Trends in Pharmacological Sciences

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Spotlight

Designer Drugs for Designer Receptors: Unlocking the Translational Potential of Chemogenetics

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Chemogenetic techniques allow selective manipulation of neurons by activating engineered actuator proteins with otherwise inert effector molecules. A recent study (Magnus *et al*. *Science* 2019;364: eaav5282) describes the coevolution of highly potent actuator–effector pairs based on a clinically approved antismoking drug. These tools allow selective excitation or inhibition of neurons in the living brain with high specificity and no detectable side-effects.

Naturally occurring receptor proteins, such as ligand-gated ion channels, have evolved over billions of years to be activated efficiently and exclusively by highly selective small-molecule ligands. The discovery and synthesis of exogenous ligands that target these receptors has massively influenced biomedical research and strongly boosted development of efficient medical treatments. This has spurred the race for the discovery of novel, highly selective compounds. However, even the most selective drug will always affect all cells and tissues expressing its target receptor, often leading to undesired side-effects. This is a particularly difficult problem in treating disorders of the central nervous system (CNS), with its incredible diversity of cell types and circuits and the semipermeable blood brain barrier (BBB). Many drugs do not efficiently pass the BBB and instead exert off-target effects elsewhere in the body.

Chemogenetic tools are two-component actuator systems designed to overcome these limitations, allowing pharmacological modulation of specific cell populations through a combination of genetic expression of an engineered receptor and delivery of a specific ligand designed to only activate the exogenous receptor [1]. Chemogenetic receptors typically constitute ligand-gated ion channels or G protein-coupled receptors that have been designed to lose affinity to their natural ligand and instead become activated by a highly specific, but otherwise inert, small-molecule agonist that can be delivered either locally or systemically.

Although chemogenetic tools have only been used in basic research to date, their translational appeal is clear: if genetic expression of the engineered receptor is feasible in the targeted cell population, systemic delivery of its ligand can circumvent any side-effects associated with conventional pharmacotherapy. To fulfill this promise, a clinically relevant chemogenetic actuator system needs to satisfy several requirements. It should: (i) allow high-affinity activation of the engineered receptor; (ii) trigger minimal activation of off-target receptors; (iii) easily cross the BBB; and (iv) ideally be based on an FDA-approved drug, thus reducing the 'energy barrier' for drug approval and translation to the clinic. Putting aside the relatively limited experience with long-term expression of heterologous transgenes in the human CNS, currently available ligands fall short of meeting these requirements, often suffering from low potency [2] or limited selectivity [3]. Moreover, some chemogenetic ligands have been shown to trigger side-effects due to metabolic conversion to other neuroactive compounds [4,5].

To address this challenge, Magnus *et al*. went back to the drawing board (or chemical bench) and redesigned the so-called pharmacologically selective actuator molecules (PSAMs) together with highly specific and selective pharmacologically selective effector molecules (PSEMs) [2], yielding a highly specific and extremely potent chemogenetic system for selective control of neuronal excitability in the mammalian CNS [6]. The study, which includes proof-of-concept experiments in mice and a non-human primate, constitutes a major leap in the race for clinically applicable strategies to control the activity of particular populations of neurons with high selectivity and precision.

PSAMs are chimeric receptors consisting of a ligand-binding domain (LBD) of the α7 nicotinic acetylcholine receptor (α7-nACh) fused to either the chloride-conducting pore of a glycine receptor (α7-GlyR) for neuronal inhibition or a cation-conducting pore of the serotonin (5HT) receptor 3 (α7-5HT3) for neuronal excitation (Figure 1A). Using structure-guided mutagenesis of the LBD, the authors reduced the potency of its natural agonist ACh 13-fold while increasing it 390-fold for varenicline, an FDA-approved cholinergic agonist used to treat nicotine addiction (Figure 1B). This yielded potent PSAMs, termed PSAM^4-5HT3 and PSAM^4-GlyR, that are fully activated by low-nanomolar concentrations of varenicline, but not by physiological concentrations of acetylcholine (Figure 1A,B).

Using PSAM^4-GlyR, Magnus *et al*. were able to continuously inhibit spiking of cultured neurons for more than 2 weeks (Figure 1C). Moreover, varenicline could easily be washed-out to restore normal spiking without any obvious long-lasting change of neuronal excitability. This is remarkable, given that chronic manipulations of neuronal activity typically lead to homeostatic adaptations working against the manipulation [7]. Conversely, a depolarized membrane potential was maintained with PSAM^4-5HT3 for more than 2 weeks (Figure 1C). Due to its highly efficient brain penetrance and long lifetime,

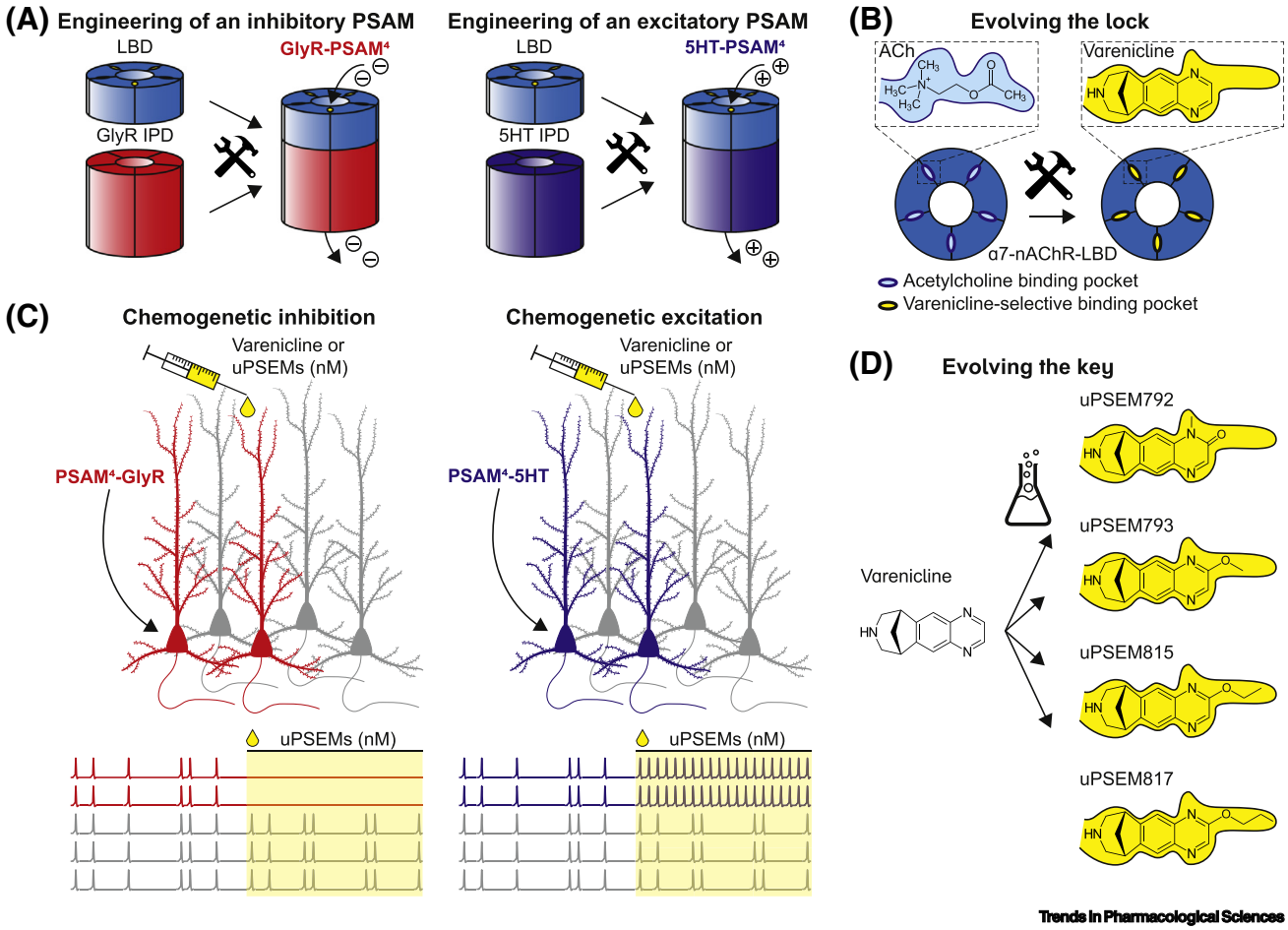

Figure 1. A Novel, Highly Selective and Ultrasensitive Chemogenetic Toolkit for Manipulation of Neuronal Activity. (A) Pharmacologically selective actuator molecules (PSAMs) are chimera consisting of a ligand-binding domain (LBD) from the α7-nicotinic acetylcholine (ACh) receptor and an ion pore domain (IPD) of the glycine receptor (GlyR, anion-conducting) or the serotonin receptor (5HT, cation-conducting). (B) The ACh binding pocket of the α7-nAChR-LBD (symbolizing the lock) is mutated to become selective for the pharmacologically selective effector molecule (PSAM) varenicline, a clinically approved antismoking drug. (C) Expression of PSAM^4-GlyR and PSAM^4-5HT in defined neuronal populations allows selective down- or upregulation of neuronal activity with nanomolar concentrations of varenicline and ultrapotent PSEMs (uPSEMs). (D) uPSEMs (symbolizing the keys to fit the binding pocket of the LBD) are derived from varenicline and optimized to fit the PSAM^4 binding pocket with higher specificity than varenicline.

systemically injected varenicline displayed robust neuronal silencing in PSAM^4-GlyR-expressing mice or a primate at doses as low as 0.1 mg/kg bodyweight.

Although the two PSAM^4 variants were potently activated by varenicline at low doses, the authors set out to generate PSEMs with improved PSAM^4-selectivity over varenicline's 'natural' targets. For this, they used again the molecular structure of the LBD as a blueprint to evolve molecular analogs of varenicline with improved binding to the LBD of PSAM^4 and reduced binding to natural receptors (Figure 1D). These ultrapotent PSEMs (uPSEMs) were active in the sub-nanomolar range and showed no detectable agonism for typical varenicline targets such as α2β4-, 5HT3-, or α7-nACh-receptors. These uPSEMs retained their water solubility and brain penetrance upon intraperitoneal injections, making systemic brain delivery *in vivo* straightforward. This was confirmed by a lowest effective dose of 0.03 mg/kg for the variant uPSEM^{793}. Since uPSEMs are based on the clinically approved anti-smoking drug varenicline, they constitute a promising step towards clinical chemogenetic applications.

As proof-of-concept of the robustness of their approach, the authors demonstrate that their new PSAM^4-uPSEM system is suitable to reliably suppress neuronal

activity of place cells (neurons that fire action potentials when an animal visits a particular spatial location) in the mouse hippocampus. Fixing mice on a treadmill studded with spatial marks, these cells can be directly visualized under a microscope. When uPSEM^{792} was injected systemically, spiking of such place cells was completely abrogated, demonstrating directly the strong potency of this chemogenetic toolset.

The high potency and selectivity of the PSAM^4-uPSEM system will make it an indispensable research tool for many neuroscientific questions. It appears especially well suited for chronic circuit manipulations, where other systems fail due to side-effects or low potency. Nonetheless, there may be cases where the use of PSAMs is limited. Since PSAM^4-GlyR is a chloride channel, neuronal silencing depends on the electrochemical chloride gradient, which in most cases is indeed inhibitory at the somatodendritic compartment of mature neurons. However, activating PSAM^4-GlyR at axonal terminals might lead to a depolarization and subsequent neurotransmitter release due to a depolarizing axonal chloride gradient [8]. Furthermore, the cellular homeostasis of chloride ions could be altered in certain neurological disorders [9]. In these cases, G protein-coupled effectors that belong to the family of 'designer receptors exclusively activated by designer drugs' (DREADDs), such as hM4D [10], could still prove to be a better choice. Ultimately, development of a K^+-selective PSAM might overcome all remaining limitations, allowing efficient and compartment-independent neuronal silencing.

In summary, the new addition to the chemogenetic toolbox presented by Magnus *et al*. will open up new avenues for cell-type specific manipulations of neuronal activity, particularly in intact animals. Building on the clinically approved drug varenicline, which is well-tolerated, water-soluble, has a long half-life, and penetrates the brain efficiently, the new uPSEMs tailored to the highly selective LBD of the next-generation PSAMs might be the first of the chemogenetic actuators to reach clinical testing, once transgene delivery to the CNS becomes feasible in a clinical setting.

Acknowledgments

We thank Mauro Pulin for comments on this manuscript. This work was supported by grants from the Adelis Foundation (to O.Y.), the European Research Council (ERC-2016-StG 714762 to J.S.W., ERC-2018-CoG 819496 to O.Y.) and the Deutsche Forschungsgemeinschaft (DFG FOR 2419, DFG SPP 1926 to J.S.W.).

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https://doi.org/10.1016/j.tips.2019.04.010

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