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Review

Acetylcholine: Future research and perspectives

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ABSTRACT

Ever since the initial description of chemical transmission in the early part of the 20th century and the identification of acetylcholine (ACh) as the first such transmitter, interests grew to define the multiple facets of its functions. This multitude is only partially covered here, but even in the areas preselected for this special issue, research on the cholinergic system is still thriving. Notwithstanding an impressive amount of knowledge that has been accumulated, partly triggered by the cholinergic hypothesis of Alzheimer's disease (AD [1]), the different reviews in this issue not only summarise our current state of the art, they also highlight that this field has still large potential for future development. Taken from these reviews, we here pinpoint several topics fit for future attention.

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Contents

1. Visualization of ACh

Visualization of the cholinergic system has a long history, starting with the histochemical detection of the ACh degrading enzyme AChE by Koelle and Friedenwald [2]. Nevertheless, progress can still be made on the detection of ACh itself. Earlier attempts to localize ACh *in situ* by either ACh histochemistry or immunocytochemistry have met with little success and therefore deserve revisit. Measuring and anatomically localizing ACh release likely represents the most direct indicator of the presynaptic component of cholinergic neurotransmission, as stated by Fadel [3]. Advanced tools for cholinoceptive structures would be helpful for better localization studies (see for example [4]), and these should be combined with the monitoring of intracellular signalling cascades set in motion by the selective activation of cholinergic receptors. Such a combination of ACh visualization with highly sensitive ACh release measurements will comprise a powerful future strategy to examine the cholinergic contribution to functional aspects in high resolution.

2. Pharmacology of ACh neurotransmission

A long-standing, still unresolved and recurrent issue for the understanding of the pharmacology of ACh neurotransmission is the search for more selective ligands both in terms of receptors but also vis-à-vis catabolising and metabolising enzymes and transporter proteins. According to Wevers [4], novel cholinergic receptor ligands are also warranted to be used for *in vivo* imaging techniques such as PET or SPECT. Clearly, we seek highly selective and specific ligands for the in-depth examination of the role of a single receptor subtype or subunit. This may shed particular light on the poorly understood M5 muscarinic acetylcholine receptor (mAChR) subtype, or further elucidate the specific functions of hypothalamic

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M3 mAChRs, which seem pivotal in keeping an individual's internal milieu constant in terms of nutrient and fluid intake [5]. At the same time, better and more selective drugs for the distinction of homomeric and heteromeric nicotinic receptors are a prerequisite if we are to fully comprehend their function in memory processes [6,7] and synaptic plasticity as its possible cellular correlate [8]. Such new pharmacological tools are urgently required in terms of halting or slowing down disease progression in dementia. It becomes increasingly clear that a polypharmacy approach for AD may be the most promising and efficacious tool for palliative therapies. Contestabile [9] argues that cholinergic drugs as the only clinically approved treatment for enhancement of cognition to date should still play a considerable role in the polypharmacological approach. The future here lies in the integration of cholinergic drugs in novel therapies and treatments.

3. Functional issues

The functional consequences of ACh-mediated responses in the brain are not yet fully understood and scrutinized. For example, non-synaptic ACh release remains somewhat obscure and is even contested by some. For new insights in the role of tonic ACh release *versus* phasic ACh release, more sensitive microdialysis techniques should be developed as suggested by Klinkenberg et al. [10]. This also relates to a gap in our knowledge of the cholinergic system: (sub)region-specificity of ACh functions is still not fully mapped to an extent that would instruct treatment approaches (although they will be rather complex in nature) to manipulate different subsystems simultaneously in different ways. Successful treatment approaches also await further distinction between direct and indirect involvement of the cholinergic system. Here, the field has still a long way to go but benefits are intuitively obvious.

The role of ACh in learning and memory is a classic topic, as is made clear by several contributions in this special issue. Several chapters of this special issue highlight blind spots in need of future research. For example, it is stressed by Micheau and Marighetto [11] that the role of ACh in learning and memory is still not fully understood (and even "chaotic"). It should be taken into account that ACh affects not only one, but possibly all memory systems in different ways (compare contributions [6,7,12] in this issue). Moreover, ACh modulates the distinct phases of learning and memory differentially: favouring memory encoding and attentional efforts while hampering memory consolidation and retrieval. Much is still to be learned (and validated) about this biphasic function of ACh. Considering attention, Klinkenberg et al. [10] make clear that future research on the role of attention needs to focus more on parietal and somatosensory cortices next to the (pre)frontal cortex, preferably combined with pharmaceutical manipulations. Similarly, Robinson et al. as well as Deiana et al. [6,7] highlight the need for a dissociation of function of cholinergic mechanisms that are intrinsic to specific brain structures against those that depend on long-distance projections arising from the basal forebrain for example.

More fundamental insights are needed with respect to cholinergic contributions to neuroplasticity in general and synaptic plasticity in particular. Woolf and Butcher [13] link cholinergic signalling to intracellular responses of cytoskeletal filaments and view this as a fundamental principle of neuroplasticity underlying cognition. According to Drever et al. [8], future studies on the complex role played by ACh signalling in synaptic plasticity would aid the development of new treatments for those affected by disorders involving cholinergic dysfunction. This would at the same time be instructive to studies on sleep and vigilance staging when cholinergic activation undergoes strong modulations [14].

Part of the intracellular cholinergic signal transduction cascade can be linked to sigma-1 receptors. The application of sigma-1 receptor agonists in the treatment of memory deficits and cognitive disorders has considerable potential as made clear by van Waarde et al. [16]. Another functional role of ACh more indirectly related to neuronal processes is the dynamic regulation of cerebral blood flow and cerebral cortical oxygenation as stated by Van Beek and Claassen [17]. Combined recording of these direct and indirect actions of ACh in the brain would undoubtedly help a more integrative data interpretation to create a richer understanding of ACh functioning. Taken together, diverse routes for future research on the intracellular modulation of cholinergic pathways are expressed in various contributions here, hopefully inspiring researchers to follow up on these themes.

4. Ontogeny and ageing

Although numerous publications have addressed brain development and ageing-related retardation linked to the cholinergic system, some issues on this topic still deserve additional attention. Abreu-Villaça et al. [18] point out the relevance of ontogenic examination of different cholinergic compartments (subregions) and how they eventually mature into one functional system. This type of information may help clarifying influences of environmental conditions and agents (e.g. neurotoxins) on cholinergic development, and may explain better how cholinergic imbalances occur. These may originate from progenitor cell proliferation as highlighted by Bruel-Jungerman et al. [19], who also stress the need for additional experiments to clarify all cellular pathways providing trophic support and physiological regulation for the survival and functional network-integration of newborn cholinergic neurons on one hand, and to determine the effect of ACh on emerging cells in olfactory bulb or hippocampus. Newborn neurons are still present in some areas of the (young) adult brain and a link to AD has recently been made [20]. Adult neurogenesis is severely hampered in some pathophysiological conditions such as major depressive disorder [21], possibly explaining the cognitive endophenotypes observed in this disease. Future research on cholinergic regulation of adult neurogenesis should pinpoint molecular mechanisms and pathways aiding the survival of newborn cells.

Havekes et al. [12] touch on the balance between cholinergic hippocampal and striatal functioning and how it is altered during ageing. Important future work should determine whether ageing-related changes in ACh release indeed parallel shifts in behavioural strategy as already reported for young and old individuals. An understanding of the balance between striatal and hippocampal cholinergic activity controlling behaviour and memory may result in novel approaches to rescue ageing-related cognitive decline. In general, it can be expected that ageing characteristics of the cholinergic system relate to its original ontogeny. A developmentally compromised cholinergic system will potentially lead to earlier (and more severe?) cholinergic dysfunctions during subsequent ageing. From this point of view, the relationship between development and ageing deserves greater attention.

5. Animal models

Naturally, several animal models are in use in the study of the cholinergic and cholinoceptive system. Van der Zee and Keijser [22] emphasize that rats and mice, the two most common rodent models, appear to differ in basal cholinergic tone: higher basal activity occurs in the mouse relative to the rat as deduced from mAChR immunocytochemistry. Moreover, considerably more muscarinic receptors are in an activated and subsequently internalized state in mice. Hence, the outcome of functional cholinergic drug testing in mice will differ from those performed in rats and it remains elusive which rodent matches more closely the human basal cholinergic activity. This is an important issue in terms of treatment strategies

when choosing a rodent model for translational pharmacological or genetic purposes. In general, more care should be taken in selecting the best animal model (also at the level of strain within each species) for preclinical model development.

6. The interaction of the cholinergic and circadian system

Circadian and ultradian rhythms regulate, amongst other, sleep/wake cycles and undergo severe changes in ageing and neurodegenerative diseases [23,24]. Circadian rhythms depend on the suprachiasmatic nucleus (SCN), referred to as the master clock. Although not with extensive neuronal projections to memory-related or cholinergic brain structures, SCN and circadian rhythms exert important influences on cognitive processes [25] and different sleep stages may support in particular the development of memory consolidation [26]. A better understanding of the interaction between the circadian rhythms generated and modulated by the cholinergic system is needed as argued by Hut and Van der Zee [27]. This interaction should be studied in more detail at both anatomical and molecular levels with a particular emphasis on functional studies how clock genes may regulate cholinergic signalling. Certain types of episodic-like memory depend on intact circadian rhythms driven by specific clock genes [28]. Yet, cholinergic signalling seems crucial for circadian time stamping, a feature highly sensitive to neurodegeneration and thus a key feature of AD. A novel and exciting finding is the presence of local cholinergic cells within rat SCN, and follow-on studies are now needed to determine whether this also holds for human SCN or peri-SCN. Therefore, cholinergic regulation in the hypothalamus in general deserves renewed attention [5].

Within the different vigilance stages occurring in parallel to circadian rhythms, high cholinergic background activity occurs during wakefulness and rapid eye movement (REM) sleep. Sleep staging relies on quantitative electroencephalogram (EEG) and controversy still surrounds the specific role of the different sleep stages for memory formation. The strongest support for a link between REM sleep and episodic memory has come from REM deprivation studies and it appears that pharmacological blockade of cholinergic receptors interferes with REM sleep. Consequently, restoration of cholinergic activity may yield normalisation of sleep wake patterns. What remains to be confirmed however is whether such improved sleep patterns may directly counteract memory deterioration, i.e. whether cholinergically controlled sleep disruptions contribute to the progressive cognitive decline or are merely an additional disease biomarker [14].

7. Acetylcholine and neural pathology

It goes without saying that many of the above mentioned hypotheses and future directions ultimately lead to a richer understanding of cholinergic (mal-)function in brain pathology. Dementia, and in particular AD, is the most striking example, but many other brain functions go astray if parts of the cholinergic signal transduction machinery is compromised. Niewiadomska et al. [15] stress the relevance of better insights into the role of NGF in ACh signalling and related plasticity. Research into the physiology of neurotrophin receptor transport and its significant contribution to cholinergic cell death corroborates this view. Preventing cholinergic cell death by increasing neurotrophic signalling might be one of the ways to combat cholinergically mediated learning and memory deficits [29]. Loss of cholinergic cells (especially in the nucleus basalis of Meynert (nbM)) has also been observed in Parkinson disease, most notably in cases of Parkinsonism with dementia. Additional research is needed to further evaluate the relationship between nbM neurodegeneration and the presence of Parkinsonian amyloidpathology and Parkinsonian dementia [30]. It is therefore important to note that cholinergic memory deficits in other pathophysiological conditions can potentially be improved by cholinergic drug treatment, independent of the causal relationship between disease and cholinergic deficit. Consequently, improved and highly specific third and forth generation cholinomimetics may prove useful in psychiatric disorders, including depression [21], for which reductions in cholinergic baseline activity has been small. These disorders certainly deserve another look.

More attention should also be attributed to the interaction between cholinergic and other transmitter and neuromodulatory systems. Examples include the glutamatergic overexcitation theory of AD progression summarised by Nyakas et al. [31] or the seemingly strong interaction with the cannabinoid system [32,33]. Dual or even multiple transmitter approaches via polypharmacology may hold benefit in future therapy but is at present under-researched.

Finally, little attention in this compendium has been paid to glial cells and their interaction with cholinergic signal transduction. Astrocytes bear ample functional mAChRs and their expression undergoes age-related decline [34,35]. Furthermore, astrocytes play crucial roles in regulating synaptic neurotransmission, including cholinergic signalling [36] but an in-depth understanding of this interplay still remains elusive.

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