Impact of acetylcholine on sebocyte biology *in vitro*

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Extraneuronal acetylcholine (ACh) has been demonstrated to influence a plethora of cutaneous cell functions in an autocrine, paracrine and endocrine fashion. Previously, we could demonstrate a differentiation-specific expression of its nicotinic (nAChR) and muscarinic receptors (mAChR) in human epidermis and its adnexal structures including sebaceous glands. Using an immortalized human sebaceous gland cell line (SZ95 sebocytes), we examined the AChR expression pattern *in vitro*. In proliferating and confluent SZ95 sebocytes, a wide range of AChR subunits could be detected using RT-PCR. In particular, we detected mRNA coding for the α3, α5, α6, α7, α10, β1, β2 and β4 nAChR as well as the M_1, and M_{3-5}. The α1 and M_2 and M_5 subunits could only be detected in confluent SZ95 sebocytes, while in proliferating ones these subunits remained negative. The α2, α4, α9 and β3 nAChR could not be detected. Using functional assays, we assessed the impact of cholinergic agonists and antagonists on sebocyte proliferation and differentiation as evidenced by lipid production. Atropine (inhibition of all mAChR) and himbacine (inhibition of M_2 and M_4) potently inhibited SZ95 sebocyte proliferation in a dose-dependent

manner with a maximum effect at milimolar concentrations, while glycopyrrolate (inhibition of M_1 and M_3) inhibited proliferation only at high concentrations (100 µM). Interestingly also muscarine inhibited SZ95 sebocyte proliferation, however, with a maximum effect (50%) at nanomolar concentrations. The inhibitory effect of mecamylamine was less pronounced (20%). Nicotine strongly induced SZ95 sebocyte proliferation to more than 200% in a dose-dependent manner. Lipid production was increased by nicotine and muscarine in the micromolar range. Inhibition of nAChR by mecamylamine did not significantly influence lipid production, while even nanomolar concentrations of atropine significantly increased lipid production. In conclusion, we could demonstrate highly potent effects of the cholinergic system on sebocyte proliferation and lipid production *in vitro*, mediated by wealth of different AChR subunits present at least at the mRNA level. In particular, promotion of sebocyte proliferation seems to be an attractive explanation for the exacerbation of sebaceous gland–related disorders like acne under the influence of chronic nicotine ingestion. In addition, seborrhea observed after treatment with anticholinergic drugs is well in line with an increase in lipid production that we found after treatment of sebocytes with atropine.