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Opioid receptor types involved in the development of nicotine physical dependence in an invertebrate (*Planaria*) model

Robert B. Raffa [a,*], Steve Baron [a,b], Jaspreet S. Bhandal [a,b], Tevin Brown [a,b], Kevin Song [b], Christopher S. Tallarida [a,b], Scott M. Rawls [b]

[a] Department of Pharmaceutical Sciences, Temple University School of Pharmacy, Philadelphia, PA, USA
[b] Department of Pharmacology & Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA, USA

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ABSTRACT

Recent data suggest that opioid receptors are involved in the development of nicotine physical dependence in mammals. Evidence in support of a similar involvement in an invertebrate (*Planaria*) is presented using the selective opioid receptor antagonist naloxone, and the more receptor subtype-selective antagonists CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2) (μ, MOR), naltrindole (δ, DOR), and *nor*-BNI (norbinaltorphimine) (κ, KOR). Induction of physical dependence was achieved by 60-min pre-exposure of planarians to nicotine and was quantified by abstinence-induced withdrawal (reduction in spontaneous locomotor activity). Known MOR and DOR subtype-selective opioid receptor antagonists attenuated the withdrawal, as did the non-selective antagonist naloxone, but a KOR subtype-selective antagonist did not. An involvement of MOR and DOR, but not KOR, in the development of nicotine physical dependence or in abstinence-induced withdrawal was thus demonstrated in a sensitive and facile invertebrate model.

1. Introduction

The abuse of nicotine (continued use despite negative health consequences) is a multifaceted phenomenon involving several neurotransmitter systems. Proximal mediation of nicotine-induced effects occurs *via* the activation of ligand-gated nAChRs (nicotinic acetylcholine receptors) –– primarily homomeric α_7 and heteromeric $\alpha_4\beta_2$ subtypes (Millar and Gotti, 2009). Distal (behavioral) effects are mediated *via* downstream receptors for several neurotransmitters, such as catecholamines (*e.g.*, norepinephrine and dopamine), 5-HT (5-hydroxytryptamine, serotonin), GABA (γ-aminobutyric acid), glutamate, cannabinoids, hypothalamic hypocretin peptides, and endogenous opioids (Clarke and Reuben, 1996; De Vries and Schoffelmeer, 2005; Di Matteo et al., 1999; Fu et al., 2000; Hollander et al., 2008; Isola et al., 2009; Liechti and Markou, 2008; Maldonado et al., 2006; Marty et al., 1985; McGehee et al., 1995; Pontieri et al., 1996; Scherma et al., 2008; Wilkie et al., 1993; Yang et al., 1996).

A recent review of neurobiological mechanisms underlying nicotine dependence places particular emphasis on the endogenous opioid system (Berrendero et al., 2010). Evidence that this system has an important role in nicotine abuse includes: nicotine stimulates the release of endogenous opioid peptides, alters the expression of endogenous opioid peptides, and induces the dopamine release in nucleus accumbens, which is attenuated by opioid antagonists and in β-endorphin or enkephalin knockout mice (Berrendero et al., 2005; Britt and McGehee, 2008; Dhatt et al., 1995; Goktalay et al., 2006; Maisonneuve and Glick, 1999; Tanda and Di Chiara, 1998; Trigo et al., 2009). Of the opioid-receptor subtypes μ (MOR), δ (DOR), and κ (KOR), the μ-subtype has been most closely associated with nicotine-induced effects (reviewed in (Berrendero et al., 2010)). The involvement of κ- and δ-subtypes is less clear (Berrendero et al., 2010; Hahn et al., 2000; Heidbreder et al., 1996). Nicotine activates μ-opioid receptors in human anterior cingulate cortex (Scott et al., 2007), and smoking initiation, reward, and dependence have been linked to μ-opioid receptor polymorphisms (Perkins et al., 2008; Zhang et al., 2006). Studies in vertebrates (*e.g.*, (Balerio et al., 2004; Biala et al., 2005; Goktalay et al., 2006; Ise et al., 2000; Malin et al., 1993)), including knockout animals (Berrendero et al., 2002, 2005; Galeote et al., 2009; Trigo et al., 2009), suggest that abstinence-induced withdrawal from nicotine involves opioid receptors and that changes in locomotor activity are a manifestation of nicotine-induced modulation of opioid and other neurotransmitter systems related to nicotine physical dependence (Decker et al., 1995).

Although models of nicotine physical dependence and withdrawal in vertebrates are available (as reviewed in (Berrendero et al., 2010)), they are relatively effort- and time-intensive and they often require antagonists to precipitate quantifiable withdrawal. A simpler *in vivo* model would be advantageous. Following several pioneering contributions that paved the way to the recognition of planarians as a suitable animal in different experimental conditions (*e.g.*, (Carolei et al., 1975; Venturini et al., 1981, 1983)), we have shown that planarians

* Corresponding author at: Department of Pharmaceutical Sciences, Temple University School of Pharmacy, Philadelphia, PA 19140, USA. Tel.: +1 215 707 4976.
E-mail address: robert.raffa@temple.edu (R.B. Raffa).

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offer a simple model to study neurotransmitter processes related to drug use and abuse, including development of physical dependence and abstinence-induced or antagonist-induced withdrawal (reviewed in monograph (Raffa and Rawls, 2008)). We recently published the use of this model to study nicotine pharmacology (Rawls et al., 2011), including abstinence-induced nicotine withdrawal. Planarians have been established as a model to study opioid behavior pharmacology (Raffa and Rawls, 2008). We now describe the use of this model to study the involvement of opioid-receptor subtypes in the development of nicotine physical dependence and abstinence-induced withdrawal.

2. Methods

2.1. Subjects and compounds

Planarians (*Dugesia dorotocephala*) were purchased from Carolina Biological Supply (Burlington, NC, USA) and were tested on the same day or the day following receipt. Nicotine, mecamylamine, scopolamine, naloxone, CTAP, naltrindole, and *nor*-BNI were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Solutions were prepared daily in water (1 ml Amquel® per 1 gal water). All of the experiments were conducted using plastic Petri dishes that contained water or test compound(s) under standard laboratory conditions. Each of the experiments used independent groups of planarians and each planarian was used only once.

2.2. Behavioral experiments

Individual planarians (n = 5–13 per group) were pretreated in nicotine (100 μM), nicotine (100 μM) plus antagonist (scopolamine, 10 μM; mecamylamine, 50 μM; naloxone, 10 μM; CTAP, 10 μM; naltrindole, 10 μM; *nor*-BNI, 10 μM), previously shown to antagonize an agonist in this preparation, or water for 60 min. They were then placed individually into a Petri dish containing nicotine (100 μM), antagonist (the same concentration as used in pretreatment phase) or water for 5 min, and spontaneous locomotor velocity (*p*LMV) was quantified as the number of gridlines (0.5 cm apart) crossed or re-crossed over the five-minute observation interval (Raffa and Valdez, 2001).

2.3. Data analysis

Comparisons of group means ± the standard error of the mean (S.E.) were first evaluated by two-way ANOVA and then, if appropriate, by *post-hoc* analysis. Slopes were calculated using linear regression analysis [with 95% confidence limits]. In all cases, a value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Abstinence-induced nicotine withdrawal

Consistent with several previous reports (for example, (Raffa et al., 2001, 2003; Raffa and Valdez, 2001)), drug-naïve planarians displayed nearly constant (linear) *p*LMV of about 13–16 gridlines per minute when they were tested in water and nicotine-exposed planarians displayed an abstinence-induced withdrawal (*i.e.*, a significant reduction in *p*LMV when they were tested in water (slope = 16.69 [16.34–17.03]), but not when they were tested in the same concentration of nicotine (100 μM) (slope = 12.09 [10.62–13.55]) ($P < 0.05$) (Fig. 1)).

3.2. Nicotinic vs muscarinic AChRs

Co-incubation of planarians for 60 min with nicotine (100 μM) with the nicotinic AChR antagonist mecamylamine (50 μM) (slope = 9.50 [9.09–9.91]), which had no effect of its own, significantly ($P < 0.05$) reduced nicotine abstinence-induced withdrawal (slope = 15.13 [13.76–16.49]) (Fig. 2). Importantly, these planarians still displayed a linear *p*LMV over the five-min observation period. They resume normal *p*LMV after a short recovery period (Raffa and Rawls, 2008). Possible confounding interpretations, such as change in pH, osmolarity, *etc.*, were eliminated in previous studies (Raffa and Valdez, 2001).

In contrast to the nicotinic AChR antagonist, co-incubation with the muscarinic AChR antagonist scopolamine (10 μM) had no effect ($P > 0.05$) on subsequent abstinence-induced nicotine withdrawal (Fig. 3).

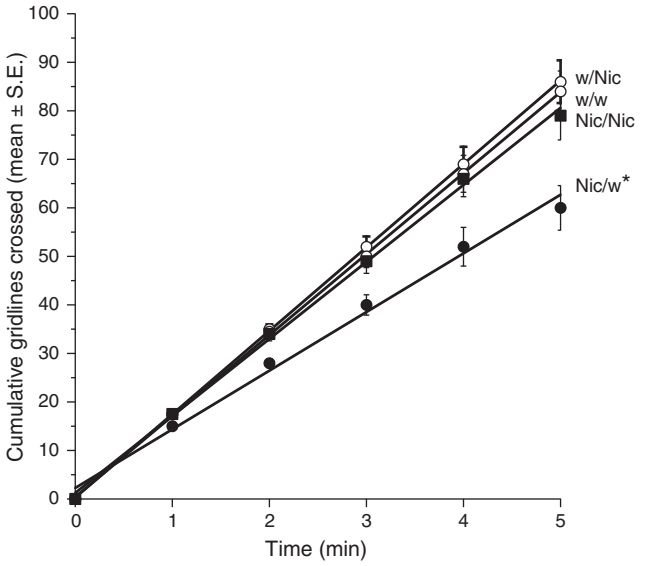

Fig. 1. Spontaneous planarian locomotor velocity (*p*LMV) measured as the mean number of gridlines crossed (± S.E.) during a 5-min observation period. Planarians were pretreated either in water (w) or nicotine (Nic, 100 μM) then tested either in water or nicotine at the same concentration used during the pretreatment. Nicotine-naïve animals displayed no difference in *p*LMV when tested in water or nicotine. Nicotine-pretreated animals displayed no difference *p*LMV from nicotine-naïve animals when tested in nicotine. However, planarians pretreated in nicotine for 60 min and then tested in water displayed significantly reduced *p*LMV. *$P < 0.05$ compared to w/w.

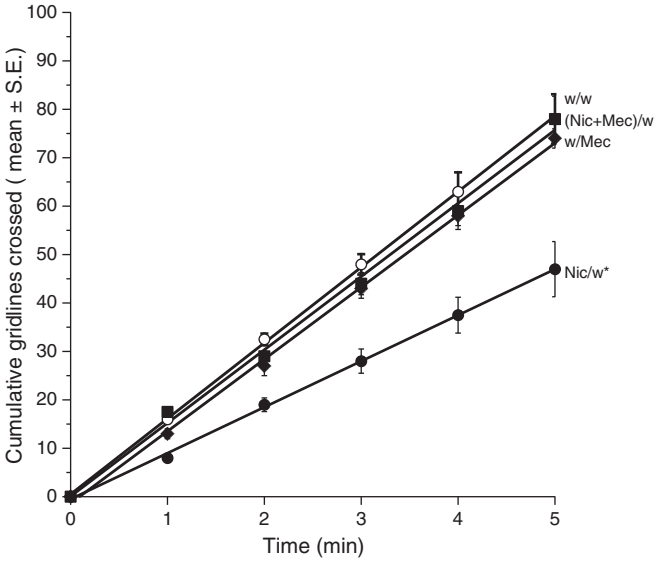

Fig. 2. Abstinence-induced nicotine (Nic) withdrawal in water (w) was attenuated when the planarians were coincubated with 50 μM mecamylamine (Nic + Mec/w) for 60 min and then tested in water. *$P < 0.05$ compared to w/w.