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Amphetamine activates/potentiates a ligand-gated ion channel

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Amphetamine (Amph) is a psychostimulant illegally used as a performance enhancer and widely prescribed to treat attention deficit hyperactive disorder (ADHD). Despite its therapeutic use for nearly a century to treat different mental disorders, the mechanism of Amph action is still not fully understood. Similarly to other drugs of abuse, Amph increases the amount of dopamine (DA) at the dopaminergic synapses. And this increase, which occurs by blocking DA reuptake and increasing DA release, is believed to initiate its behavioral outcomes. Notably, Amph increases DA through more than one mechanism[1]. First, as Amph is a substrate for the DA transporter (DAT) it competes for DA reuptake and then, upon entering the neuron via DAT, Amph acts on the vesicular monoamine transporters causing redistribution of DA from the vesicles to the cytoplasm. Consequently, Amph decreases vesicle-dependent DA release and induces DA reverse transport through the DAT. However, recent publications have challenged the assertion that Amph acts solely through DAT to mediate Amph-induced behaviors, by suggesting that behavioral effects may come from Amph action on targets other than DAT. Support for this hypothesis comes from DAT knockout mice where Amph can still generate reward responses.[2] Moreover, in DAT knockout animals that are pharmacologically treated to inhibit DA synthesis, administration of Amph derivatives reverses the severe akinesia caused by lack of DA, suggesting that Amph acts on other targets than the DAT and DA itself.[3] Given these intriguing findings, we decided to look for new protein targets of Amph by using the model system *C. elegans*. Although *C. elegans* is phylogenetically distant from humans, several studies have demonstrated that genes involved in dopaminergic transmission including DA synthesis, packaging, release and reuptake are highly conserved from worm to man suggesting structural and functional similarity between mammals and *C. elegans* for this process. The use of *C. elegans* was also justified by the fact that Amph generates a unique behavior in the worms termed swimming induced paralysis (SWIP).[4] By screening a number of mutants lacking expression of proteins involved in the DA transmission, we ultimately found that Amph requires DAT and the amine-gated ion channel LGC-55 to elicit SWIP.[5] In fact, while DAT or LGC-55 null mutants generated partial reduction of Amph-induced SWIP, only animals lacking both proteins (double null) completely lost the SWIP response upon Amph treatment. Moreover, our data show that in oocytes injected with LGC-55, Amph directly activates LGC-55 channels,[5] demonstrating that Amph itself is an agonist for LGC-55 capable of generating behavioral effects independent of DA. These findings represent the first experimental evidence of direct Amph binding and activation to an ionotropic receptor.

The Amph activated LGC-55 receptors are chloride channels belonging to the cys-loop ligand-gated channel super family, which includes the 5-HT_3, $GABA_A$ and glycine receptors. The apparent affinity of Amph for the LGC-55 (K_m = 150μM) is lower than the endogenous ligand tyramine (K_m = 4μM) or β-phenylethylamine (βPEA) (K_m = 9μM).[5,6] Our in vitro

experiments show that the endogenous trace amine βPEA, like Amph, increases extracellular DA via a DAT dependent manner,[7] however, βPEA is more effective at activating LGC-55 as it induces larger currents and generates stronger SWIP behaviors than Amph.[6] The observation that the LGC-55 is strongly activated by βPEA and relatively poorly activated by Amph suggests that βPEA and Amph could act as full and partial LGC-55 agonists, respectively. Interestingly, both our in vitro and in vivo data demonstrate that Amph potentiates the activation of the LGC-55 by βPEA.[6] This intriguing finding suggests that Amph and βPEA bind separate sites on LGC-55. The phenomenon of channel potentiation has been reported for several other members of the cys-loop family. For example, the $GABA_A$ and glycine receptors are potentiated by benzodiazepines and alcohol, respectively. The ability of Amph to enhance LGC-55 channel activation by endogenous amines is likely to have important physiological consequences if LGC-55 homologs exist in humans. In fact, because neuronal excitability is rapidly achieved by fine-tuning of the membrane potential, the activation of a chloride conductance would cause hyperpolarization which ultimately results in an inhibitory effect in the LGC-55 expressing cells. Therefore, the LGC-55 might have an important role in regulating neuronal transmission.

Although LGC-55 homologs have not yet been identified in mammals, the existence of amine-gated channels in mammals has been suggested given the finding that histamine generates fast IPSPs in the brain through the activation of an as-of-yet unidentified chloride channel.[8] Moreover, our analysis of the human protein database uncovered four orphan proteins that share 30-45% identity with LGC-55 receptors suggesting these proteins may be functionally homologous to LGC-55 and constitute new molecular targets for therapeutic intervention in Amph addiction.

References

1. Sulzer D, Sonders MS, Poulsen NW, Galli A. Mechanisms of neurotransmitter release by amphetamines: a review. Prog Neurobiol 2005; 75:406-33; PMID:15955613; http://dx.doi.org/10.1016/j.pneurobio.2005.04.003
2. Sotnikova TD, Beaulieu JM, Barak LS, Wetsel WC, Caron MG, Gainetdinov RR. Dopamine-independent locomotor actions of amphetamines in a novel acute mouse model of Parkinson disease. PLoS Biol 2005; 3:e271; PMID:16050778; http://dx.doi.org/10.1371/journal.pbio.0030271
3. Sotnikova TD, Budygin EA, Jones SR, Dykstra LA, Caron MG, Gainetdinov RR. Dopamine transporter-dependent and -independent actions of trace amine beta-phenylethylamine. J Neurochem 2004; 91:362-73; PMID:15447669; http://dx.doi.org/10.1111/j.1471-4159.2004.02721.x
4. Carvelli L, Matthies DS, Galli A. Molecular mechanisms of amphetamine actions in Caenorhabditis elegans. Mol Pharmacol 2010; 78:151-6; PMID:20410438; http://dx.doi.org/10.1124/mol.109.062703
5. Safratowich BD, Lor C, Bianchi L, Carvelli L. Amphetamine activates an amine-gated chloride channel to generate behavioral effects in Caenorhabditis elegans. J Biol Chem 2013; 288:21630-7; PMID:23775081; http://dx.doi.org/10.1074/jbc.M113.484139
6. Safratowich BD, Hossain M, Bianchi L, Carvelli L. Amphetamine potentiates the effects of β-phenylethylamine through activation of an amine-gated chloride channel. J Neurosci 2014; 34:4686-91; PMID:24672014; http://dx.doi.org/10.1523/JNEUROSCI.3100-13.2014
7. Hossain M, Wickramasekara RN, Carvelli L. β-Phenylethylamine requires the dopamine transporter to increase extracellular dopamine in Caenorhabditis elegans dopaminergic neurons. Neurochem Int 2014; 73:27-31; PMID:24161617; http://dx.doi.org/10.1016/j.neuint.2013.10.010
8. Hatton GI, Yang QZ. Ionotropic histamine receptors and H2 receptors modulate supraoptic oxytocin neuronal excitability and dye coupling. J Neurosci 2001; 21:2974-82; PMID:11312281