# Proteins for increased surface expression of the α6β4 nicotinic acetylcholine receptor: nothing but good news?

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**Useful animal models of disease in neuroscience can make accurate predictions about a therapeutic outcome, a feature known as predictive validity. In this issue of the *JCI*, Knowland et al. provide an improved model to assess nicotinic acetylcholine receptor (nAChR) ligands for treating chronic pain. The authors identify two proteins, the voltage-dependent calcium channel auxiliary subunit BARP and the unfolded protein response sensor IRE1α, that are required for robust heterologous expression of α6β4, an nAChR subtype in dorsal root ganglia (DRG). This nAChR is a candidate for the analgesic effects of nicotine as well as the frog toxin epibatidine. Now researchers can efficiently screen for α6β4 nAChR–selective agonists using heterologous expression systems. Candidates that emerge will enable researchers to test the predictive validity of mouse models for chronic pain in the nAChR context. If all these steps work, one can envision a class of non-opioid nAChR-targeted analgesics for chronic pain.**

*...Nothing but good news*
*There is a frog in South America*
*Whose venom is a cure*
*For all the suffering that mankind*
*Must endure*
*More powerful than morphine*
*And soothing as the rain*
*A frog in South America*
*Has the antidote for pain...*
*— (Paul Simon, "Senorita with a Necklace of Tears")*

## The opioid epidemic

Paul Simon's comparison of epibatidine — the compound secreted by those frogs — with morphine has contemporary relevance. The opioid epidemic continues to ravage, causing approximately one hundred deaths per day in the United States (1). A majority of these opioid overdose deaths come from misused pharmaceutical opioids (2). Unfortunately, 30% of Americans suffer from some form of pain; for thousands of years, chronic pain has been relieved by opioids. Society continues to struggle to balance several competing issues. (a) Ethical: Some companies encouraged opioid use, a transgression that led to over-prescription. (b) Biological: Opioid use disorder has neurobiological effects ranging from cell biology to behavior. (c) Clinical: Successfully tapering a patient off opioids remains an unsolved problem arising from variations in individual pharmacokinetics and a lack of objective biomarkers for chronic pain. (d) Social: The chemical ease of synthesizing heroin and fentanyl derivatives has enabled people to obtain these opioids to maintain addiction — an issue with inputs from international relations, law enforcement, and education.

A suitable agonist within the theme of G protein–coupled opioid receptors that also allows for reduced dependence represents a long-sought goal (3, 4). One popular strategy is to develop a biased agonist. Simply stated, a biased opioid receptor agonist activates the appropriate G protein but fails to recruit β-arrestin, thus reducing tolerance and dependence. Unfortunately, efforts in revealing such an agonist have not yet led to success in the clinic.

## The analgesic target for epibatidine has posed challenges

For several decades, scientists have known that epibatidine acts on most nicotinic acetylcholine receptors (nAChRs), which comprise another signaling pathway for pain relief as well as drugs of abuse (5–7). There are three challenges that limit clinicians from optimally modulating the analgesic properties of nAChRs: (a) Identifying which nAChR constitutes the major analgesic target. (b) Developing a nicotinic agonist that activates α4β2-containing nAChRs without leading to the varied and complex dependence pathways downstream from nicotine. (c) Utilizing heterologous expression systems suitable for addressing point (b) with modern drug screening methods.

Seventeen different mammalian subunits (α1–α10, β1–β4, γ, ε, and δ) assemble to form pentameric ligand-gated ion channels that establish an unknown but large number of nAChR subtypes (8). Although many of these receptors are readily expressed in various recombinant expression systems, some require additional proteins to help assembly and trafficking to the plasma membrane. An early-researched example is the α7 nAChR, which generally requires coexpression of chaperone proteins, like Ric3 or NACHO, to conduct meaningful experiments (9, 10). Some small-molecule α4β2 ligands, such as nicotine, also enhance nAChR surface expression; these ligands act as pharmacological chaperones, binding to nascent receptors in the cytoplasm, and aiding in assembly and trafficking (11, 12).

Because the pharmacology of α6β2-containing and α6β4 nAChRs strongly

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resembles that of α4β2 nAChRs, it has also been suspected that α6-containing nAChRs are also targets for nAChR agonists' analgesic properties (13). The Gordian knot has been achieving functional expression of α6-containing nAChRs. Suboptimal methods to attain functional expression have included engineering mutations that produce hypersensitive receptors (14), that aid trafficking (15), or that enhance expression (16).

## Players revealed by cotransfection

The group at Janssen Pharmaceutical Companies of Johnson & Johnson has systematically been identifying chaperone proteins for nAChR expression over the past few years; happily, in this issue of the *JCI*, they now report a combination of proteins that yield good expression of wild-type α6β4 nAChRs in recombinant systems (17). The top two proteins that increased the nicotine-evoked $Ca^{2+}$ response in unbiased genome-wide cotransfection were sodium channel β-subunit–anchoring and –regulatory protein (BARP) and IRE1α, an unfolded protein response (UPR) sensor. The experiments did not re-identify NACHO, an otherwise promiscuous accessory protein for nAChR expression that increases α7, α4β2, α3β2, and α3β4 (18).

In Knowland et al.'s experiments, simply expressing α6 and β4 (in *Xenopus* oocyte or HEK293T cell systems) failed to induce ACh-evoked currents, but cells coexpressing BARP generated substantial currents. BARP also increased α6β4 plasma membrane (PM) expression on HEK293T cells, but failed to induce changes in epibatidine binding. In contrast, coexpression with IRE1α failed to evoke ACh currents or change PM expression, but increased [$^3$H]epibatidine binding (presumably to assembled intracellular nAChRs), suggesting that IRE1α enhances receptor assembly (17). These results suggest that BARP and IRE1α affect α6β4 through distinct mechanisms.

The IRE1α results are striking because this protein is thought to sense ER stress rather than to enhance protein levels directly. For many membrane proteins, simply improving their folding and assembly within the ER increases PM protein levels (19), as though the rate-limiting step in protein levels at the PM is ER-resident folding and assembly. Indeed, an increase in ER exit sites accompanies both pharmacological chaperoning and increased surface levels (11, 20). Although some membrane proteins, for instance some voltage-gated proton channels, spend more time in the Golgi than the ER (21), this is a rare property in the nAChR field. The Knowland et al. results (17) remind us that IRE1α acts, via its classical XBP1 splicing pathway, during the UPR to reprogram several aspects of ER function. The recent data on IRE1α present a more general context for the previous suggestion that suppressing the UPR via nicotine-nAChR interactions might benefit people with early-stage Parkinson's disease (22).

The primary motivation to search for these accessory proteins was to help identify the nAChR subunits responsible for the ameliorative pain effects (specifically, antiallodynia) following nAChR stimulation. The dorsal root ganglia (DRG) play a role in nociception and coexpress α6 and β4 mRNA (23). Consistent with the experiments performed in HEK293T cells, wild-type DRG neurons had greater α6β4 surface levels than *BARP*-KO neurons (17). Nicotine has an antiallodynic effect in rodent models of neuropathic pain (24). Consistent with these previous observations, nicotine decreased mechanical allodynia in the spared nerve injury (SNI) model for wild-type animals, but failed to provide relief to *BARP*-KO samples. To determine whether α6β4 was the chief target for nicotine's antiallodynic effects, Knowland et al. studied *NACHO*-KO mice, which would have substantially reduced nAChR expression except for α6β4. The authors found no difference between nicotine's antiallodynia properties in *NACHO*-KO mice compared to wild-type mice, consistent with the paper's viewpoint that α6β4 is the nAChR that carries out nicotine's antiallodynia effects. Inhibition of α4β2 did not prevent nicotine-induced antiallodynia (17).

## Conclusion

While α6β4 in DRG neurons plays a key role in nicotine-induced antiallodynia, dopaminergic neurons of the brain express an especially varied collection of other nAChR subtypes. The Janssen Pharmaceutical group recently identified accessory proteins that increase the protein levels of one such subtype, the α6β2β3 nAChR (25). One therefore hopes that both Parkinson's disease and nicotine dependence may also soon benefit from the ability to screen a full collection of nAChRs (17).

As a caution, we do not yet know of a nicotinic agonist that affects α6-containing AChRs more potently than α4-containing nAChRs. ABT-894, one of the synthetic ligands tested by Knowland et al., has the opposite selectivity, with higher agonist activity at α4-containing nAChRs (17). We can hope that enhanced α6β4 levels at the plasma membrane will enable experiments to find an α6β4-selective ligand, thus providing a compound to test the hypothesis that such drugs will become nonaddictive opioids. We must remember that all the indications noted here — antiallodynia, nicotine dependence, and Parkinson's disease — require chronic administration of drugs, which may present additional problems. Overcoming such intellectual and technical barriers would constitute Paul Simon's "Nothing but good news," indeed.

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