COPI polices nicotine-mediated up-regulation of nicotinic receptors

Rene Anand

Department of Pharmacology, Ohio State University College of Medicine, Columbus, OH 43210

The Journal of General Physiology

Mayans, Aztecs, and indigenous Americans cultivated tobacco for medicinal and religious purposes well over 2,000 years ago. The subsequent trade and industrial-scale production of tobacco have led to its global recreational use with devastating health consequences. It is currently responsible for the greatest number of preventable deaths worldwide by any single agent, estimated to be 5 million per year by the Center for Disease Control and Prevention. The active ingredient of tobacco, nicotine, efficiently permeates the blood brain barrier and activates neuronal nicotinic acetylcholine receptors (nAChRs) in the brain. In addition, nicotine exposure of the brain during childhood and adolescence is likely to increase susceptibility to neuropsychiatric and addiction disorders.

A multitude of distinct nAChR subtypes is formed by the assembly of α1–α7, α9, and β1–β4 subunits in mammals. These nAChR subtypes are expressed in different populations of neurons and other types of cells. The nAChRs most relevant to the study of nicotine addiction are expressed in dopaminergic, glutamatergic, and GABAergic neurons, where they modulate the probability of neurotransmitter release at presynaptic terminals (Wonnacott, 1997). Studies in rodents show that nicotine use affects various behaviors (Russo et al., 2010), including (a) impulse control and attention by modulating prefrontal cortex functions, (b) reward salience by modulating the ventral tegmental area (VTA)-striatum functions, and (c) aversive salience by modulating the medial habenula-interpenducular nucleus functions.

Multiple nAChR subtypes mediate nicotine's actions in the brain at the nanomolar concentrations found in smokers' serum. The α4β2 nAChRs in VTA projections to the nucleus accumbens (Picciotto et al., 1998; Tapper et al., 2004), the α6α4β2β3 and α6β2β3 in substantia nigra pars compacta projections to the striatum (Quik et al., 2011), and the α3α5β4 and/or α4α5β2 in medial habenula projections to the interpeduncular nucleus (Fowler et al., 2011) collectively mediate nicotine's complex behavioral effects. These nAChRs exhibit different channel kinetics, rates of desensitization, and affinities for the endogenous neurotransmitter, acetylcholine, and nicotine.

Nicotine exposure, unlike exposure to most other drugs of abuse, results in the "up-regulation" of its cognate nAChRs mediated by an increase in their abundance at the cell surface membrane in human, rodent, and primate brains (Schwartz and Kellar, 1983; Breese et al., 1997; Marks et al., 1998; Mamede et al., 2007; Nashmi et al., 2007). Both the α4β2 nAChRs (Kuryatov et al., 2005; Sallette et al., 2005; Lester et al., 2009) and the α6* nAChRs (Walsh et al., 2008; Henderson et al., 2014) are up-regulated when nAChR-expressing cells are exposed to nanomolar nicotine concentrations as found in smokers' brains. In rodent brains, up-regulation appears to be a posttranscriptional effect: mRNA for neither the α4 nor the β2 nAChR subunits change after chronic nicotine exposure. The mechanism(s) by which nicotine up-regulates the α4β2 nAChRs, in particular, has attracted substantial attention because of the profound effect nAChR up-regulation has on the functional circuitry in which these nAChRs are expressed.

Based on experiments primarily done in vitro, which to a large degree mimic the up-regulation observed in vivo, many different mechanisms for up-regulation have been proposed (Govind et al., 2009). These include (a) decreased nAChR turnover at the plasma membrane, (b) increased nAChR affinity for nicotine itself caused by an induced conformational change in the receptor, (c) increased trafficking of nAChR to the plasma membrane, and (d) chemical chaperoning by nicotine to catalyze subunit assembly in the ER. Among the various mechanisms proposed, a preponderance of evidence supports the intracellular chemical chaperoning effects of nicotine.

In this issue, Henderson et al. showed that nicotine up-regulates α6* nAChRs in dopaminergic, medial habenula, and superior colliculus neurons of a knock-in mouse expressing a GFP-labeled α6 nAChR subunit. They then used normalized Förster resonance energy transfer (FRET) between the fluorescent labeled α6* nAChR subunit and intracellular transport vesicle and organelle proteins to study mechanistic aspects of nicotine-dependent up-regulation of recombinant α6β2β3 nAChRs expressed in vitro in Neuro-2a cells. By following FRET signals between the α6 nAChR subunit and proteins at the ER exit site, in coat protein complex I (COPI)- and COPII-coated vesicles, and the cis-Golgi, they found something quite unexpected about the ability of nicotine to up-regulate α6β2β3 nAChRs. Blocking the

Correspondence to Rene Anand: anand.20@osu.edu

The Rockefeller University Press
J. Gen. Physiol. Vol. 143 No. 1 49–50
www.jgp.org/cgi/doi/10.1085/jgp.201311136

retrograde transport of proteins from the cis-Golgi by COPI-coated vesicles completely blocked the nicotine-dependent up-regulation of α6β2β3 nAChRs at the plasma membrane. They demonstrated this by using a low concentration of the COPI inhibitor, CI-976, which selectively inhibits retrograde but not anterograde transport. They augmented this finding by showing that a mutant α6β2β3 nAChR, in which a canonical COPI-binding site motif, KKK, within the long intracellular loop of the β3 subunit is mutated to AAA, did not up-regulate in response to nicotine but rather showed normal basal expression at the plasma membrane. They also demonstrated that CI-976 blocked the up-regulation of the α4β2 nAChR in response to nicotine exposure. Thus, the retrieval of nAChRs from the cis-Golgi by COPI for effecting nicotine-dependent up-regulation is not idiosyncratic to the α6β2β3 nAChRs but instead represents a ubiquitous process by which nicotine up-regulates high sensitivity nAChRs.

The findings of Henderson et al. (2014) suggest that in the presence of nicotine, rapid assembly of α4β2 nAChRs and α6β2β3 nAChRs occurs in the ER, resulting in incomplete posttranslational processing of receptors that overwhelms the ER quality control machinery. These immature nAChRs, however, are quarantined by the quality control machinery of the cis-Golgi and policed by a COPI-mediated mechanism back to the ER for proper posttranslational reprocessing. In short, the chemical chaperoning mediated by nicotine requires retrograde trafficking between the Golgi and ER to effect up-regulation of high sensitivity nAChRs at the plasma membrane of cells.

The latest Nobel Prize for Physiology or Medicine was awarded to a trio of scientists—James Rothman, Randy Schekman, and Thomas Sudhof—for their significant contributions to the understanding of the cellular mechanisms for vesicle transport, protein machinery for vesicle fusion, and signals that regulate vesicles to release their cargo with precision at synapses. The timely study by Henderson et al. (2014) builds on these findings to elucidate how nicotine hijacks the cellular machinery of the ER and cis-Golgi to up-regulate nAChRs at the plasma membrane and thus nonphysiologically alter the dynamics of vesicle release at synapses to drive nicotine addiction. Finding ways to therapeutically intervene at the level of COPI–nAChR interactions, as suggested by the authors, represents a rather novel strategy to prevent nAChR up-regulation, nicotine addiction, and the deadly consequences of tobacco use on human health.

Edward N. Pugh Jr. served as editor.

REFERENCES

Breese, C.R., M.J. Marks, J. Logel, C.E. Adams, B. Sullivan, A.C. Collins, and S. Leonard. 1997. Effect of smoking history on [3H] nicotine binding in human postmortem brain. *J. Pharmacol. Exp. Ther.* 282:7–13.

Fowler, C.D., Q. Lu, P.M. Johnson, M.J. Marks, and P.J. Kenny. 2011. Habenular α5 nicotinic receptor subunit signalling controls nicotine intake. *Nature.* 471:597–601. http://dx.doi.org/10.1038/nature09797

Govind, A.P., P. Vezina, and W.N. Green. 2009. Nicotine-induced upregulation of nicotinic receptors: underlying mechanisms and relevance to nicotine addiction. *Biochem. Pharmacol.* 78:756–765. http://dx.doi.org/10.1016/j.bcp.2009.06.011

Henderson, B.J., R. Srinivasan, W.A. Nichols, C.N. Dilworth, D.F. Gutierrez, E.D.W. Mackey, S. McKinney, R.M. Drenan, C.I. Richards, and H.A. Lester. 2014. Nicotine exploits a COPI-mediated process for chaperone-mediated up-regulation of its receptors. *J. Gen. Physiol.* 143:51–66.

Kuryatov, A., J. Luo, J. Cooper, and J. Lindstrom. 2005. Nicotine acts as a pharmacological chaperone to up-regulate human alpha-4beta2 acetylcholine receptors. *Mol. Pharmacol.* 68:1839–1851.

Lester, H.A., C. Xiao, R. Srinivasan, C.D. Son, J. Miwa, R. Pantoja, M.R. Banghart, D.A. Dougherty, A.M. Goate, and J.C. Wang. 2009. Nicotine is a selective pharmacological chaperone of acetylcholine receptor number and stoichiometry. Implications for drug discovery. *AAPS J.* 11:167–177. http://dx.doi.org/10.1208/s12248-009-9090-7

Mamede, M., K. Ishizu, M. Ueda, T. Mukai, Y. Iida, H. Kawashima, H. Fukuyama, K. Togashi, and H. Saji. 2007. Temporal change in human nicotinic acetylcholine receptor after smoking cessation: 5IA SPECT study. *J. Nucl. Med.* 48:1829–1835. http://dx.doi.org/10.2967/jnumed.107.043471

Marks, M.J., K.W. Smith, and A.C. Collins. 1998. Differential agonist inhibition identifies multiple epibatidine binding sites in mouse brain. *J. Pharmacol. Exp. Ther.* 285:377–386.

Nashmi, R., C. Xiao, P. Deshpande, S. McKinney, S.R. Grady, P. Whiteaker, Q. Huang, T. McClure-Begley, J.M. Lindstrom, C. Labarca, et al. 2007. Chronic nicotine cell specifically upregulates functional alpha 4* nicotinic receptors: basis for both tolerance in midbrain and enhanced long-term potentiation in perforant path. *J. Neurosci.* 27:8202–8218. http://dx.doi.org/10.1523/JNEUROSCI.2199-07.2007

Picciotto, M.R., M. Zoli, R. Rimondini, C. Léna, L.M. Marubio, E.M. Pich, K. Fuxe, and J.P. Changeux. 1998. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature.* 391:173–177. http://dx.doi.org/10.1038/34413

Quik, M., X.A. Perez, and S.R. Grady. 2011. Role of α6 nicotinic receptors in CNS dopaminergic function: relevance to addiction and neurological disorders. *Biochem. Pharmacol.* 82:873–882. http://dx.doi.org/10.1016/j.bcp.2011.06.001

Russo, S.J., D.M. Dietz, D. Dumitriu, J.H. Morrison, R.C. Malenka, and E.J. Nestler. 2010. The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends Neurosci.* 33:267–276. http://dx.doi.org/10.1016/j.tins.2010.02.002

Sallette, J., S. Pons, A. Devillers-Thiery, M. Soudant, L. Prado de Carvalho, J.P. Changeux, and P.J. Corringer. 2005. Nicotine upregulates its own receptors through enhanced intracellular maturation. *Neuron.* 46:595–607. http://dx.doi.org/10.1016/j.neuron.2005.03.029

Schwartz, R.D., and K.J. Kellar. 1983. Nicotinic cholinergic receptor binding sites in the brain: regulation in vivo. *Science.* 220:214–216. http://dx.doi.org/10.1126/science.6828889

Tapper, A.R., S.L. McKinney, R. Nashmi, J. Schwarz, P. Deshpande, C. Labarca, P. Whiteaker, M.J. Marks, A.C. Collins, and H.A. Lester. 2004. Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. *Science.* 306:1029–1032. http://dx.doi.org/10.1126/science.1099420

Walsh, H., A.P. Govind, R. Mastro, J.C. Hoda, D. Bertrand, Y. Vallejo, and W.N. Green. 2008. Up-regulation of nicotinic receptors by nicotine varies with receptor subtype. *J. Biol. Chem.* 283:6022–6032. http://dx.doi.org/10.1074/jbc.M703432200

Wonnacott, S. 1997. Presynaptic nicotinic ACh receptors. *Trends Neurosci.* 20:92–98. http://dx.doi.org/10.1016/S0166-2236(96)10073-4