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Effect of the α4β2* nicotinic acetylcholine receptor partial agonist varenicline on dopamine release in β2 knock-out mice with selective re-expression of the β2 subunit in the ventral tegmental area

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ABSTRACT

We studied the effects of 1 mg/kg doses of nicotine and the α4β2* nicotinic acetylcholine receptor (nAChR) partial agonist, varenicline, on extracellular dopamine (DA) levels in the nucleus accumbens (NuAcc) of lentivirally vectorized male mice. Three separate experimental groups were injected with a lentiviral vector transducing the ventral tegmental area (VTA): wild-type C57BL/6J mice with a vector expressing eGFP only, β2 knock-out mice (β2KO) with the eGFP-only vector, and β2KO mice with a bicistronic vector reintroducing β2 and eGFP into the VTA as recently described (Maskos et al., 2005). Our results suggest that the neurochemical effects of varenicline as measured by using microdialysis in awake, freely moving mice are mainly mediated via β2* nAChR subunits localized in the VTA.

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1. Introduction

The recently introduced α4β2* neuronal nicotinic acetylcholine receptor (nAChR) partial agonist varenicline has demonstrated increased efficacy for smoking cessation over the other first line treatments bupropion and nicotine replacement therapy (Lancaster et al., 2008). The improved efficacy is likely due to its dual mechanism of action: when quitting smoking, varenicline can reproduce the subjective effects of smoking by partially activating α4β2* nAChRs, and can prevent full activation of these receptors by nicotine when smoking again (Rollema et al., 2007b). Since varenicline binds selectively with sub-nanomolar affinity to α4β2* nAChRs, it is believed that the varenicline-induced increase in dopamine (DA) release from rat nucleus accumbens (Coe et al., 2005; Rollema et al., 2007a) is mediated via interactions with α4β2* nAChRs in the ventral tegmental area (VTA), similar to nicotine-evoked DA release. Convincing evidence for the crucial role of mesolimbic β2* nAChRs in the actions of nicotine came from our previous study in which elimination of the β2 subunit attenuated pharmacological and behavioral effects of nicotine, while targeted expression of the β2 subunit in the VTA of β2 knock-out (β2KO) mice reinstated nicotine self-administration and nicotine-induced DA release (Maskos et al., 2005). The present study examined whether the effects of varenicline on mesolimbic DA release in mice are also dependent on activation of β2 subunit containing nAChRs in the VTA, using the same protocols as in our nicotine study (Maskos et al., 2005).

2. Methods

2.1. β2KO mice and β2KO mice with re-expressed®2

2.1.1. Lentiviral vectors

The lentiviral vectors used in this work were derived from the pHR' expression vectors first described by Naldini and colleagues (Naldini et al., 1996a,b) with several subsequent modifications. Vectors are based on the previously described pTRIPΔU3 (Sirven et al., 2001), in which the U3 region of the 3′ long terminal repeat (LTR) was deleted (ΔU3), rendering the integrated viral DNA replication-incompetent. The central polypurine tract (cPPT) (Charneau et al., 1992) and the central termination sequence (CTS) (Charneau et al., 1994) of the wild-type HIV-1 were added, creating the 99-base pair central DNA "flap" (Zennou et al., 2000) that enhances infection of non-dividing cells by facilitating transport of the preintegration complex through the nuclear membrane pores. The woodchuck hepatitis B virus post-transcriptional regulatory element (WPRE) was added to increase RNA stability and transgene expression (Zufferey et al., 1999).

2.1.2. Re-expression lentivector

The re-expression lentivector [PGK-Beta2-Ires2-eGFP], see Fig. 1A, is a bicistronic β2-IRES2-eGFP construct, previously described (Maskos et al., 2005). Briefly,

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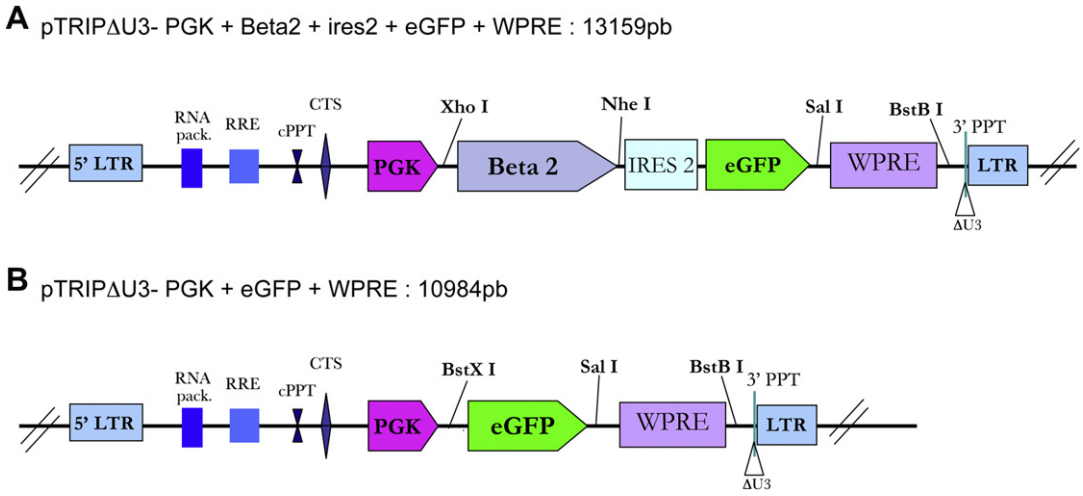

Fig. 1. Maps of lentiviral expression vectors. Diagrams of the two lentiviral vectors used in this study, between and including the LTR regions. *LTR*, long terminal repeat; *RNA pack.*, genomic RNA packaging signal; *RRE*, *rev* response element; *cPPT*, central polypurine tract; *CTS*, central termination sequence; *PGK*, promoter of the mouse phosphoglycerate kinase gene; *IRES2*, internal ribosome entry sequence; *eGFP*, enhanced green fluorescent protein; *WPRE*, woodchuck hepatitis B virus post-transcriptional regulatory element; *3'PPT*, 3′-polypurine tract; Δ*U3*, deletion of the U3 portion of 3′-LTR.

the mouse phosphoglycerate kinase (PGK) promoter was PCR amplified from a PGK-nls lacZ expression vector, M48, and ligated into pTRIPΔU3. To generate the β2-IRES2-eGFP construct a cloning site was created in the pIRES2-EGFP expression plasmid (Clontech) by mutagenesis (QuikChange, Stratagene), and the wild-type mouse β2 subunit, containing a consensus Kozak translation initiation site (Maskos et al., 2002), was then ligated into plasmid pIRES-EGFP. The β2-IRES2-eGFP cassette was then ligated into the pTRIPΔU3-PGK vector using *Xho*I-*Sal*I sites. Finally, the WPRE sequence was added. The PGK-eGFP control lentivector, Fig. 1B, is identical to the bicistronic version, but lacks the β2-IRES2 portion.

2.1.3. Lentivirus production

HEK-293T cells (at 80–85% confluence) were co-transfected with the vector plasmid (either the pTRIPΔU3 [PGK-Beta2-Ires2-EGFP] or the pTRIPΔU3 [U6-shRNA-Ubiq-EGFP]), together with a packaging plasmid (CMVΔ8.9) and an envelope plasmid (CMV-VSVg), using Lipofectamin Plus (Invitrogen) according to the manufacturer's instructions. Two days after transfection, viral particles were harvested from the supernatant, treated with DNaseI (1/5000; 0.2 μl/ml) and $MgCl_2$ 2 M (1 μl/ml), filtered through 0.45 μm pores and concentrated by ultracentrifugation at 24.000 rpm for 2 h ($\omega^2t = 4.55$). Viruses were stored in 10 μl aliquots at −80 °C.

Viral titers were determined by quantification of the p24 capsid protein using an HIV-1 p24 antigen immunoassay (Beckman Coulter, Villepinte, France). As all lentivectors contained the eGFP sequence, transfecting units were estimated by Fluorescence Activated Cell Sorting (FACS) after infection of HEK-293T cells with increasing doses of each viral production. Immediately before stereotaxic injections lentiviruses were diluted in PBS to achieve a dose of injection of $0.5–1x10^6$ TU in 2 μl (equivalent to 150 ng p24 protein for bicistronic viruses, 70–80 ng p24 for monocistronic viruses).

2.1.4. Mice

β2KO mice were backcrossed to the C57BL/6J@Ico strain for 19 generations. β2KO and WT (C57BL/6J@Ico) mice, 7 weeks old, were kept under standard laboratory conditions with ad libitum food and water and in a 12 h light/dark period (on at 8:00 AM).

2.1.5. Stereotaxic procedure

Mice aged 8–10 weeks (weight 25–30 g) were deeply anaesthetized using ketamine/xylazine and introduced into a stereotaxic frame adapted for mice (Cunningham and McKay, 1993). 2 μl of virus were injected bilaterally, for VTA at the following coordinates (Paxinos and Franklin, 2001): −3.4 mm antero-posterior, ±0.5 mm lateral, and −4.3 mm dorso-ventral. All procedures were in accordance with European Commission directives 219/1990 and 220/1990, and approved by Animalerie Centrale and Médecine du Travail, Institut Pasteur. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

2.2. In vivo microdialysis

The effects of nicotine and varenicline on basal DA release were determined by intra-cerebral microdialysis in the nucleus accumbens of awake, freely moving mice as previously described (Maskos et al., 2005). Briefly, mice were anaesthetized with chloral hydrate (400 mg/kg, i.p.), placed in a stereotaxic frame and concentric dialysis probes (cuprophan fiber, 1.0 mm, outer diameter 0.30 mm) were implanted unilaterally into the ventral striatum. Twenty hours after surgery the probe was continuously perfused with an artificial CSF solution (composition in mmol/L: NaCl 147, KCl 3.5, $CaCl_2$ 1.26, $MgCl_2$ 1.2, NaH_2PO_4 1.0, pH7.4) at a flow rate of 1.5 μL/min using a CMA/100 pump (Carnegie Medicin, Stockholm, Sweden) and dialysates were collected at 15 min intervals. Measurements of dialysate DA concentrations ([DA]ext) were made by HPLC (XL-ODS 4.6 × 75 mm, 3 μm column, Beckman) with amperometric detection (1049A, Hewlett–Packard, Les Ulis, France). Eight samples were collected to determine basal values (means ± SEM) before systemic administration of nicotine or varenicline. The limit of sensitivity for [DA]ext was 0.5 fmol per sample (signal-to-noise ratio = 2). Mice were injected *i.p.* with drugs dissolved in 0.9% NaCl (10 ml/kg) and control animals received an i.p. injection of 0.9% NaCl (10 ml/kg). Responses to drug administration were determined over a 120-min period. Probe placement was verified histologically after completion of the microdialysis experiment according to Bert et al. (2004)

2.3. Drugs and chemicals

Varenicline tartrate (MW: 361; MW base: 211; Pfizer, Groton, CT, USA) and nicotine bitartrate (MW: 462; MW base:162; Sigma–Aldrich, St Quentin Fallavier, France) were dissolved in 0.9% NaCl. Both compounds were dosed at 1 mg/kg active drug (free base). All chemicals were analytical grade and purchased from Sigma–Aldrich, St. Quentin, Fallavier, France.

2.4. Statistical analyses

Data were analyzed by repeated measures ANOVAs using StatView version 5.0 (Abacus Concepts, Berkeley, CA, USA), followed by post-hoc Student-T test. The level of statistical significance was set at $P < 0.05$.

3. Results

Three groups of mice were vectorized with a lentiviral vector as previously described (Maskos et al., 2005; Mameli-Engvall et al., 2006; Pons et al., 2008): WT mice obtained an injection of the eGFP-only expressing vector, Fig. 1B, and β2 knock-out (β2KO) mice

Table 1
Basal extracellular levels of dopamine (fmol/sample; Bo at t0) in the nucleus accumbens of wild-type (WT), β2 knock-out (KO) and β2 vectorized β2 knock-out (KO) mice (6–9 mice per group). No statistically significant differences were observed in the three groups of mice ($P > 0.05$). Stereotaxic coordinates from Bregma (in mm): anterior: +3.4; lateral: 0.7; ventral: −5.4.

	C57BL6 WT	β2KO	β2vect. KO
Bo	9.7	6.5	6.0
±SEM	1.1	1.5	1.0

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