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In vivo interactions between α7 nicotinic acetylcholine receptor and nuclear peroxisome proliferator-activated receptor-α: Implication for nicotine dependence

Asti Jackson [a, *], Deniz Bagdas [a, b], Pretal P. Muldoon [a], Aron H. Lichtman [a], F. Ivy Carroll [c], Mark Greenwald [d], Michael F. Miles [a], M. Imad Damaj [a]

[a] Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, United States
[b] Experimental Animals Breeding and Research Center, Faculty of Medicine, Uludag University, Bursa, Turkey
[c] Center for Organic and Medicinal Chemistry, Research Triangle Institute, Research Triangle Park, NC, United States
[d] Substance Abuse Research Division, Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, 2761 East Jefferson Ave., Detroit, MI 48207, United States

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ABSTRACT

Chronic tobacco use dramatically increases health burdens and financial costs. Limitations of current smoking cessation therapies indicate the need for improved molecular targets. The main addictive component of tobacco, nicotine, exerts its dependency effects *via* nicotinic acetylcholine receptors (nAChRs). Activation of the homomeric α7 nAChR reduces nicotine's rewarding properties in conditioned place preference (CPP) test and i.v. self-administration models, but the mechanism underlying these effects is unknown. Recently, the nuclear receptor peroxisome proliferator-activated receptor type-α (PPARα) has been implicated as a downstream signaling target of the α7 nAChR in ventral tegmental area dopamine cells. The present study investigated PPARα as a possible mediator of the effect of α7 nAChR activation in nicotine dependence. Our results demonstrate the PPARα antagonist GW6471 blocks actions of the α7 nAChR agonist PNU282987 on nicotine reward in an unbiased CPP test in male ICR adult mice. These findings suggests that α7 nAChR activation attenuates nicotine CPP in a PPARα-dependent manner. To evaluate PPARα activation in nicotine dependence we used the selective and potent PPARα agonist, WY-14643 and the clinically used PPARα activator, fenofibrate, in nicotine CPP and we observed attenuation of nicotine preference, but fenofibrate was less potent. We also studied PPARα in nicotine dependence by evaluating its activation in nicotine withdrawal. WY-14643 reversed nicotine withdrawal signs whereas fenofibrate had modest efficacy. This suggests that PPARα plays a role in nicotine reward and withdrawal and that further studies are warranted to elucidate its function in mediating the effects of α7 nAChRs in nicotine dependence.

1. Introduction

The homomeric α7 nicotinic acetylcholine receptor (nAChR) has unique features of high calcium permeability, rapid desensitization and low probability of channel opening (Séguéla et al., 1993; Williams et al., 2011), and has been shown to play a role in cognition, inflammation, immunity and neuroprotection (Corradi and Bouzat, 2016). Recent findings suggest this low-affinity α7 nAChR modulates nicotine reward and reinforcement in rodents (Brunzell and McIntosh, 2012; Harenza et al., 2014). The α7 nAChR selective agonist PNU282987 infused locally into the nucleus accumbens (NAc) shell reduced intravenous (i.v.) self-administered nicotine in rats. In contrast, ArIB, an α7 selective nAChR antagonist, infused in the NAc increased nicotine intake (Brunzell and McIntosh, 2012). Similarly, the genetic deletion of α7 nAChRs in mice enhances nicotine reward as measured in the conditioned place preference (CPP) test, whereas α7 knock-in (producing mice heterozygous for a Leu250-to-Thr substitution in the channel domain of α7 subunit which creates a gain-of-function mutation) abolishes nicotine preference. In addition, the selective α7 agonist PHA-543613 blocked the development of nicotine CPP in mice (Harenza et al.,

* Corresponding author.
E-mail address: jacksonab2@mymail.vcu.edu (A. Jackson).

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2014). Attenuation of nicotine reward and reinforcement by α7 nAChR agonists seems to be associated with a decreased nicotine-induced dopaminergic transmission in the brain, as PNU282987 blocks nicotine-induced increased firing activity of the ventral tegmental area (VTA) dopamine neurons in rats (Melis et al., 2013).

This important effect of α7 nAChR modulation of nicotine reward has prompted studies of the underlying mechanism. It has been suggested that α7 nAChR activation regulates VTA dopaminergic cells *via* the peroxisome proliferator activated receptor α (PPARα) in the rat. The α7 nAChR agonist PNU282987 induced synthesis of two fatty acid PPARα endogenous ligands, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), that in turn activate PPARα and phosphorylate β2-containing nAChRs on dopamine neurons *via* a tyrosine kinase pathway (Melis et al., 2013). These findings suggest a pathway by which α7 nAChR pharmacological stimulation indirectly inactivates β2-containing nAChRs via PPARα receptors. However, the above-noted study did not directly investigate this mechanism using a nicotine reward paradigm which is imperative because β2-containing nAChRs are required for nicotine reward (Picciotto et al., 1998; Walters et al., 2006).

PPARα is a nuclear ligand-activated transcription factor that when activated, enhances transcription of various genes involved in modulating many peripheral physiological responses such as inflammation and lipolysis (Zhu et al., 2000). Importantly, PPARαs, which are located in brain regions associated with reward (Moreno et al., 2004; Plaza-Zabala et al., 2010; Smaga et al., 2014), have been shown to modulate the rewarding properties of abused substances such as alcohol and nicotine (Bilbao et al., 2015; Melis et al., 2008). Acute administration of PPARα agonists attenuates nicotine (Mascia et al., 2011; Muldoon et al., 2013; Panlilio et al., 2012) and alcohol reinforcement (Bilbao et al., 2015), alcohol intake (Blednov et al., 2016a, 2016b) and nicotine-induced dopamine firing in rodents (Melis et al., 2008). For example clofibrate, a lipid-lowering agent and PPARα agonist (Staels et al., 1998), was shown in rats to block acquisition of nicotine seeking, decrease nicotine i.v. self-administration and block nicotine-induced dopamine release into the NAc shell (Panlilio et al., 2012).

Therefore, we hypothesize that PPARα may serve as a downstream mediator of α7 nAChR activation in nicotine reward. To test this hypothesis the present study investigated the interaction of the α7 nAChR and PPARα in a preclinical mouse model of reward (nicotine CPP). Furthermore, we examined PPARα activation in nicotine CPP and nicotine withdrawal, a behavioral outcome not measured before in preclinical studies with PPARα activators. We compared effects of the selective and potent PPARα agonist WY-14643 (Lo Verme et al., 2005; Willson et al., 2000) with a commonly used lipid lowering fibrate medication that activates PPARα fenofibrate (Keating, 2011). Results from these experiments may provide insight into the roles of α7 nAChR and PPARα in nicotine dependence.

2. Materials and methods

2.1. Animals

ICR male mice (8 weeks upon arrival; Harlan Laboratories, Indianapolis, IN) served as subjects. Mice were housed four per cage with *ad libitum* access to food and water on a 12-h light cycle in a humidity and temperature controlled vivarium that was approved by the Association for Assessment and Accreditation of Laboratory Animal Care. Experiments were performed during the light cycle and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2. Drugs

(−)-Nicotine hydrogen tartrate [(−)-1-methyl-2-(3- pyridyl) pyrrolidine (+)-bitartrate] and mecamylamine HCl (non-selective nAChR antagonist) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). PNU282987 (α7 nAChR agonist) and cocaine HCl were provided by the Drug Supply Program of the National Institute on Drug Abuse (Rockville, MD). Drugs were dissolved in physiological saline and administered systemically (s.c. for nicotine, mecamylamine, PNU282987 and i.p. for cocaine). Fenofibrate (PPARα agonist), WY-14643 (PPARα agonist), and GW6471 (PPARα antagonist) were purchased from Tocris (Minneapolis, MN) and dissolved in a mixture of 1:1:18 [1 vol ethanol/1 vol Emulphor-620 (Sanofi-Aventis, Bridgewater, NJ) and 18 vol saline] and administered i.p. Drug solutions were prepared in 10 ml solutions (i.e. 3 mg of drug in 10 ml of vehicle indicates 3 mg/kg dose). Freshly prepared solutions were injected at a total volume of 1 ml/100 g of body weight. Doses are expressed as the free base of the drug.

2.3. Nicotine and cocaine conditioned place preference studies

An unbiased CPP paradigm was performed as we previously described (Kota et al., 2007; Sanjakdar et al., 2015). Briefly, the CPP apparatus consisted of three chambers in a linear arrangement (ENV3013; Med Associates, St Albans, VT). The external white and black chambers (20 × 20 × 20 cm each) differed in overall color and floor texture (white mesh or black rod), and were separated by a smaller gray chamber with a smooth PVC floor. Partitions could be removed to allow access from the gray chamber to the black and white chambers. On day 1 animals were confined to the middle chamber for a 5 min habituation and then allowed to freely move between all three chambers for 15 min. Time spent in each chamber was recorded and these data were used to populate groups of approximately equal bias in baseline chamber preference. Twenty-minute conditioning sessions occurred twice a day (days 2–4). During conditioning sessions mice were confined to one of the larger chambers. The saline groups received saline in one large chamber in the morning and saline in the other large chamber in the afternoon. The nicotine group received nicotine in one large chamber and saline in the other large chamber. Treatments were counterbalanced to ensure some mice received the unconditioned stimulus in the morning and others received it in the afternoon. The nicotine-paired chamber was randomized across groups. Sessions were 4 h apart and were conducted by the same investigator. On test day (day 5) mice could access all chambers for 15 min in a drug free state. The preference score was calculated by determining the difference between time spent in the drug paired side on the test day versus the time in drug paired side on the baseline day. Any mouse showing preference for one side higher than 65% was not used in the study.

2.4. Nicotine precipitated withdrawal studies

A well-established and validated nicotine withdrawal model was performed (Bagdas et al., 2014; Damaj et al., 2003; Muldoon et al., 2015; Salas et al., 2007) Mice were infused with 24 mg/kg/day nicotine or saline for 14 days using s.c. osmotic minipumps (model 2000; Alzet Corporation, Cupertino, CA) implanted under isoflurane anesthesia (Jackson et al., 2008). Nicotine concentration was adjusted according to animal weight and mini pump flow rate. On the morning of day 15 mice were pretreated with vehicle, WY-14643 (0.3, 1 and 5 mg/kg, i.p.; 15 min prior) or fenofibrate (50 and 100 mg/kg, i.p.; 1 h prior) before challenge with the non-selective