Protein kinase C epsilon modulates nicotine consumption and dopamine reward signals in the nucleus accumbens

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Nicotine addiction and alcohol use disorders are very widespread and often occur together. Currently, there is no single drug approved for the simultaneous treatment of both conditions. Although these conditions share common genetic factors, the molecular mechanisms underlying their comorbidity are unknown. We have previously shown that mice lacking protein kinase C epsilon (PKCε) show decreased ethanol self-administration and reward as well as increased aversion to ethanol. Here we find that *Prkce*$^{-/-}$ mice self-administer less nicotine and show decreased conditioned place preference for nicotine compared with wild-type mice. In *Prkce*$^{-/-}$ mice, these behaviors are associated with reduced levels of α_6 and β_3 nicotinic receptor subunit mRNA in the ventral midbrain and striatum as well as a functional deficit in cholinergic modulation of dopamine release in nucleus accumbens. Our results indicate that PKCε regulates reward signaling through α_6-containing nicotinic receptors and suggest that PKCε could be a target for the treatment of comorbid nicotine and alcohol addictions.

Tobacco addiction remains the leading cause of premature mortality in the world, and half of all tobacco users will die of tobacco-related disease (1). Nicotine is the primary, if not the only, compound that maintains addiction to tobacco (2), and smokers will adjust their level of cigarette smoking to maintain constant levels of plasma nicotine (3). Current approved pharmacotherapies for smoking cessation all target nicotinic acetylcholine receptors (nAChRs) and include nicotine-replacement therapy, bupropion (a nicotinic antagonist) (4), and varenicline (a partial nicotinic agonist) (5). Although this approach is effective in the short term, long-term abstinence rates are low, underscoring the need for the discovery of new drug targets and development of new treatments.

It is striking that, among all drug addictions, alcohol and tobacco addictions are highly comorbid with more than 60% of smokers in the US reporting concurrent binge drinking or heavy use of alcohol (6). Likewise, the prevalence of smoking among those with alcohol use disorders has been reported to be as high as 88–96% (7, 8). In humans, a single exposure to alcohol can increase the urge to smoke and increase cigarette consumption (9, 10). Conversely, in rats, s.c. injections of nicotine can increase ethanol self-administration (11) and promote reinstatement for ethanol responding after extinction (11, 12). In rats that self-administer i.v. nicotine and oral alcohol, the extinction of alcohol responding is prolonged if nicotine remains available (13), suggesting that combined use of both substances may make it more difficult to successfully quit the use of nicotine or alcohol alone. Nicotine and alcohol addictions appear to share common genetic factors (14–16), but the molecular mechanisms underlying their comorbidity are unknown. Currently, no pharmaceutical agent has been approved to treat comorbid nicotine and alcohol addictions.

The protein kinase C (PKC) family of serine/threonine kinases transduces signals carried by lipid second messengers, and some members of the family modulate behaviors in rodents that model aspects of drug addiction (17). One family member, PKCε, is widely expressed in the central nervous system and belongs to the novel subclass of PKCs that are activated by *sn*-1,2-diacylglycerols and phosphatidylserine but not by calcium (18). Our previous studies have demonstrated a major role for PKCε in behavioral responses to ethanol (17). We have found that PKCε knockout (*Prkce*$^{-/-}$) mice show decreased ethanol self-administration (19–21) and reward (22) and increased sensitivity to the aversive effects of ethanol (22). Because nicotine and alcohol addictions are highly comorbid, we tested the hypothesis that PKCε also regulates behavioral responses to nicotine. We found that *Prkce*$^{-/-}$ mice show decreased nicotine consumption and conditioned place preference in association with impaired cholinergic modulation of reward signals mediated by α_6-containing (α_6*) nAChRs in the nucleus accumbens (NAc).

Results

We measured voluntary nicotine self-administration by using a continuous-access, two-bottle choice paradigm, a widely used method to measure nicotine consumption and preference in mice (23–25). *Prkce*$^{-/-}$ and wild-type mice were given a choice between a bottle of 15 μg/mL nicotine with 2% saccharin and a bottle of tap water with 2% saccharin for 4 wk. Wild-type and *Prkce*$^{-/-}$ mice showed similar levels of nicotine consumption (Fig. 1*A*) and preference (Fig. 1*B*) during the first week. Thereafter, wild-type mice progressively increased their consumption of nicotine by 26% over the final 3 wk, whereas *Prkce*$^{-/-}$ mice maintained a constant level of intake [F_{genotype} (1, 135) = 17.39, P = 0.0001; F_{week} (3, 135) = 6.13, P = 0.006; $F_{\text{genotype} \times \text{week}}$ (3, 135) = 3.37, P = 0.02]. *Prkce*$^{-/-}$ mice also showed lower nicotine preference than wild-type mice did during this time [F_{genotype} (1, 135) = 4.46, P = 0.04; F_{week} (3, 135) = 2.26, P = 0.08; $F_{\text{genotype} \times \text{week}}$ (3, 135) = 1.91, P = 0.13]. We measured nicotine clearance in *Prkce*$^{-/-}$ and wild-type mice to assess whether differences in metabolism contributed to differences in nicotine consumption. There was no difference in plasma levels of nicotine (Fig. 1*C*) between genotypes after a s.c. injection of 3.0 mg/kg nicotine [F_{genotype} (1, 20) = 0.019, P = 0.89; F_{time} (3, 20) = 12.66, P < 0.0001; $F_{\text{genotype} \times \text{time}}$ (3, 20) = 0.34, P = 0.80], indicating that genotype differences in voluntary nicotine intake were not related to differences in nicotine pharmacokinetics.

We next used a conditioned place preference paradigm to determine whether *Prkce*$^{-/-}$ mice show altered nicotine reward (Fig. 1*D*). In this paradigm, mice are given nicotine and saline, each paired with a distinct context. After pairing, the mice are allowed to explore both contexts. If the mice spend more time in the nicotine-paired context, then nicotine is interpreted as being rewarding. When we paired saline with both contexts in a group of *Prkce*$^{-/-}$ and wild-type mice, both wild-type and *Prkce*$^{-/-}$ mice did not show a preference for either context (P > 0.05, one-

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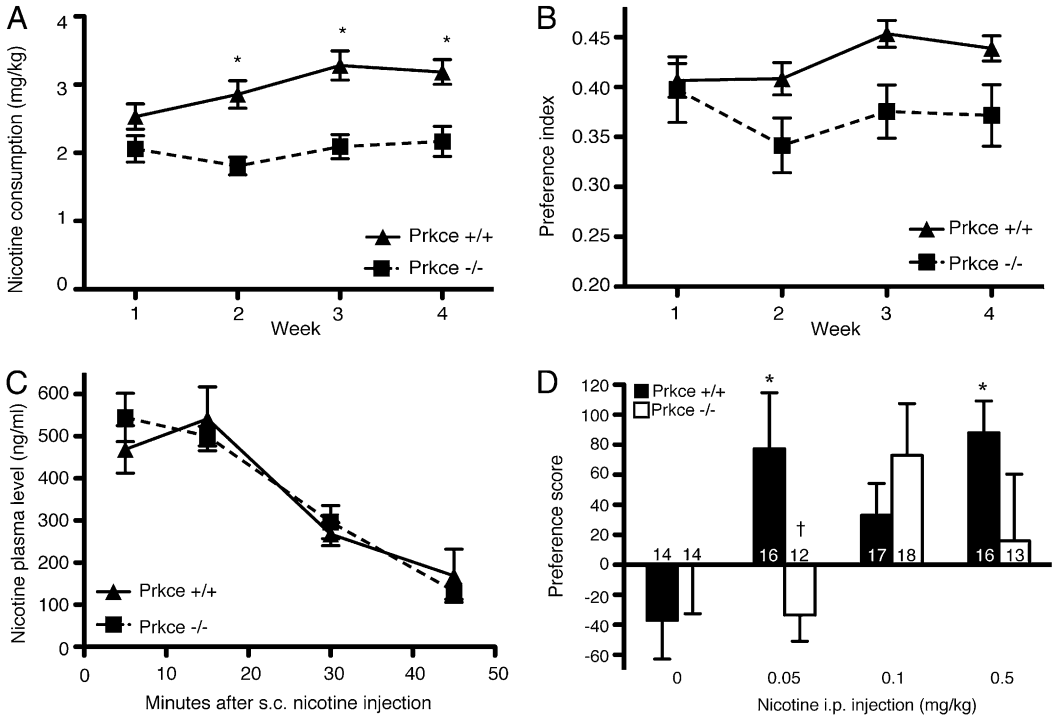

Fig. 1. Reduced nicotine consumption and preference in mice lacking PKCε. (*A*) *Prkce*$^{-/-}$ mice (n = 23) consumed less 15 μg/mL nicotine solution than wild-type mice did (*Prkce*$^{+/+}$; n = 24) during weeks 2–4. *$P < 0.001$ compared with wild-type mice on same week. (*B*) *Prkce*$^{-/-}$ mice showed decreased preference for nicotine compared with wild-type mice. (*C*) Plasma nicotine clearance after a s.c. injection of 3 mg/kg nicotine did not differ between genotypes (n = 2–6 mice per genotype per time point). (*D*) Wild-type mice developed nicotine conditioned place preference for 0.05 mg/kg i.p. nicotine, whereas *Prkce*$^{-/-}$ mice did not. *$P < 0.05$ compared with saline-treated wild-type mice by one-way ANOVA and Dunnett's post hoc analysis; $^{\dagger}P < 0.05$ compared with *Prkce*$^{-/-}$ mice at the same dose by two-way ANOVA and Tukey's post hoc analysis. Numbers in the bars indicate the number of mice in each group.

sample t test). When we conditioned separate groups of mice with 0.05, 0.1, or 0.5 mg/kg nicotine and saline, wild-type mice showed a significant preference for the chamber paired with 0.05 mg/kg nicotine [$F_{genotype}$ (1, 112) = 1.47, P = 0.228; F_{dose} (3, 112) = 2.36, $P < 0.076$; $F_{genotype \times dose}$ (3, 112) = 3.04, P = 0.032]. When analyzed separately by genotype, wild-type mice showed a nicotine preference [$F(3, 59) = 4.07, P = 0.01$] that was significant at 0.05 and 0.5 mg/kg nicotine ($P < 0.05$ by Dunnett's test compared with vehicle control). By contrast, *Prkce*$^{-/-}$ mice failed to show a significant place preference for nicotine at any of the doses tested [$F(3, 53) = 1.78, P = 0.16$]. These data suggest that *Prkce*$^{-/-}$ mice experience less nicotine reward than wild-type mice do.

Nine different nAChR subunits are found in the mammalian brain (α_{2-7} and β_{2-4}), and nAChRs can be either heteromeric, containing α- and β-subunits, or homomeric, comprising α_7-subunits only (26). Heteromeric nAChRs, but not homomeric α_7 nAChRs, are critical for nicotine consumption (27) and reward (28) in mice. Using quantitative PCR, we measured mRNA levels of common subunits in heteromeric nAChRs in the ventral midbrain and striatum. We found that, compared with wild-type mice, *Prkce*$^{-/-}$ mice showed a 26–28% reduction in α_6 (P = 0.03) and β_3 (P = 0.01) mRNA in the striatum and a 22% decrease in both (P = 0.04) transcripts in the ventral midbrain (Table 1). There were no genotype differences in α_3, α_4, α_5, and β_2 transcripts in the ventral midbrain or in α_4, α_5, and β_2 transcripts in the striatum (Table 1).

To assess whether the decrease in α_6 and β_3 mRNA in *Prkce*$^{-/-}$ mice resulted in a functional deficit, we measured cholinergic control of dopamine release in the NAc core, which depends on α_6* nAChRs. Striatal cholinergic interneurons facilitate dopamine release in the NAc when ventral tegmental area (VTA)

Table 1. Relative gene expression levels of nAChR subunits in wild-type and *Prkce*$^{-/-}$ mouse brain regions by quantitative PCR

nAChR subunit	β_2	β_3	α_3	α_4	α_5	α_6
Ventral midbrain						
Prkce$^{+/+}$	1.00 ± 0.11	1.00 ± 0.08	1.00 ± 0.15	1.00 ± 0.07	1.00 ± 0.07	1.00 ± 0.07
Prkce$^{-/-}$	0.96 ± 0.08	0.78 ± 0.06*	0.82 ± 0.07	0.90 ± 0.07	0.91 ± 0.06	0.78 ± 0.05*
Striatum						
Prkce$^{+/+}$	1.00 ± 0.05	1.00 ± 0.06	—	1.00 ± 0.12	1.00 ± 0.06	1.00 ± 0.08
Prkce$^{-/-}$	1.03 ± 0.06	0.74 ± 0.06*	—	0.94 ± 0.13	0.95 ± 0.09	0.72 ± 0.09*

Results for each subunit were normalized to GAPDH mRNA detected in the same sample and then were normalized to the average value for wild-type (*Prkce*$^{+/+}$) samples from the same brain region. n = 5–8 mice per condition.

*$P < 0.05$ by unpaired, two-tailed t tests. α_3 mRNA was not assessed in the striatum because α_3-subunits are not found in nAChRs on dopaminergic terminals in that brain region (45).

dopamine neurons fire at a low frequency, but they limit release when dopamine neurons fire at a high frequency because of short-term synaptic depression (29). During presentation of a reward or reward-related cue, cholinergic neurons, which are tonically active, decrease their rate of firing, thereby alleviating postsynaptic depression at dopaminergic terminals and permitting increased dopamine release. A similar increase in dopamine release can be produced by desensitizing all nAChRs on dopamine terminals with nicotine, antagonizing all nAChRs with mecamylamine (29), antagonizing $\alpha_4\beta_2$* nAChRs with dihydro-β-erythroidine (DHβE), or specifically blocking α_6* nAChRs with the antagonist α-conotoxin MII (αCTXMII) in the NAc (30). We used in vitro fast-scan cyclic voltammetry in the NAc core to determine whether there was a deficit in α_6* regulation of dopamine release in *Prkce*$^{-/-}$ mice. As expected (30), when αCTXMII was applied to brain slices from wild-type mice, there was a reduction in the average peak extracellular dopamine concentration ($[DA]_o$) in the NAc core during low-frequency electrical stimulation (one pulse) and an increase in $[DA]_o$ during high-frequency stimulation (four pulses at 100 Hz) (Fig. 2 *A* and *D*). Addition of αCTXMII was substantially less effective in raising this percentage in *Prkce*$^{-/-}$ slices (Fig. 2 *B* and *D*) compared with wild-type slices. To compare across treatment groups, we calculated the percentage increase in $[DA]_o$ evoked by four pulses versus one pulse (Fig. 2*E*). The percentage increase was greater in wild-type slices than in *Prkce*$^{-/-}$ slices after treatment with αCTXMII, whereas addition of the nicotinic antagonist DHβE, which primarily inhibits $\alpha_4\beta_2$* nicotinic receptors, further increased this difference in *Prkce*$^{-/-}$ but not in wild-type slices [F_{genotype} (1, 42) = 4.14, P = 0.05; $F_{\text{treatment}}$ (2, 42) = 96.66, $P < 0.0001$; $F_{\text{genotype} \times \text{treatment}}$ (2, 42) = 9.97, $P = 0.0003$]. Because α_6* nAChRs are more sensitive to nicotine than other nAChR subtypes are (31), we investigated whether nicotine is less effective in increasing $[DA]_o$ with high-frequency stimulation in *Prkce*$^{-/-}$ compared with wild-type slices. We found that low doses of nicotine (100–500 nM) produced less of a difference in $[DA]_o$ measured after one and four pulses in *Prkce*$^{-/-}$ than wild-type slices did [Fig. 2*F*; $F_{\text{nicotine dose}}$ (4, 56) = 162.15, $P < 0.001$; F_{genotype} (1, 56) = 2.03, $P = 0.18$; $F_{\text{genotype} \times \text{nicotine dose}}$ (4, 56) = 8.30, $P < 0.001$]. These data indicate that the reduced level of nAChR α_6-subunit mRNA in *Prkce*$^{-/-}$ mice is func-

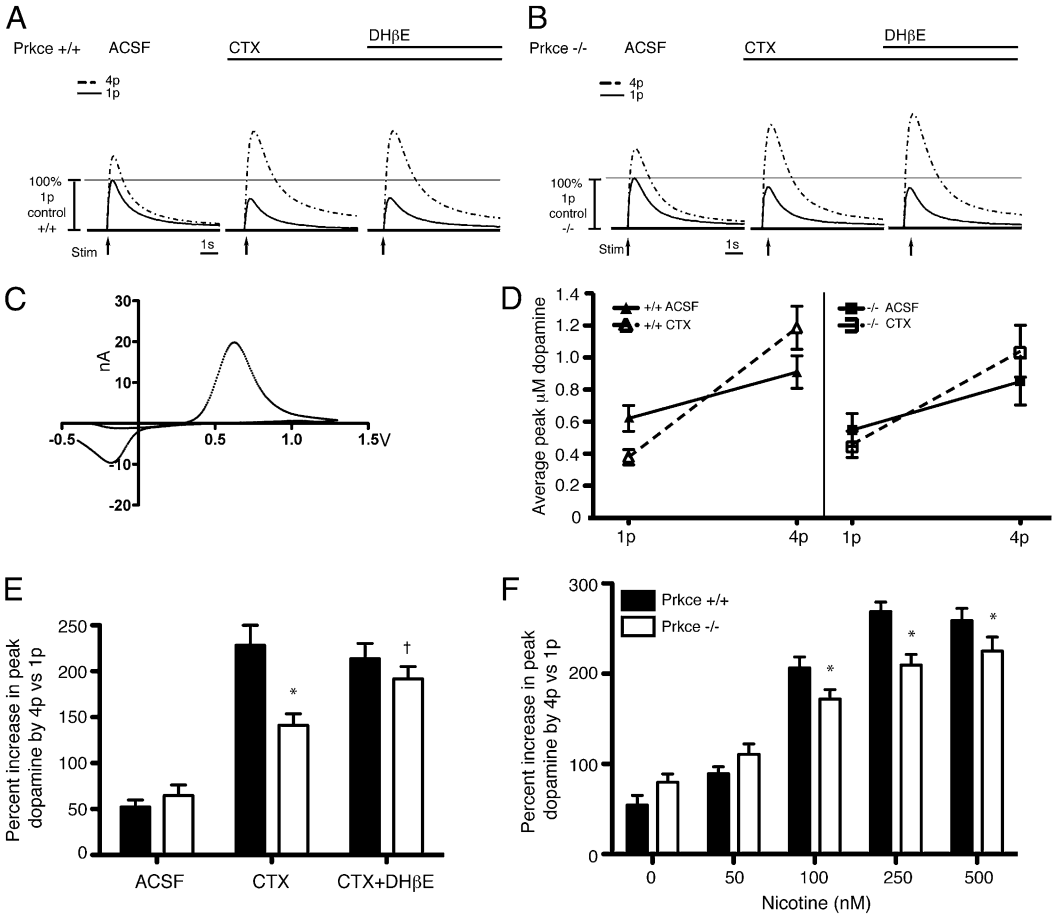

Fig. 2. Decreased function of α_6* nAChRs in *Prkce*$^{-/-}$ NAc core. (*A* and *B*) Average profiles of $[DA]_o$ after stimulation by one pulse (1p) and four pulses (4p) (100 Hz, 0.6 mA) in control ACSF (left traces), after addition of αCTXMII (30 nM, center traces), and after αCTXMII plus DHβE (100 nM, right traces) for wild-type (*A*; n = 11) and *Prkce*$^{-/-}$ (*B*; n = 12) brain slices. (*C*) Typical dopamine voltammogram elicited by one 0.6-mA pulse of 250-μs duration. (*D*) Peak $[DA]_o$ levels after 1p and 4p stimulation in control ACSF (solid lines) or in the presence of 30 nM αCTXMII (dashed lines) in wild-type (+/+) and *Prkce*$^{-/-}$ (–/–) NAc core. The slopes of the lines for αCTXMII versus ACSF were different ($P < 0.05$) for wild-type but not for *Prkce*$^{-/-}$ slices. (*E*) Enhancement of peak $[DA]_o$ by αCTXMII or by αCTXMII plus DHβE shown as the percentage increase in peak $[DA]_o$ evoked by 4p over 1p stimulation. αCTXMII increased peak $[DA]_o$ evoked by 4p to a lesser extent in *Prkce*$^{-/-}$ than in wild-type NAc. *$P < 0.05$ compared with αCTXMII-treated wild-type slices by Bonferroni post hoc analysis. Compared with αCTXMII alone, addition of DHβE further increased peak $[DA]_o$ evoked by 4p in *Prkce*$^{-/-}$ but not in wild-type NAc. †$P < 0.05$ compared with αCTXMII-treated *Prkce*$^{-/-}$ slices by Bonferroni post hoc analysis. (*F*) Low doses of nicotine (100–500 nM) increased peak $[DA]_o$ evoked by 4p versus 1p stimulation to a lesser extent in *Prkce*$^{-/-}$ than in wild-type NAc. *$P < 0.05$ compared with nicotine-treated wild-type slices by Tukey's post hoc analysis.

tionally significant and reduces the ability of nicotine to enhance dopamine release.

Discussion

In this study, we found that *Prkce*$^{-/-}$ mice show decreased nicotine consumption and preference compared with wild-type littermates. These findings are similar to those we have previously reported for self-administration of ethanol (21, 22, 32). We previously found that *Prkce*$^{-/-}$ mice do not show altered preference for saccharin or quinine solutions compared with wild-type mice (32), indicating that differences in nicotine and ethanol consumption are not attributable to differences in taste reactivity between wild-type and *Prkce*$^{-/-}$ littermates. We also found that *Prkce*$^{-/-}$ mice failed to develop a conditioned place preference for nicotine. In contrast, wild-type mice developed a conditioned place preference at 0.05 and 0.5 mg/kg nicotine, although not at an intermediate (0.1 mg/kg) nicotine dose. A similar biphasic dose–response for nicotine in place-conditioning studies has been reported previously in mice across different studies (28, 33, 34).

In addition to impaired nicotine intake and conditioned place preference, we found that α_6* nAChR mRNA was down-regulated in the VTA and NAc of *Prkce*$^{-/-}$ mice. This down-regulation is functionally significant because it was associated with decreased α_6* nAChR regulation of evoked dopamine release in the NAc of *Prkce*$^{-/-}$ mice. Given that dopaminergic neurons of the VTA that extend projections to the NAc are an integral part of the reward system (35), our findings suggest that decreased α_6* nAChR function in *Prkce*$^{-/-}$ mice is causally related to their reduced nicotine self-administration and reward.

α_6* nAChRs are expressed by the cell bodies of dopaminergic neurons in the VTA and by their terminals in the NAc. Because of this highly localized pattern of expression, these receptors are poised to play a key role in regulating drug intake and reward (36, 37). Targeted gene deletion of α_6 abolishes and selective reexpression of α_6 in the VTA restores nicotine self-administration in mice (27). In addition, inhibition of α_6* nAChRs on the terminals of VTA dopaminergic neurons through administration of αCTXMII into the NAc decreases the breaking point for nicotine responding in rats (38). As demonstrated here, down-regulation of α_6* nAChRs impairs nicotine enhancement of evoked dopamine release in mouse NAc slices, whereas in vivo administration of αCTXMII into the VTA of rats attenuates nicotine-induced dopamine release in the NAc (39). Altogether, these results indicate that α_6* nAChRs expressed by cell bodies of VTA dopaminergic neurons and their terminals in the NAc regulate both nicotine self-administration and nicotine-stimulated dopamine release.

α_6* nAChRs have also been implicated in self-administration of ethanol. Injection of αCTXMII into the VTA decreases ethanol consumption in rats and ethanol-induced locomotor stimulation in mice and attenuates the increase in $[DA]_o$ in the NAc resulting from a 1.75 g/kg i.p. injection of ethanol (40). We previously found that systemic administration of 1–2 g/kg ethanol does not increase $[DA]_o$ in the NAc of *Prkce*$^{-/-}$ mice (21). We propose that down-regulation of α_6-subunit mRNA and diminished α_6* nAChR function in *Prkce*$^{-/-}$ mice could explain this finding.

α_6* nAChRs in the NAc modulate reward signaling by regulating dopamine release from terminals of VTA dopaminergic neurons (30). During presentation of rewards or reward-related cues, cholinergic interneurons of the NAc decrease their firing, resulting in decreased cholinergic tone at dopaminergic terminals. This event amplifies the contrast in peak $[DA]_o$ levels achieved during low- versus high-frequency firing of VTA dopaminergic neurons, which is thought to signal salience for rewards and associated cues (41). Inhibition of α_6* nAChRs by nicotine-induced desensitization also diminishes cholinergic tone at these terminals and is postulated to be a critical factor in nicotine reward (29). We found that *Prkce*$^{-/-}$ mice show reduced nicotine regulation of dopamine release, which may contribute to their reduced nicotine conditioned place preference and nicotine intake. The impairment in α_6* nAChR function in *Prkce*$^{-/-}$ mice is not severe enough to affect all rewards because *Prkce*$^{-/-}$ mice do not show decreased operant responding for sucrose. It is also unlikely to affect self-administration of drugs that increase $[DA]_o$ by mechanisms that do not depend on increased firing of dopaminergic neurons, such as cocaine, which we previously found increases accumbal $[DA]_o$ similarly in *Prkce*$^{-/-}$ and wild-type mice (21).

In summary, we find that deletion of PKCε decreases nicotine self-administration and reward, reduces α_6-subunit mRNA in the ventral midbrain and striatum, and impairs control of dopamine release by α_6* nAChRs in the NAc. These findings suggest that inhibitors of PKCε could be useful in reducing nicotine intake. Moreover, given that α_6* nAChRs play an important role in regulating ethanol self-administration and ethanol-induced increases in $[DA]_o$ in NAc, inhibiting PKCε could provide a strategy for the treatment of comorbid nicotine and alcohol addictions.

Materials and Methods

Animals. PKCε knockout (*Prkce*$^{-/-}$) mice were generated as previously described (42). The mutant PKCε null allele was maintained on an inbred 129S4/SvJae background. *Prkce*$^{+/-}$ 129S4/SvJae mice were crossed with C57BL6/J mice to generate *Prkce*$^{+/-}$ C57BL/6J × 129S4/SvJae F1 hybrid breeding pairs, which were used to generate F2 hybrid *Prkce*$^{-/-}$ and wild-type littermates for experiments. Male mice were a minimum of 8 wk old and a maximum of 18 wk old for all studies. Mice were housed in a 12 h/12 h light/dark cycle room in groups of five or fewer per cage. Food and water were provided ad libitum. All mice were drug-naïve for each experiment. All procedures were conducted under guidelines established by the Gallo Center Institutional Animal Care and Use Committee and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (43).

Reagents. Nicotine hydrogen tartrate salt was purchased from Sigma-Aldrich and dissolved in 0.9% normal saline. All concentrations of nicotine reported are for the free base. DHβE and αCTXMII were purchased from Tocris Bioscience and dissolved in 0.9% normal saline. All other reagents were purchased from Sigma-Aldrich.

Nicotine Metabolism. Mice were injected s.c. with 3.0 mg/kg nicotine, and trunk blood was collected. Plasma was extracted and analyzed for nicotine concentration at the Clinical Pharmacology Laboratory at San Francisco General Hospital, University of California, San Francisco. Nicotine concentration was determined by gas chromatography with nitrogen-phosphorus detection (44). The internal standard, 5-methylnicotine, was obtained from Peyton Jacob, III, Division of Clinical Pharmacology of the Department of Medicine, University of California, San Francisco. Data were analyzed by ANOVA with a repeated measure for time and a between-subjects factor for genotype.

Oral Nicotine Consumption. Voluntary nicotine was assessed with a two-bottle choice paradigm, a widely used method to measure consumption and preference in mice (23–25). Mice were individually housed and given 24-h access to a water bottle with 2% saccharin and a water bottle with 15 μg/mL nicotine and 2% saccharin for 31 d. Bottles were weighed every 2 d, and the positions of the bottles were reversed after each weighing. The solutions were refreshed every week, and both bottles were covered with aluminum foil to prevent photodegradation of nicotine. Mice were weighed every 4 d and were never deprived of food or water. Nicotine consumption was calculated as mg/kg per d, and preference was calculated as the volume of nicotine solution consumed divided by the volume of total fluid consumed multiplied by 100. Data were analyzed by ANOVA with a repeated measure for week and a between-subjects factor for genotype. Where there was a significant genotype × week interaction, differences between pairs of means were analyzed post hoc by a Bonferroni multiple-comparisons test.

Conditioned Place Preference. The experimental design was unbiased and was based on the method of Brunzell et al. (33). We used two-chamber apparatuses, each consisting of a 27.3 × 27.3 cm Med Associates open-field chamber with custom acrylic floors of differing textures and a central partition with a manual door. On the Wednesday to Friday of the week before

the start of the conditioning procedure, mice were habituated to the experimental room for 4–5 h per d. During these three sessions, mice were handled ~5 min per d and were held and turned on their backs as if to receive an i.p. injection. The following Monday, mice were given an i.p. saline injection and placed individually in one apparatus with access to both floor textures for 15 min to determine baseline floor preference. Mice with a baseline preference greater than 70% for one side were removed from the study. A total of 133 mice were tested, and 13 (7 wild type and 6 knockout) were removed because of baseline preferences greater than 70%. On Tuesday through Friday, mice underwent two conditioning sessions, one in the morning and one in the afternoon. During each 30-min session, mice received either i.p. saline or nicotine and were immediately confined to one chamber. Mice received the alternate injection and were confined to the other chamber in the afternoon. The pairing of treatment condition and floor texture was randomized and counterbalanced. On Friday, mice were given i.p. saline and given access to both chambers for 15 min. Data are presented as preference scores calculated as time spent in the nicotine-paired chamber after conditioning minus time spent in the nicotine-paired chamber during baseline.

Quantitative PCR. Mice were killed, and brain regions were immediately dissected and frozen in liquid nitrogen until processing. RNA was isolated with an RNeasy Plus Miniprep Kit (Qiagen). RNA samples were treated with RQ1 DNase (Promega) according to the manufacturer's instructions. cDNA was synthesized from 500 to 1,000 ng of total RNA with reverse transcription reagents from Applied Biosystems. TaqMan quantitative PCR was performed with standard thermal-cycling conditions on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems). The predesigned probe and primer sets for the mouse α_3 (Mm00520145_m1), α_4 (Mm00516561_m1), α_5 (Mm00616329_m1), α_6 (Mm00517529_m1), β_2 (Mm00515323_m1), and β_3 (Mm00532602_m1) nicotinic subunits were purchased from Applied Biosystems. A mouse GAPDH probe and primer set (Applied Biosystems) was used as a housekeeping gene control. Negative controls with no cDNA were performed for all reactions, and all reactions were repeated three to six times. Relative amplicon quantification was calculated by normalizing target Ct values to GAPDH. Relative gene expression between PKCε knockout and wild-type mice was calculated by normalizing these values to the average of PKCε wild-type values for each receptor subunit in each brain region. Data were analyzed by two-tailed *t* tests.

Fast-Scan Cyclic Voltammetry. Mice were deeply anesthetized with 50 mg/kg i.p. sodium pentobarbital and perfused with ice-cold sucrose solution (87 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 0.50 mM $CaCl_2$, 7 mM MgCl, and 75 mM sucrose). The brains were removed, and 300-μm coronal slices were cut with a Leica Microsystems VT1200S Vibratome. Slices were maintained at 32 °C in bicarbonate-buffered artificial cerebrospinal fluid (ACSF) containing 2.4 mM $CaCl_2$. $[DA]_o$ was monitored with 7-μm carbon fiber (Goodfellow Corporation) microelectrodes (tip length 50–100 μm, manufactured in house). Voltammetric data were obtained by using TarHeel CV data-acquisition hardware and software (University of North Carolina, Chapel Hill, NC). The scanning voltage was a triangular waveform (−0.4 to +1.3V versus Ag/AgCl) generated at a scan rate of 400 V/s and a sampling frequency of 10 Hz. Stimulus pulses (250-μs duration, 0.6 mA) were generated out of phase with voltammetry scans by tungsten matrix electrodes placed ~100–200 μm dorsal to the recording electrode (115-μm spacing; FHC). Recording sites were in the NAc core immediately ventral to the anterior commissure. Dopamine release evoked by this type of stimulation has been shown to be tetrodotoxin-sensitive and calcium-dependent (29, 30). Stimulus pulses were repeated at 3-min intervals. Stimuli consisted of alternating between single pulses and four pulses at 100 Hz (29, 30). αCTXMII and DHβE were bath-applied for 15 min, and nicotine was bath-applied for 5 min before collecting data.

Dopamine was identified by its characteristic oxidation and reduction peak potentials at 0.6 and −0.2 mV versus Ag/AgCl, respectively. Evoked peak oxidation and reduction currents were converted to $[DA]_o$ by using calibration curves generated by scanning known concentrations of dopamine (0.5–2.0 μM) in ACSF at the end of each experimental day. Sample size, *n*, represents the number of recording sites collected from a minimum of seven mice per genotype, with a maximum of two slices per mouse. The change in peak $[DA]_o$ measured after one and four stimulation pulses was calculated by linear regression, and slope values for each drug treatment (ACSF versus αCTXMII) were compared within each genotype by two-tailed *t* tests. The ratios of peak $[DA]_o$ evoked by four pulses versus one pulse after the different drug treatments were compared by two-way ANOVA and a post hoc Bonferroni or Tukey's multiple-comparisons test with a between-subjects factor for genotype and a repeated measure for drug treatment.

Statistical Analysis. All data are shown as mean ± SEM. Results were examined for normality by using a D'Agostino–Pearson omnibus normality test. Outliers were identified by the Grubb's test. Data were analyzed by ANOVA with indicated post hoc comparisons, Student's *t* test, or linear regression with Prism 5.0c (GraphPad Software) and SigmaPlot 11 (Systat Software Inc.). Differences were considered significant when $P < 0.05$.

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