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REVIEW

Acetylcholine beyond neurons: the non-neuronal cholinergic system in humans

I Wessler and CJ Kirkpatrick

Institute of Pathology, University Hospital, Johannes Gutenberg-University, Mainz, Germany

Animal life is controlled by neurons and in this setting cholinergic neurons play an important role. Cholinergic neurons release ACh, which via nicotinic and muscarinic receptors (n- and mAChRs) mediate chemical neurotransmission, a highly integrative process. Thus, the organism responds to external and internal stimuli to maintain and optimize survival and mood. Blockade of cholinergic neurotransmission is followed by immediate death. However, cholinergic communication has been established from the beginning of life in primitive organisms such as bacteria, algae, protozoa, sponge and primitive plants and fungi, irrespective of neurons. Tubocurarine- and atropine-sensitive effects are observed in plants indicating functional significance. All components of the cholinergic system (ChAT, ACh, n- and mAChRs, high-affinity choline uptake, esterase) have been demonstrated in mammalian non-neuronal cells, including those of humans. Embryonic stem cells (mice), epithelial, endothelial and immune cells synthesize ACh, which via differently expressed patterns of n- and mAChRs modulates cell activities to respond to internal or external stimuli. This helps to maintain and optimize cell function, such as proliferation, differentiation, formation of a physical barrier, migration, and ion and water movements. Blockade of n- and mAChRs on non-innervated cells causes cellular dysfunction and/or cell death. Thus, cholinergic signalling in non-neuronal cells is comparable to cholinergic neurotransmission. Dysfunction of the non-neuronal cholinergic system is involved in the pathogenesis of diseases. Alterations have been detected in inflammatory processes and a pathobiologic role of non-neuronal ACh in different diseases is discussed. The present article reviews recent findings about the non-neuronal cholinergic system in humans.
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Keywords: non-neuronal cholinergic system; ACh; ChAT; nicotinic and muscarinic receptors; epithelial, endothelial, mesenchymal cells; immune cells; signal transduction; phenotype cell functions; inflammation

Abbreviations: mAChRs, muscarinic ACh receptors; nAChRs, nicotinic ACh receptors; OCT, organic cation transporter; TCR/CD3, T-cell receptor complex; VCAM, vascular cellular adhesion molecule

Introduction

ACh is regarded as a classical neurotransmitter. Nicotinic ACh receptors (nAChRs) are recognized as binding and effector proteins to mediate chemical neurotransmission at neurons, ganglia, interneurons and the motor endplate. Muscarinic ACh receptors (mAChRs) are recognized as binding and effector proteins to mediate chemical neurotransmission at neurons and effector organs such as heart, smooth muscle fibres and glands. This traditional view of ACh acting solely as neurotransmitter has to be revised based on the findings published both early and late in the last century, demonstrating the non-neuronal cholinergic system. Cholinergic communication and regulation have been established from the beginning of life; that is, in primitive uni- and multicellular organisms such as bacteria, algae, protozoa, sponge and primitive plants and fungi (Wessler *et al*., 1999; 2001a, 2003a; Horiuchi *et al*., 2003). The ubiquitous synthesis of ACh and the expression of n- and mAChRs in mammalian cells give an impressive example of the complexity of biological systems. All receptor subtypes and signal-transduction pathways used by cholinergic neurons are also used by single non-neuronal cells to communicate among each other and to maintain their phenotypic functions and thus organ homoeostasis. The present review will focus on the following topics, with particular emphasis on the situation in humans:

(1) Synthesis of ACh outside the nervous system;
(2) expression of n- and mAChRs on non-neuronal cells;
(3) release mechanisms;
(4) cellular functions of non-neuronal ACh;
  (a) signal transduction;
  (b) regulation of phenotype cell functions;
  (c) epithelial cells;
  (d) endothelial cells;

Correspondence: Professor I Wessler, Institute of Pathology, Johannes Gutenberg University, University Hospital Mainz, Langenbeckstr. 1, Mainz D-55101, Germany.
E-mail: wessler@mail.uni-mainz.de
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(e) immune cells and the cholinergic anti-inflammatory pathway;
(f) mesenchymal cells;
(5) non-neuronal cholinergic system involved in the pathophysiology of diseases.

Synthesis of ACh in non-neuronal cells

In the last decades, several reviews have focused on this topic (Sastry and Sadavongvivad, 1978; Grando, 1997; Wessler *et al*., 1998, 1999, 2001a, 2003a; Kawashima and Fujii, 2000, 2004; Eglen, 2006; Grando *et al*., 2006, 2007; Kurzen *et al*., 2007). ACh is synthesized by practically all living cells and can play an intermediary role in the interactions of non-neuronal cells with the external environment, hormones, growth factors, cytokines and also the neural system. On the other hand, all of these factors can also affect the expression and function of the non-neuronal cholinergic system. Table 1 gives a summary of the synthesis of ACh in various human, non-neuronal cells. Evidence for ACh synthesis is not only provided by positive anti-ChAT immunoreactivity, but ChAT enzyme activity and/or ACh content have also been determined in the majority of the cells indicated in Table 1

Table 1 Positive anti-ChAT immunoreactivity or HPLC detection of ACh in human cells[a]

Epithelial cells	
Airways	Basal, ciliated and secretory cells
Alimentary tract	Buccal mucosa, oesophagus, stomach, jejunum, ileum, colon, sigmoid and gall bladder
Skin	Keratinocytes, eccrine and sebaceous glands
Kidney	Tubuli
Urogenital tract	Urothelium, vaginal mucosa and granulosa cells
Placenta	Trophoblast
Glandular tissue	Female breast and thymus
Eye	Cornea
Endothelial cells	
Skin, umbilical vein and pulmonary vessels	
Immune cells	
Mononuclear leukocytes, bone marrow-derived dendritic cells, macrophages, skin mast cells	
Mesothelial cells	
Pleura, pericardium	
Mesenchymal cells	
Adipocytes (skin)	
Smooth muscle fibres (skin, airways)	
Fibroblasts (airways[b])	
Tendon (tenocytes)	
Brain	
Astrocytes[b]	

[a]Data from Sastry and Sadavongvivad, 1978; Grando *et al*., 1993; Grando, 1997; Klapproth *et al*., 1997; Kawashima *et al*., 1998; Kawashima and Fujii, 2000, 2004; Wessler *et al*., 1998, 1999, 2001a; Wessler and Kirkpatrick, 2001a, b; Danielson *et al*., 2007).
[b]Unpublished observation (Wessler and Kirkpatrick, 2007).

(epithelial cells in airways, intestine, skin, urothelium, vagina, placenta, cornea; granulosa cells; endothelial and immune cells; subcutaneous fat). In addition, it is noteworthy that embryonic stem cells (mice) also synthesize considerable amounts of ACh (Paraoanu *et al*., 2007). In the urothelium (mice and humans), positive ChAT immunoreactivity using a polyclonal antibody has been demonstrated, but this polyconal anti-ChAT antibody could be successfully preabsorbed with carnitine acetyltransferase (CarAT), another enzyme generating ACh (Lips *et al*., 2007b). In this latter paper, ChAT-specific mRNA was not detected, whereas in rat urothelial cells both ChAT- and CarAT-mRNA have been demonstrated (Hanna-Mitchell *et al*., 2007). Moreover it is known that monoclonal antibodies, also available for anti-ChAT immunoreactivity studies, operate more specifically than polyclonal antibodies.

The widespread synthesis of ACh beyond the nervous system has changed the paradigm of ACh acting merely as a neurotransmitter. Consequently, it has been repeatedly questioned whether ACh present in non-neuronal cells can be explained by neuronal contamination. This possibility can be categorically ruled out on the basis of several findings:

(1) ChAT mRNA and ChAT protein have been demonstrated in isolated human epithelial and immune cells (Grando *et al*., 1993; Klapproth *et al*., 1997; Fujii *et al*., 1998; Ogawa *et al*., 2003; Kawashima and Fujii, 2004).
(2) Multiple papers (for references, see review articles indicated above) have described specific labelling of human non-neuronal cells by anti-ChAT antibodies.
(3) Isolated human airway epithelial cells or cultured human keratinocytes show ChAT enzyme activity and contain ACh (Grando *et al*., 1993; Reinheimer *et al*., 1996; Klapproth *et al*., 1997).
(4) The human placenta—free of cholinergic neurons—synthesizes, stores and releases ACh (Olubadewo and Rama Sastry, 1978; Wessler *et al*., 2001b).
(5) Human blood cells (mononuclear cells, lymphocytes) and human leukaemic cell lines as well as embryonic stem cells (mice) show ChAT enzyme activity and/or contain ACh, even under culture conditions (Fujii *et al*., 1998; Wessler *et al*., 2003a; Kawashima and Fujii, 2004; Neumann *et al*., 2007; Paraoanu *et al*., 2007).
(6) *In vivo* release of ACh from the human skin as measured by dermal microdialysis maintained after pretreatment with botulinum toxin, which is known to block the exocytotic release of neuronal ACh (Kao *et al*., 1976; Schlereth *et al*., 2006).

Taken together, there exists overwhelming evidence that cells outside the cholinergic neuronal network synthesize, contain and release ACh. This property has been identified not only for the abundant majority of human cells but also in other mammals (rat), lower invertebrates (sponge, coral, sea squirt, sea urchin, tubellaria), protozoa, plants, fungi, blue-green algae and even in bacteria (*Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Lactobacillus plantarum*; for references, see Wessler *et al*., 1999; Horiuchi *et al*., 2003).

It should also be considered that both precursors for ACh synthesis—choline and acetyl-CoA—are present in nearly all cells. Acetyl-CoA is the major product of carbohydrate,

protein and lipid catabolism in aerobic organisms, and is thus present in more or less all cells. Choline originates from the intracellular breakdown of choline-containing phospholipids or from the uptake of extracellular choline via low- or high-affinity choline transporter (CHT1). The latter has been demonstrated in the rat and human epithelial, urothelial, endothelial and vascular smooth muscle cells (Haberberger *et al*., 2002; Lips *et al*., 2003; Pfeil *et al*., 2003; Hanna-Mitchell *et al*., 2007). Thus, the equipment for ACh synthesis corresponds to that of neurons, even with respect to different ChAT mRNA species. In the human brain, R, N0, N1, N2 and M types have been found and most of these subtypes are detected, for example, in human T-lymphocytes (Oda, 1999; Ogawa *et al*., 2003). Likewise, AChE has been detected as nuclear protein very recently in both neuronal and non-neuronal cells (Santos *et al*., 2007).

Expression of nicotinic and muscarinic receptors on non-neuronal cells

Table 2 gives an overview of the expression of n- and mAChRs on non-neuronal cells. It is evident that most cell types—not innervated by cholinergic neurons at all—express n- and mAChRs that are part of the auto- and paracrine regulatory loop of non-neuronal ACh released from these cells. The specificity of antibodies raised against subtype-specific n- and mAChRs has recently been questioned (Moser *et al*., 2007; Zarghooni *et al*., 2007). When commercial antibodies directed against α3, α4, α7, β2 and β4 nAChR subtypes were applied, the respective immunoreactivity in the brain did not differ between knockout and wild-type mice (Moser *et al*., 2007). However, in this context it has to be considered that in most of the references given in Table 2 more than one method had been applied to demonstrate the expression of n- and mAChRs. The different methods used are indicated in the legend of Table 2.

The expression pattern of the nicotinic ion channel complex and of the metabotrophic mAChRs varies according to phenotypic cell functions, as well as internal and external environmental conditions. For example, immature basal keratinocytes express predominantly α3β2/4-nAChRs and M2 and M3 receptors, transitional keratinocytes contain more α5, α9 subtypes and M4 and M5 receptors, whereas the mature keratinized surface keratinocyte expresses mainly α7-nAChRs and M1 receptors (Grando *et al*., 2006). Steroids (glucocorticosteroids, oestrogen) modify the expression of n- and mAChRs (Batra, 1990; Carrasco-Serrano and Criado, 2004). Thus, cholinergic input varies with cell type, state of cell differentiation and activity, as well as with cell environmental conditions.

Release mechanisms

In contrast to the release of neuronal ACh via exocytosis, our knowledge about the release mechanisms of non-neuronal ACh is scarce. Most details have been obtained from release studies using the human placenta as a model of the non-neuronal cholinergic system (Wessler *et al*., 2001d). Inhibitors (quinine, corticosterone) of organic cation transporters (OCT)) markedly suppressed ACh release, as well as substrate inhibitors (amiloride, cimetidine, verapamil, noradrenaline) and antisense oligonucleotides directed against subtypes 1 and 3. Experiments with transfected ooyctes (*Xenopus laevis*) showed ACh transport by OCT1 and OCT2 but not by OCT3 (Lips *et al*., 2007b). This latter finding differed from the results in the human placenta where OCT3 contributes to the release of ACh (Wessler *et al*., 2001d). Most likely, the subtypes involved differ between cells and organs. In the airway epithelium, OCT subtypes 1 and 2 appear to mediate the release of non-neuronal ACh (Lips *et al*., 2005; Kummer *et al*., 2006). The content of epithelial ACh was substantially enhanced in OCT1/2 double knockout mice compared with wild type (Kummer *et al*., 2006). These transporter proteins (OCTs) are very widely expressed on more or less every cell and therefore represent appropriate candidates to mediate the release of non-neuronal ACh. In addition, packing of ACh into secretory vesicles or endosomes may occur for intermediate intracellular storage and subsequent release of non-neuronal ACh, when these organelles fuse with the cell membrane. With the technique of immunogold electron microscopy the synthesizing enzyme ChAT has been found in endosomes of the human placenta (Wessler *et al*., 2001c).

Cellular functions of non-neuronal ACh

Signal transduction

Non-neuronal ACh is released from living cells, for example, from the human skin (Schlereth *et al*., 2006) and binds to n- and mAChRs of its source and neighbouring cells to mediate auto- and paracrine regulatory loops. Additional endogenous compounds can stimulate cholinergic receptors, for example, choline stimulates the α9 subtype and bile acid M3 receptors (Raufman *et al*., 2003; Alexander *et al*., 2006). In this way, cells may receive cholinergic input not only by local but also by hormone-like pathways. Moreover, endogenous allosteric modulators of n- and mAChRs play an essential role as an extremely sophisticated tool to fine-tune the cholinergic input to a cell. For example, progesterone operates as an allosteric modulator on the neuronal α4 subtype (Valera *et al*., 1992). SLURP (secreted mammalian *Ly-6*/urokinase plasminogen activator receptor-related protein 1 and 2), a modulator of nAChRs, has recently been found to be co-expressed with nAChRs not only in the brain but also on immune cells and epithelial cells in the skin and lung (Sekhon *et al*., 2005; Grando *et al*., 2006; Kawashima *et al*., 2007).

The most detailed analysis of the signal-transduction machinery involved has been evaluated for keratinocytes of the human skin (Grando *et al*., 2006). Table 3 summarizes some examples of the biochemical signal-transduction pathways that are triggered by auto- and paracrine actions or applied ACh on non-neuronal cells. It has to be considered that ACh can modify more or less all known signalling pathways via n- and mAChRs. Classical ionic channels as well as non-selective cation channels transporting Na^+ and Ca^{2+} inside the cells can be affected. On granulosa cells ACh

Table 2 Expression of n- and mAChRs on non-neuronal cells

Cell type	*Tissue*	*Muscarinic*	*Nicotinic*	*References*
Epithelial cells	*Airway (human)*			
	Surface epithelium	M1 (small airways) M3 (M2; BEAS-2B cell line)	α1, α3, α5, α7, α9, β1, β2, β4, δ, ε	1–11
	Alveolar type 2 cells		α4, α7, β1, β2 (rhesus monkey)	
	Glands	M1, M3	α4 (rhesus monkey)	
	Skin (human)			
	Keratinocytes	M1, M2, M3, M4, (M5 mRNA)	α3, α5, α7, α9, α10, β1, β2, β4	
	Pilosebaceous unit	All subtypes	α3, α4, α5, α7, α9, α10, β1, β2, β4	12–17
	Sweat glands			
	Myoepithelial	M2–M5	α3, α4, α5, α7	
	Acinar cells	M1, M3, M4	α9, β2	
	Melanocytes	All subtypes	α1, α3, α5, α7, β1, β2, γ, δ	
	Intestine			
	Surface epithelium	M1, M3	α3	18–25
	Colonic epithelial cell line		α4, α5, α7, β1	
	Glands (salivary cells, gastric cells, pancreatic acinar cells)	M1, M3	α2, α3, α4, α5, α7, β2 (beta cell line, rat)	
	Ovary			
	Granulosa (human)	M1, M3, M5		26,27
	Urothelium			
	Human	M1, M2, M3, M4, M5	α7, α9, α10	
	Rat, mouse	M1, M2, M3, M4, M5	α2, α4, α5, α6, α7, α9, α10, β3, β4	28–30
Endothelial cells	*Aorta/pulmonary vessels*			
	Human	M1, M2, M3, M2, M4, M5 (corneal endothelium)	α3, α5, α7, β2, β4, β4	
	Rat		α2, α3, α4, α5, α6, α7, α10, β2, β4	6,7,31–37
	Mouse	M3, M5 (brain vessels)	α3, α5, α7, β2 (cerebral microvasculature)	
	Bovine	m1, m2, m3 (mRNA)		
Immune cells				
	MNLs (human)	M1–M5 (variable expression)	α2, α4, α5, α6, α7, α9, α10, β2, β4 (variable expression with dominant expression of α2, α5, α7)	
	Eosinophiles (human)		α3, α4, α7	
	Macrophages human, airways	M2, M3	α1, α7, α10	38–43
	Mouse	M1–M5	α2, α4, α5, α6, α7, α10, β2, β4	
	DC cells (mouse)	M1–M5	α2, α5, α6, α7, α10, β2, β4	
	Mast cells (human) mucosal-type skin	M1	α3, α5, α10	
Mesenchymal cells	*Fibroblasts*			
	Sclera (human)	m3 > m2 > m4 > m5 > m1 (mRNA)		
	Lung (human)	M2 > M1 > M3 > M4	α1, α3, α4, α6, α7, α9	
	Skin (human)	M2, M4, M5	β1, β2, β3, β4, δ, ε	
	Tenocytes	M2 (human)	α7 (rat)	
	Adipocytes (rat)		α1–7, α9, α10, β1–4, δ, ε	5,10,36, 44–53
	Smooth muscle fibres			
	Lung	M2, M3 (human)	α6, α7, β2, β4 (rhesus monkey); α4, α7 (mouse)	
	Vasculature	M3 (human)	α2–7, α10 (rat); α6, β2, β4 (rhesus monkey)	
Mesothelial cells	Mesothelioma		α7	54
Others	Insulinoma cells (mouse)		α3, α4, β4	55

Abbreviations: B, binding experiments; F, functional experiments with agonists and antagonists; I, immunoreactivity; KO, knockout mice; R, detection of subtype-specific mRNA.

(1) Zia *et al*. (1997) (F, I); (2) Wessler and Kirkpatrick (2001b) (F, I, R); (3) Metzen *et al*. (2003) (F); (4) Gosens *et al*. (2006) (F, I, R); (5) Carlisle *et al*. (2004, 2007) (F, R); (6) Maus *et al*. (1998) (F); (7) Wang *et al*. (2001) (R); (8) Proskocil *et al*. (2004) (I, R); (9) Plummer *et al*. (2005) (R); (10) Sekhon *et al*. (2005) (I); (11) Gwilt *et al*. (2007) (F, I, R); (12) Grando (1997) (B, F, I, R); (13) Buchli *et al*. (2001) (B, R); (14) Grando *et al*. (2006) (F, KO, I, R); (15,16) Kurzen *et al*. (2004, 2007) (F, KO, I, R); (17) Hagforsen (2007) (I); (18) Richardson *et al*. (2003) (I, R); (19) Hirota and McKay (2006) (F, KO); (20) Gautam *et al*. (2006) (KO); (21) Haberberger *et al*. (2006) (F, I, R); (22, 23) Gautam *et al*. (2004, 2005) (I, KO, R); (24) Xie *et al*. (2005) (KO); (25) Yoshikawa *et al*. (2005) (B, F); (26) Fritz *et al*. (2001) (F, R); (27) Mayerhofer and Fritz (2002) (F, I); (28) Beckel *et al*. (2006) (F, R); (29) Bschleipfer *et al*. (2007) (I, R); (30) Zarghooni *et al*. (2007)(I, R); (31) Grueb *et al*. (2006) (I); (32) Tracey and Peach (1992) (R); (33) Khurana *et al*. (2004) (KO); (34) Walch *et al*. (2001) (F); (35) Yamada *et al*. (2001) (KO); (36) Bruggmann *et al*. (2003) (I, R); (37) Hawkins *et al*. (2005) (F, I); (38) Kawashima and Fujii (2003) (B, F, R); (39) Kawashima *et al*. (2007) (R); (40) Reinheimer *et al*. (2000) (F); (41) Profita *et al*. (2005) (F, I); (42) Wang *et al*. (2003) (F, KO, R); (43) Blanchet *et al*. (2007) (F, I, R); (44) Qu *et al*. (2006) (R); (45) Roman *et al*. (2004) (F); (46) Sekhon *et al*. (2002) (B, F, I, R); (47) Racké *et al*. (2006) (F, R); (48) Matthiesen *et al*. (2006) (F, R); (49) Danielson *et al*. (2007) (I, R); (50) Romano *et al*. (1997) (I, R); (51) Liu *et al*. (2004) (F, R); (52) Dorion *et al*. (2005) (F, I); (53) Buchli *et al*. (1999) (F); (54) Trombino *et al*. (2004) (B, R); (55) Ohtani *et al*. (2006) (B, F).

Table 3 Signal-transduction pathways triggered by n- and mAChRs on non-neuronal cells

Cell type	*Nicotinic receptor*	*Muscarinic receptor*	*References*
Epithelial cells keratinocytes	Increase in Ca_{in} (for example, mediated by α7): Ras/Raf/MEK/ERG CaMKII, PKC, PI3K α9: PLC, Src, PKC, Rac, Rho	M1: increase in Ca_{in} Ras/Raf/MEK/ERG CaMKII, PKC, PI3K M3: $G_{q/11}$/guanylyl cyclase/cGMP/PKG; increase in Ca_{in} M4: $G_{i/o}$/adenylyl cyclase/cAMP/PKA; decrease in Ca_{in}	Grando *et al.* (2006); Chernyavsky *et al.* (2007)
Lung cell line (BEAS2B)	Muscle type (α1, β1, δ, ε): increase in Ca_{in}/PKC/MAPK(p38)		Carlisle *et al.* (2004)
NSLC	MAPK-ERK1/2, PKC, PKA, PI3K, Bad		Jin *et al.* (2004)
Skin fibroblasts	α3β2: increase in Ca_{in} p21, cyclin D1, PCNA, Ki67, caspase 3	M2/M4: $G_{i/o}$/MAPK-ERK1/2 (p42/p44)	Kurzen *et al.* (2007); Matthiesen *et al.* (2007)
Lung fibroblasts	Muscle type (α1, β1, δ, ε) or α7: increase in Ca_{in}PKC/MAPK (p42/44)	M2: $G_{i/o}$/MAPK-ERK1/2 (p42/p44)	Carlisle *et al.* (2004); Matthiesen *et al.* (2007)
Lung smooth muscle fibres (cultured)		M2, M3: MAPK (p42/p44) increase in Ca_{in}	Gosens *et al.* (2007)
Immune cells (T and B cells)	α3, α7 subtypes: increase in Ca_{in} Jak2-STAT3	M3, M5: $G_{q/11}$/guanylyl cyclase/cGMP/PKG/PLC/DAG/IP3/ increase in Ca_{in}/cfos	Kawashima and Fujii (2003); De Jonge *et al.* (2005)
Endothelial cells	α7 subtype: increase in Ca_{in} PI3K/Akt/MAPK-ERK1/2/p38		Heeschen *et al.* (2002); Wang *et al.* (2006)
Differentiating embryoid cells	MAPK-ERK1/2 (p42/p44)		Serobyan *et al.* (2007)

Abbreviations: Akt, protein kinase B (signalling molecule in the phosphatidyl inositol 3-kinase pathway regulating energy metabolism, apoptosis, proliferation and migration); BAD, pro-apoptotic protein; Ca_{in}, intracellular Ca; CaMKII, calmodulin-dependent protein kinase; DAG, diacylglycerol; ERG, extracellular signal-regulated kinase; IP3, inositol 1,4,5-trisphosphate; Jak/STAT, signal transducer and activator of transcription; MAPK, mitogen-activated protein kinase; NSLC, non-small lung cancer cells; PI3K, phospatidylinositol-3-kinase; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; PCNA, proliferating cell nuclear antigen; PLC, phospholipase C; p21, inducer of cell arrest; Rac and Rho, small GTPases; Src, family of tyrosine kinases.

activates the calcium-dependent potassium channels (BK_{Ca}) and inhibits outward potassium channels such as the six transmembrane potassium (KCNQ) channels (Kunz *et al.*, 2007), an effect that corresponds to the so-called M-current in neurons. In the heart, ACh causes hyperpolarization due to the activation of the inward-rectifier current. Proliferation and differentiation of lymphocytes are strongly associated with the expression and activation of potassium channels (Lee *et al.*, 1986; DeCoursey *et al.*, 1987) and the regulatory role of non-neuronal ACh on lymphocytes may be linked to its action on potassium channels. Recently, it has been shown that the α7 subtype on T cells fails to form a typical ligand-gated Ca^{2+} channel but requires functional TCR/CD3 (T-cell receptor complex) and leukocyte-specific tyrosine kinase (Razani-Boroujerdi *et al.*, 2007). Thus, via auto-paracrine loops ACh may represent a co-stimulatory pathway for T-cell activation.

In conclusion, a large body of evidence indicates that all elements of the cholinergic system (ChAT and ACh synthesis, release mechanisms, receptors) are functionally expressed independently of cholinergic innervation and can modify or even control phenotypic cell functions.

Regulation of phenotype cell functions

Primary cultures of human keratinocytes or bronchial epithelial cells are excellent examples to demonstrate the regulatory effect of auto- and paracrine ACh. Grando (1997) showed profound morphological alteration induced by n- and mAChR antagonists, that is, within a couple of minutes the cells lose their polygonal shape, retract their intermediate filament bundles, show substantial shrinkage and detach from each other (see Figure 1a, from Grando, 1997). A comparable but less extensive effect has been reported with 10μM tubocurarine and human bronchial epithelial cells, showing shrinkage of the cells and separation from neighbouring cells, thus increasing the intercellular space (see Figure 1b; Klapproth *et al.*, 1994). Cultured gingival keratinocytes responded to 0.1mM mecamylamine in a comparable manner (Nguyen *et al.*, 2000). Importantly, these alterations are reversible either by waiting for recovery, by washing or by the application of agonists (Grando, 1997). The difference in the degree of cell alterations between skin and airway cells may be linked to the more than 100-fold higher ACh content in keratinocytes (Reinheimer *et al.*, 1996, Klapproth *et al.*, 1997) and to the considerable differences in the concentrations of the applied antagonists. All these culture preparations are free of cholinergic neurons, enabling the following conclusions to be drawn:

(1) The effects of the antagonists indicate an endogenous tone, that is, non-neuronal ACh is released and exerts auto-/paracrine effects via stimulation of n- and mAChRs.
(2) These receptors are strongly involved in the control of the cytoskeleton and cell–cell contact. In transfected cells, it has already been shown that M3 receptors

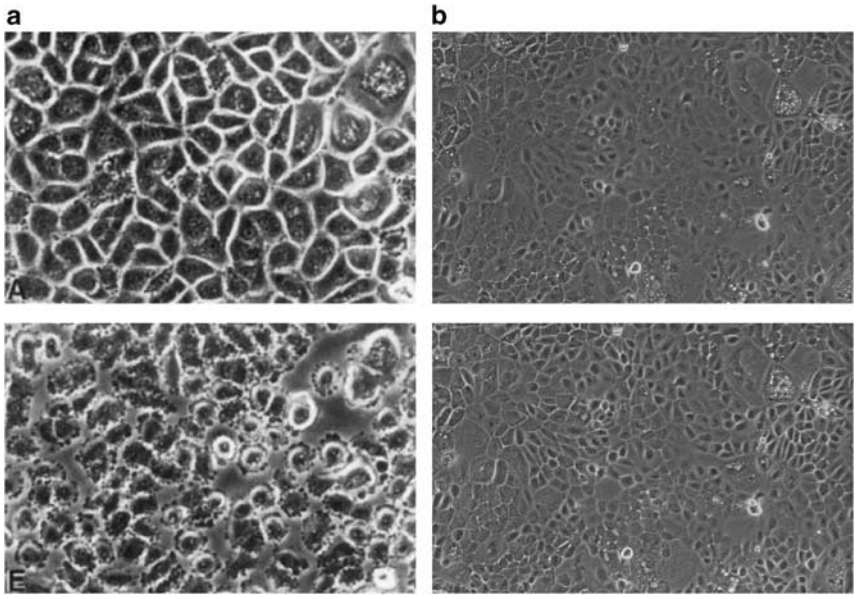

Figure 1 (**a**) Confluent keratinocyte monolayer; effect of 1mM atropine after 5min. Upper figure: cells immediately before atropine (Grando, 1997). (**b**) Confluent monolayer of human airway epithelial cells; effect of 10μM tubocurarine after 20min. Upper figure: cells immediately before tubocurarine (Klapproth *et al*., 1994).

Table 4 Examples of cellular effects mediated by identified subtypes of n- and mAChRs (↑ increase; ↓ decrease)

Cell type	*Receptor*	*Functions*	*References*
Epithelial cells			
Skin	α7	Terminal differentiation, apoptosis ↑, random migration ↓, directed migration ↑	Grando *et al*. (2006)
	α3	Cell–cell contact ↑, random migration ↑	Tournier *et al*. (2006)
	α9	Cornification, cell shape, cytoplasm mobility, cell adhesion	Metzen *et al*. (2003); Chernyavsky *et al*. (2007)
	M3	Random migration ↓, wound healing ↓	
Airways	M4	Random migration ↑, wound healing ↑	
	M4	Regulation of hair follicle cycle	
	α3,α5,β2	Wound healing ↑	
	M1	Proliferation ↑	
Macrophages, mononuclear cells	α7	Anti-inflammatory effects, release of proinflammatory cytokines ↓	Shytle *et al*. (2004); Pavlov and Tracey (2005)
T cells	α7	Interaction with TRC, Ca_{in} ↑	Razani-Boroujerdi *et al*. (2007)
B cells	α7	Regulation of antibody production	Kawashima *et al*. (2007)
Mucosal mast cells	M1	Histamine release ↓	Reinheimer *et al*. (2000)
Endothelial cells	α7	Angiogenesis ↑	Heeschen *et al*. (2002); Cooke (2007)
	M1	Release of NO ↑	Evora *et al*. (2007)
Fibroblasts (airway)	α7	Collagen gene expression ↑	Sekhon *et al*. (2002); Matthiesen *et al*. (2006)
	M2	Proliferation ↑	Racké *et al*. (2008)

regulate cytoplasmic myosin via the small GTPase RhoA and PKC (Strassheim *et al*., 1999).

(3) Epithelial cells establish a 'cholinergic' micromilieu to control their homoeostasis. In the present experiments, blockade of the non-neuronal cholinergic system induces profound, but reversible cellular alterations.

In the following sections, further regulatory mechanisms are presented to demonstrate cellular effects mediated by non-neuronal ACh. Some examples mediated by identified subtypes of n- or mAChRs on distinct specialized cells are indicated in Table 4. Some caution is addressed to the interpretation of the results from knockout experiments. Co-segregating background genes may contribute to the observed effects unless a 100% backcrossing of the knockout mice onto isogenic backgrounds has been achieved and demonstrated.

Epithelial cells

Epidermis. The most detailed analysis of the regulatory role of non-neuronal ACh on cellular functions has been

established for the epidermis by the investigation of intact skin, wound healing and cultured keratinocytes using different techniques (knockout mice, anti-sense RNA, selective agonists and antagonists; Grando *et al*., 1995, 2006; Chernyavsky *et al*., 2003, 2004, 2005; Kurzen *et al*., 2004, 2007).

Stimulation of mAChRs on isolated human gingival keratinocytes induces proliferation markers such as PCNA (proliferating cell nuclear antigen), Ki67 (nuclear antigen) and cyclin D1. Elimination of α7-nAChRs signalling slowed down terminal differentiation and reduced the amounts of proapoptotic proteins Bad and Bax (Grando *et al*., 2006). Thus, nicotinic pathways appear to facilitate the progress of cell cycle and differentiation. In organotypic epidermal co-cultures, combined blockade of n- and mAChRs completely prevented epidermal proliferation and differentiation (Kurzen *et al*., 2007). Physiological skin regeneration, as well as wound healing, is strongly dependent on proliferation, cell–cell detachment with assembling and disassembling processes involving metalloproteases, migratory and linker proteins, locomotion, migration and finally, reassembling of first adherens junctions and subsequently desmosomal junctions (Grando *et al*., 2006). M4 and α3-nAChRs appear to facilitate random migration (chemokinesis), whereas M3 and α7-nAChRs mediate the opposite effects. It has also been published that ACh and carbachol stimulate wound healing (see Grando *et al*., 2006). Wound healing is impaired in M4−/− knockout mice but enhanced in M3−/− (Grando *et al*., 2006), an observation that is consistent with the role of muscarinic receptor subtypes on chemokinesis. M4−/− knockout mice also showed an impaired hair follicle cycling with a prolonged telogen phase and failed to produce pigmented hair shafts (Hasse *et al*., 2007). Directed migration (chemotaxis) is promoted by α7-nAChRs. Moreover, keratinocytes exposed in a chamber to an electrical field re-orientated with their leading edge (lamellipodium) to the cathode and increased their expression of α7-nAChRs and M1 receptors (Chernyavsky *et al*., 2005). Long-term blockade of α3-, α9-nAChRs and M3 receptors by antisense oligonucleotides induces cell–cell detachment and changes in the expression of E-cadherin and catenins (Grando *et al*., 2006). Cadherin–catenin complexes are involved in cell–cell aggregation and essential morphoregulatory processes, and a change in their expression or structure is associated with cell migration and invasion. All these findings indicate that the non-neuronal cholinergic system is intimately involved in numerous cell functions including cell cycle, control of differentiation, apoptosis, organization of the cytoskeleton, cell–cell contact and migration (see also Figure 1).

Mucosa/glandular tissue. ACh stimulates proliferation of human and rat airway epithelial cells via mAChRs (M1 in rat) and nAChRs (Klapproth *et al*., 1997; Wessler and Kirkpatrick, 2001a, b; Metzen *et al*., 2003). Nicotine in nanomolar concentrations activates the serine/threonine kinase Akt and at high concentration (100μM) the mitogen-activated protein kinase (MAPK) p38 kinase pathway, thereby affecting the cell cycle and apoptosis (West *et al*., 2003; Carlisle *et al*., 2004). These different agonist sensitivities allow an extremely sophisticated modulation of cell cycle and survival by auto- and paracrine effects of non-neuronal ACh. Proliferation, cell spreading and migration are important repair mechanisms also in the airway mucosa. Wound repair of bronchial surface epithelium (*in vivo* and *in vitro*) was significantly improved by nicotine and ACh but delayed by nicotinic receptor antagonists such as mecamylamine and κ-bungarotoxin (Tournier *et al*., 2006). These functional experiments correspond with the enhanced expression of α3α5β2 nAChRs on migrating bronchial epithelial cells involved in wound repair (Tournier *et al*., 2006).

Stimulatory effects of ACh on ciliary activity, mucociliary clearance and apical chloride secretion are well-established actions mainly mediated via mAChRs (Acevedo, 1994). ACh inhibits apical sodium conductance and stimulates basolateral potassium conductance, thus modifying ion and fluid movements of mucosal and glandular epithelial cells. Similar effects are mediated by ACh in the mammalian intestine, as recently summarized by Hirota and McKay (2006). Baseline activity of ion and water transports remains despite the removal of the myenteric plexus and the application of tetrodotoxin that gives some evidence for local effects of auto- and paracrine ACh. However, the effect of atropine on baseline activities varies among different intestinal regions and species. Therefore, a modulatory role of ACh on baseline activity has been questioned in contrast to the effect of applied ACh on ion transport (Hirota and McKay, 2006).

Permeability of contact surfaces (skin, airways and gastrointestinal tract) is controlled by trans- and paracellular mechanisms. Blockade of n- and mAChRs in cultures of airway epithelial cells and keratinocytes reduces cell–cell contact, increases the intercellular space and therefore enhances permeability and impairs the physical barrier function in these cell layers (see Figures 1a and b). In the pancreas, ACh increases paracellular permeability by interfering with tight junctions (Jansen *et al*., 1980). mAChRs affect the activity of gap junctions in gastric epithelial cells (rat) and in granulosa cells of the ovary (Ueda *et al*., 1995; Fritz *et al*., 2002), most likely an extremely old regulatory target on the evolutionary timescale. Very recently it has been demonstrated that ACh is the transmitter responsible for regulating gap-junction activity of the central synapse of *Drosophila* (Allen and Murphey, 2007).

Finally, the release of mediators such as prostanoids, leukotrienes and chemokines (granulocyte macrophage-CSF; neutrophil and monocyte chemoattractants, interleukin (IL)-8) from airway epithelial cells is regulated by n- and mAChRs (Koyama *et al*., 1992, Brunn *et al*., 1995; Klapproth *et al*., 1998; Profita *et al*., 2008). This mechanism of recruiting immune cells together with the effect of ACh on the epithelial barrier function and the stimulation of mucus and mucociliary activity may represent local anti-infectious pathways to protect mucosal boundaries from invasion by pathogens.

With respect to the extremely wide distribution of nAChRs the role of active and passive smoking and nicotine has been re-considered, particularly with respect to the situation on public areas. Passive smoking transfers sufficient amounts of nicotine to stimulate nAChRs on non-neuronal cells. Nicotine induces proliferation of epithelial cells, including

tumour cell lines, and angiogenesis (Cattaneo *et al*., 1997; Heeschen *et al*., 2002; Cooke, 2007). The underlying molecular mechanisms are comparable to the effect of growth factors, that is, the involvement of Src kinase, Raf-1 kinase, cyclins D and E and proliferative promoters (Dasgupta and Chellappan, 2006). However, the involvement of the non-neuronal cholinergic system in tumorigenesis remains to be elucidated.

Endothelial cells

Convincing evidence has been published in recent years that the cholinergic system is strongly involved in angiogenesis. nAChRs mediate proliferation, survival, migration and tube formation *in vitro* as well as angiogenesis *in vivo* (Heeschen *et al*., 2002; Cooke, 2007). Nicotine and neostigmine facilitate migration of endothelial cells; nicotine promotes the generation of growth factors and autocoids such as endothelin and prostacyclin (Cooke, 2007). Most importantly, capillary network formation *in vitro* is blocked by specific nicotinic receptor antagonists indicating an endogenous tone by non-neuronal ACh (Heeschen *et al*., 2002). This effect is mediated by the α7 subtype. nAChR antagonists reduce neovascularization in response to inflammation, ischaemia and neoplasia (Heeschen *et al*., 2002). Moreover, nicotine stimulates interactions between endothelial cells and monocytes and thereby facilitates arteriogenesis (Heeschen *et al*., 2003). All these recent findings demonstrate that the non-neuronal cholinergic system is involved in the regulation of angiogenesis, a mechanism which may become impaired by smoking or other disease conditions.

Endothelial cells contribute to the regulation of perfusion. In vascular tissue, ACh via activation of mAChRs (M_3 and M_1 subtypes) is a well-known mediator for the release of nitric oxide (NO), endothelium-derived hyperpolarizing factor and prostanoids. Blood flow, shear stress, body temperature and local blood pressure may affect endothelial ACh synthesis and release and as a consequence may modulate the release of vasoactive mediators. Gradual hypothermia induces NO-dependent vasodilatation of canine isolated coronary, femoral and renal arteries, an effect which was blocked by 1μM pirenzepine (Evora *et al*., 2007). This recent finding indicates an endogenous regulatory loop mediated most likely by non-neuronal ACh. Milner *et al*. (1990) have shown the release of endothelial ACh in response to an increased flow. Immunocompetent cells must penetrate the vascular wall before their migration into inflammatory tissue. Adhesion molecules mediate the cross-talk between immune and endothelial cells. Nicotine at low concentrations did not affect the expression of VCAM (vascular cellular adhesion molecule) and E-selectin, but slightly enhanced that of ICAM-1 (intercellular adhesion molecule-1) (Kirkpatrick *et al*., 2003). In contrast to these results, it was reported that nicotine substantially stimulated the expression of VCAM1, ICAM and E-selectin in human umbilical vein endothelial cells (HUVECs) via calcium influx, an effect sensitive to mecamylamine and MAPK inhibitors (Albaugh *et al*., 2004; Wang *et al*., 2004, 2006). Nicotine increased the blood–brain barrier permeability and paracellular permeability and reduced connexin 43 expression and gap-junctional communication (Abbruscato *et al*., 2002; Tsai *et al*., 2004; Hawkins *et al*., 2005). All these findings open new and highly important insights into the fine-tuning of endothelial homoeostasis by non-neuronal cholinergic mechanisms.

Immune cells

In recent years, several review articles have focused on the expression and biological role of the non-neuronal cholinergic system in immune cells, particularly in T and B cells (Kawashima and Fujii, 2000, 2003, 2004). Activation of the T-cell receptor by phytohaemagglutinin or by anti-CD11a antibodies triggers the expression of ChAT and M5 receptors and enhances the synthesis of ACh. Likewise, stimulation of dendritic cells with lipopolysaccharide causes expression of ChAT (Kawashima *et al*., 2007). ACh is involved in the induction of $CD4^+$ T-cell maturation as well as in the generation of cytolytic $CD8^+$ T-lymphocytes under *in vitro* conditions (Kawashima and Fujii, 2000, 2003; Zimring *et al*., 2005). In addition, it has been demonstrated that ACh can modify immune responses. Thus, for example, nAChRs appear to play a role in the generation of antibodies with a possible inhibitory regulatory role of the α7 subtype (Kawashima *et al*., 2007; Skok *et al*., 2007). Stimulation of the same nAChR subtype (α7) by neuronal or non-neuronal ACh inhibits the release of proinflammatory mediators (tumour necrosis factor, IL1-β) from immune cells as part of the so-called cholinergic anti-inflammatory pathway (Borovikova *et al*., 2000; Pavlov and Tracey, 2005; see below). Most interestingly, a comparable regulatory pathway has been described for brain mononuclear phagocytic cells, the microglia, as stimulation of α7-nAChRs suppresses lipopolysaccharide-induced tumour necrosis factor-α release (Shytle *et al*., 2004).

All these observations indicate that ACh modulates the activity of immune cells via auto- and paracrine loops. The α7 subtype expressed on T cells requires functional TRC/CD3 to raise $[Ca^{2+}]_{in}$ (Razani-Boroujerdi *et al*., 2007), suggesting a co-stimulatory function of non-neuronal ACh in T cells. Interestingly, the components of the non-neuronal cholinergic system are expressed very early during fetal haematopoiesis and nicotine affects the colonization of the fetal bone with haematopoietic stem/progenitor cells (Serobyan *et al*., 2007). Finally, mucosal mast cells in human airways are controlled by a strong M1-mediated inhibitory pathway, thereby limiting the liberation of proinflammatory mediators (Reinheimer *et al*., 2000). This effect becomes impaired in advanced chronic obstructive pulmonary disease (Wessler *et al*., 2007a).

The cholinergic anti-inflammatory pathway. Borovikova *et al*. (2000) described the so-called cholinergic anti-inflammatory reflex or pathway. Stimulation of the vagal nerve during artificial lethal endotoxaemia prevented the development of shock and reduced the release of proinflammatory cytokines such as tumour necrosis factor-α, IL-1β, IL-6 and IL-18 (Borovikova *et al*., 2000). Meanwhile, the concept of a cholinergic anti-inflammatory pathway has been examined in various models of acute systemic or local inflammation (Tracey, 2002; Pavlov and Tracey, 2005; de Jonge and Ulloa,

2007). Undoubtedly, the vagal nerve plays an important role as a sensory and nociceptive system to communicate the activation state of the immune system to the brain. In addition, efferent fibres mediate regulatory integrative responses. However, it is still questionable whether the anti-inflammatory effect occurring intimate to the immune cells is mediated by vagally released, that is, neuronal ACh. Rather few neuroanatomical studies have been performed to identify the innervation pattern of the immune system by the vagal nerve. In contrast to the innervation by the sympathetic nervous system, there is no convincing neuroanatomical evidence for an innervation of the spleen, thymus, lymph nodes and bone marrow by the vagal nerve, for example in rats (Bulloch and Moore, 1981; Nance *et al*., 1987; Bellinger *et al*., 1993; for review, see Nance and Sanders, 2007). Some immune cells show close membrane apposition with neuronal elements, for example, in the area postrema (Goehler *et al*., 2006). How should a vagally mediated efferent response at macrophages, dendritic and other immune cells migrated in mucosal and parenchymatous tissues operate without a close neuroanatomical substrate? In this context, one has to consider the extremely high effectiveness of AChE at neurons, which prevents neuronal ACh from acting as a diffusing signalling molecule. Nance and Sanders (2007) have suggested that stimulation of the efferent vagal nerve caused activation of the adrenal medulla and the sympathetic nervous system which, in turn, contributes or even mediates the anti-inflammatory mechanism. In addition, the vagal nerve, particularly the subdiaphragmatic portion, modulates ascending impulse transmission projecting to the hypothalamus and is involved in the threshold regulation of the nociceptive system in abdominal visceral organs (Miao *et al*., 1997a, b). From this point of view, it is not surprising that vagotomy increases lethality of endotoxaemia. Moreover, nicotine induces complex effects depending on the route of application and dose. For example, intrathecal application of nicotine (rat) reduces bradykinin-induced plasma extravasation and this anti-inflammatory effect was substantially potentiated by subdiaphragmatic vagotomy (Miao *et al*., 1997a). In contrast, local application of a very low dose of nicotine induces proinflammatory effects when the adrenal medulla has been inactivated (Miao *et al*., 1997b, 2001). All these observations demonstrate extreme complex interactions of applied nicotine at central and peripheral neurons, nociceptors, adrenal medulla and non-neuronal cells.

Nevertheless, one should consider that anti-inflammatory effects are also observed with muscarinic agonists (at higher concentrations) and can be detected with locally applied nicotinic agonists or ACh on isolated immune cells (Borovikova *et al*., 2000; Tracey, 2002; Pavlov and Tracey, 2005; de Jonge and Ulloa, 2007). Thus, the principle of a cholinergic, anti-inflammatory mechanism mediated, for example, by nAChRs does exist. We suggest that besides the activation of the adrenal medulla, neuronal ACh released from efferent and probably also from afferent fibres is involved and triggers the release of non-neuronal ACh from neighbouring cells, passing the signal such as a wave within mucosal and parenchymatous tissues. In this context, it is noteworthy that the release of non-neuronal ACh is increased in the human placenta by nAChRs (Wessler *et al*., 2001b).

Mesenchymal cells

Our knowledge about the role of non-neuronal ACh in mesenchymal cells, such as adipocytes, fibroblasts, smooth muscle fibres and tenocytes, is very poor. At best, airway fibroblasts have been examined. All subtypes of mAChRs are expressed with a dominance of the M2 subtype. Stimulation of these receptors increased the proliferation rate and also the incorporation of proline, the latter giving evidence of an enhanced collagen synthesis (Matthiesen *et al*., 2006; Racké *et al*., 2008). Whether these effects are involved in the pathogenesis of chronic airway diseases remains to be elucidated. In human neonatal skin fibroblasts, mAChRs stimulate DNA synthesis and CD40 expression (Casanova *et al*., 2006).

ACh can modify phenotypic functions of the airway smooth muscle fibres (Gosens *et al*., 2004). For example, the expression of contractile proteins as well as smooth muscle secretory functions can be affected by ACh, and mAChRs potentiate the mitogenic effect of growth factors (Gosens *et al*., 2004). Acute or chronic inflammation can cause increased levels of ACh (see next section) and this effect may contribute to the remodelling processes induced by inflammation. Recently, the first evidence has been presented that AChE is highly expressed in fibroblasts in which the enzyme protein mediates non-enzymatic morphoregulatory effects (Anderson *et al*., 2007). AChE is prominently expressed at the leading edge of spreading and migrating fibroblasts. In this context, cell surface AChE has been proposed to contribute as a more general signalling mechanism to protrusion and migration of polarized cells (Anderson *et al*., 2007).

Non-neuronal cholinergic system involved in the pathophysiology of diseases

All components of the system—synthesis, storage, release, inactivation as well as the expression and function of the various n- and mAChRs—can be affected as a key pathogenetic event or secondary to the disease state. Particularly, the fine-tuning of cellular effects mediated by the different subtypes of n- and mAChRs may become impaired. Subtypes of n- and mAChRs act in a synergistic or antagonistic way (see Tables 3 and 4) and even a small change in the expression pattern may result in cellular stress. The scientific community has just started to investigate the non-neuronal cholinergic system. A systematic analysis of all components of the non-neuronal cholinergic system in different diseases is practically non-existent and should be established as soon as possible. In the final section some pathophysiological conditions are discussed, where substantial alterations of the non-neuronal cholinergic system have already been described.

The effect of inflammation on the non-neuronal cholinergic system and vice versa appears to be regulated in a very complex manner and is as yet only poorly understood.

Chronic inflammation may upregulate ACh synthesis. For example, in atopic dermatitis substantially enhanced levels of ACh have been detected within the skin, including the superficial 2mm of epidermis (Scott, 1962; Wessler *et al*., 2003b). In a model of acute renal allograft rejection in rats, it was found that the ACh-synthesizing machinery was substantially upregulated in intravascular leukocytes (Hecker *et al*., 2006). The opposite was observed in mice or rat lung using an acute allergic inflammation model (Lips *et al*., 2007a). Enhanced levels of ACh have been associated with pruritus, thickening of the stratum spinosum, enhanced blood flow and impaired barrier function (Wessler *et al*., 2003b). In psoriasis, enhanced expression of SLURP-2 has been demonstrated, which binds to nAChRs, thus inhibiting caspase 3 and filaggrin (Grando *et al*., 2006). AChE activity is lowered in vitiliginous skin. Acantholysis observed in patients with pemphigus is caused by autoantibodies directed against desmosomal cadherins and nAChRs (Grando, 2000). Mustard gases such as sulphur mustard cause dose-dependent injuries of skin and mucosa. These toxins interfere with the cholinergic system, for example with n- and mAChRs and produce similar effects as shown in Figure 1a (Grando, 2003).

Chronic patellar tendon tendinosis is associated with enhanced anti-ChAT and anti-M2 immunoreactivity (Danielson *et al*., 2007). A downregulation of ChAT and upregulation of M2 receptors have been found in the colon epithelium of patients with ulcerative colitis (Jonsson *et al*., 2007). As already mentioned, ACh released by the vagal nerve as well as non-neuronal ACh can induce an anti-inflammatory effect via α7-nAChRs in various models of acute systemic inflammation (Pavlov and Tracey, 2005; de Jonge and Ulloa, 2007). Interestingly, muscarinic receptor antagonists appear to mediate anti-inflammatory effects (Profita *et al*., 2005; Pahl *et al*., 2006, Bühling *et al*., 2007).

Very recently it has been shown that ACh content is substantially reduced in blood cells (leukocytes) as well as in bronchi of patients with cystic fibrosis despite a somewhat enhanced ChAT activity (Wessler *et al*., 2007b). This decrease was not caused by an enhanced esterase activity. Double labelling experiments with anti-ChAT and anti-CFTR (cystic fibrosis transmembrane regulator protein) antibodies showed a colocalization (Wessler *et al*., 2007b). This observation gives first evidence that ChAT and CFTR—a widely expressed transport and regulator protein—may be linked in a functional way, presumably to regulate the storage and transport of non-neuronal ACh within a cell. In cystic fibrosis, the storage may become impaired and in consequence cells contain less ACh. In the airways of CF patients, this cholinergic dysfunction may contribute to the deleterious alterations in ion and water movements, as ACh is known to stimulate apical Cl^- secretion and to inhibit apical Na^+ absorption.

It has been reported that ACh inhibits long-term hypoxia-induced apoptosis in mouse stem cells (Kim *et al*., 2008), demonstrating protective and trophic effects. On endothelial cells, α7-nAChRs are upregulated by hypoxia or ischaemia and nicotinic receptor antagonists block endothelial network formation *in vitro* (Heeschen *et al*., 2002). Meanwhile, it has been demonstrated that nicotine and endothelial ACh represent proangiogenic factors. Nicotine promotes the growth of atherosclerotic plaques, potentiates endothelial–monocyte interactions and the incorporation of endothelial progenitor cells into newly established vessels (Heeschen *et al*., 2003, 2006; Cooke, 2007). Lung cancers express n- and mAChRs; activation of these receptors by applied nicotine or ACh stimulates growth of tumour cell lines (Cattaneo *et al*., 1997; Song *et al*., 2003). Small cell lung carcinoma cells utilize ACh as an auto- and paracrine growth factor, and M3 receptor antagonists have been reported to reduce cell growth both *in vitro* and *in vivo* (Song *et al*., 2007). Exposure to tobacco products or nicotine alters cell cycle and can induce squamatization of oral keratinocytes and the formation of squamous cell carcinoma (Arredondo *et al*., 2008). It has been shown that environmental tobacco smoke or nicotine upregulates the expression of α5- and α7-nAChRs, raising Ca_{in} signalling and causing profound pathobiologic effects as described above (Arredondo *et al*., 2007). In this context, one has to consider differences between acute and chronic exposure as well as high and low concentrations of nicotine producing either pro- or anti-inflammatory responses. For example, smoking appears to exert protective effects against ulcerative colitis and can reduce mucosal inflammation (for references, see de Jonge and Ulloa, 2007).

In conclusion, it is important to analyse systematically the changes of the main components of the non-neuronal cholinergic system (synthesis and release of ACh, expression and function of subtypes of n- and mAChRs, expression and function of esterases) in models of acute and chronic inflammation in different organs and in human diseases. Release can be modified by alterations of synthesis (ChAT expression), intracellular storage and transporter proteins. Expression and function of n- and mAChR subtypes can be modified by various mediators, including inflammatory cytokines and autoantibodies. Numerous interactions occur and this complex scenario needs to be elucidated to learn more about the regulatory role of the non-neuronal cholinergic system in acute and chronic inflammatory processes. This will help in understanding the cholinergic properties of non-neuronal cells and in turn will lead to optimization of drug therapy.

Conflict of interest

The authors state no conflict of interest.

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