuronal neurotransmitter and neurotrophic factors in amniotic epithelial cells: expression and function in humans and monkey. Jpn J Pharmacol. 2001;85:20–23.
57 Horikoshi T, Fujii T, Kawashima K, Sakuragawa N. Acetylcholine increase in amniotic fluid of experimental rats for intrauterine growth retardation. Life Sci. 2003;72:2145–2150.
58 Sastry BV. Human placental cholinergic system. Biochem Pharmacol. 1997;53:1577–1586.
59 Lips KS, Brüggmann D, Pfeil U, Vollerthun R, Grando SA, Kummer W. Nicotinic acetylcholine receptors in rat and human placenta. Placenta. 2005;26:735–746.
60 Wessler I, Hershel S, Bittinger F, Kirkpatrick CJ. Release of non-neuronal acetylcholine from the isolated human placenta is affected by antidepressants. Life Sci. 2007;80:2210–2213.
61 Kawashima K, Ishikawa H, Mochizuki M. Radioimmunoassay for acetylcholine in the rat brain. J Pharmacol Method. 1980;3:115–123.
62 Kawashima K, Watanabe N, Oohata H, Fujimoto K, Suzuki T, Ishizaki Y, et al. Synthesis and release of acetylcholine by cultured bovine arterial endothelial cells. Neurosci Lett. 1990;119:156–158.
63 Ikeda C, Morita I, Mori A, Fujimoto K, Suzuki T, Kawashima K, et al. Phorbol ester stimulates acetylcholine synthesis in cultured endothelial cells isolated from porcine cerebral microvessels. Brain Res.1994;655:147–152.
64 Kirkpatrick CJ, Bittinger F, Unger RE, Kriegsmann J, Kilbinger H, Wessler I. The non-neuronal cholinergic system in the endothelium: evidence and possible pathobiological significance. Jpn J Pharmacol. 2001;85:24–28.
65 Tracey WR, Peach MJ. Differential muscarinic receptor mRNA expression by freshly isolated and cultured bovine aortic endothelial cells. Circ Res. 1992;70:234–240.
66 Elhusseiny A, Cohen Z, Olivier A, Stanimirović DB, Hamel E. Functional acetylcholine muscarinic receptor subtypes in human brain microcirculation: identification and cellular localization. J Cereb Blood Flow Metab. 1999;19:794–802.
67 Walch L, Gascard J-P, Dulmet E, Brink C, Norel X. Evidence for a M1-muscarinic receptor on the endothelium of human pulmonary veins. Br J Pharmacol. 2000;130:73–78.
68 Macklin KD, Maus ADJ, Pereira EFR, Albuquerque EX, Conti-Fine BM. Human vascular endothelial cell express functional nicotinic acetylcholine receptors. J Pharmacol Exp Ther. 1998;287:435–439.
69 Moccia F, Frost C, Berra-Romani R, Tanzi F, Adams DJ. Expression and function of neuronal nicotinic ACh receptor in rat microvascular endothelial cells. Am J Physiol Heart Circ Physiol. 2004;286:H486–H491.
70 Cooke JP. Angiogenesis and the role of the endothelial nicotinic acetylcholine receptor. Life Sci. 2007;80:2347–2351.
71 Pieper MP, Chaudhary NI, Park JE. Acetylcholine-induced proliferation of fibroblasts and myofibroblasts in vitro is inhibited by tiotropium bromide. Life Sci. 2007;80:2270–2273.
72 Danielson P, Andersson G, Alfredson H, Forsgren S. Extensive expression of markers for acetylcholine synthesis and of M2 receptors in tenocytes in therapy-resistant chronic painful patellar tendon tendinosis – a pilot study. Life Sci. 2007;80:2235–2238.
73 Willbold E, Layer PG. Butyrylcholinesterase regulates laminar retinogenesis of the chick embryo in vitro. Eur J Cell Biol. 1994;64:192–199.
74 Buznikov GA, Shmukler YB, Lauder JM. From oocyte to neuron: do neurotransmitter function in the same way throughout development? Cell Mol Neurobiol. 1996;16:537–559.
75 Paraoanu LE, Steinert G, Koehler A, Wessler I, Layer PG. Expression and possible functions of the cholinergic system in a murine embryonic stem cell line. Life Sci. 2007;80:2375–2379.
76 Hamann M, Chamoin MC, Portalier P, Bernheim L, Baroffio A, Widmer H, et al. Synthesis and release of an acetylcholine-like compound by human myoblasts and myotubes. J Physiol. 1995;489:791–803.