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Research article

Region-specific up-regulation of oxytocin receptor binding in the brain of mice following chronic nicotine administration

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HIGHLIGHTS

- Nicotine administration increases amygdalar oxytocin receptor binding.
- Chronic nicotine does not alter oxytocin receptor binding in the striatum.
- Oxytocin may be a potential target for the treatment of nicotine addiction.

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ABSTRACT

Nicotine addiction is considered to be the main preventable cause of death worldwide. While growing evidence indicates that the neurohypophysial peptide oxytocin can modulate the addictive properties of several abused drugs, the regulation of the oxytocinergic system following nicotine administration has so far received little attention. Here, we examined the effects of long-term nicotine or saline administration on the central oxytocinergic system using [^{125}I]OVTA autoradiographic binding in mouse brain. Male, 7-week old C57BL6J mice were treated with either nicotine (7.8 mg/kg daily; rate of 0.5 μl per hour) or saline for a period of 14-days via osmotic minipumps. Chronic nicotine administration induced a marked region-specific upregulation of the oxytocin receptor binding in the amygdala, a brain region involved in stress and emotional regulation. These results provide direct evidence for nicotine-induced neuroadaptations in the oxytocinergic system, which may be involved in the modulation of nicotine-seeking as well as emotional consequence of chronic drug use.

1. Introduction

Cigarette smoking is considered the main preventable cause of death worldwide [1]. While there is evidence suggesting that nicotine may exert antidepressant [2–5] and anxiolytic [6] effects, chronic nicotine use has been also associated with severe depression symptoms [7–9] and anxiety, which persist following abstinence [10,11]. This negative affective state following nicotine cessation might contribute to relapse [12]. Although there are currently numerous therapeutic agents and cognitive behavioral interventions for smoking cessation that are considered beneficial for the treatment of nicotine addiction, none of these therapeutic strategies has been shown to effectively prevent relapse to nicotine-seeking following abstinence [13]. In fact, among the 40% of smokers undergoing smoking-cessation interventions, only a small percentage of 4% achieve a long-term abstinence for 6–12 months [14]. Therefore, development of an optimal treatment for the effective treatment and prevention of nicotine use and relapse following abstinence requires further understanding of the mechanisms contributing to nicotine long-term abuse, which might be associated with the emergence of emotional impairment during withdrawal.

Emerging evidence indicates the involvement of the oxytocinergic system in drug addiction processes [15–17]. In particular, chronic administration of addictive substances including cocaine [18,19], methamphetamine [20], opioids [21] and alcohol [22] have been shown to induce marked alterations in the oxytocin (OT) sys-

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tem in the brain, which might be involved in the modulation of the emotional consequences of chronic drug use. Indeed, recent evidence supports an association between oxytocinergic deficiency and the negative emotional consequences of drug addiction, including depression, anxiety and social deficits [21]. OT-producing neurons located in the hypothalamus also project to several brain regions involved in drug-seeking behavior as well as emotional regulation, including the septum and amygdala, where oxytocin receptors (OTR) are expressed [23]. Few previous studies also demonstrated a role for OT in the modulation of nicotine addiction processes. In particular, acute intravenous administration of nicotine has been shown to decrease OT content in the pituitary of rats [24], and systemic administration of OT abolished physical somatic symptoms of nicotine withdrawal in rats [25]. Overall, although these studies clearly support the involvement of OT in nicotine addiction, the effects of chronic nicotine administration on the central oxytocinergic system remains largely unknown.

Based on the evidence implicating the OT neuropeptidergic system in addictive-related behaviors, we hypothesized that chronic nicotine administration might also induce alterations in the central oxytocinergic system. This is the first study to investigate the effects of chronic nicotine treatment on oxytocin receptor binding with the use of autoradiographic binding.

2. Materials and methods

2.1. Animals and chronic nicotine administration paradigm

Male C57BL/6J mice (seven-week old, Charles River Laboratories, Kingston, UK), were individually housed in a temperature-controlled environment with a 12:12-hour light/dark cycle (lights on at 06:00). Food and water were available ad libitum. Mice were given seven days to acclimatize to their new environment and were handled daily by the experimenter. Mice were treated with a nicotine administration paradigm as described previously [26]. Briefly, saline or nicotine hydrogen salt (7.8 mg/kg/day; Sigma–Aldrich, UK) were administered via osmotic mini-pumps (ALZET®2002 model, Charles River, UK). For minipump implantation, mice were anaesthetized using an isoflurane/oxygen vapor mixture (3.5%–4.5%; Isoflo, Abbott Laboratories Ltd, UK). A single incision along the midline of the back of each animal was made and osmotic mini-pumps were placed in parallel position to the spine. The flow operator was pointing away from the incision site. Nicotine was delivered for a period of 14 days at the daily dose of 7.8 mg/kg (free-base weight), at a rate of 0.5 μl per hour. This dose has been shown to induce blood nicotine levels comparable to the values measured in human smokers [26].

All animal care and experimental procedures complied with protocols approved by the University of Surrey Animal Welfare and Ethical Review Body and by the UK Home Office under Animals (Scientific Procedures) Act 1986. Mice were randomly assigned to two different drug-administration groups; control saline-treated group and chronic nicotine-treated group.

2.2. OTR autoradiography

OTR binding was carried out on sections from 14-day saline- and nicotine-treated mice as previously described [20]. Total binding was determined by incubating sections with 50 pM [^{125}I]-ornithine vasotocin (OVTA) for 1 h in an incubation buffer medium containing 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1% w/v bovine serum albumin, and 0.05% w/v bacitracin (Sigma–Aldrich, Poole, UK, pH 7.4 at room temperature). Adjacent sections were incubated with [^{125}I]-OVTA (50 pM) in the presence of 50 μM unlabelled (Thr^4,Gly^7)-oxytocin (Bachem, Germany), to determine non-specific binding (NSB). Slides were apposed to Kodak MR-1 films (Sigma–Aldrich, UK) in Hypercassettes with autoradiographic [^{14}C] microscales of known radioactive concentration (GE Healthcare Life Sciences, Amersham, U.K.) for 3 days. Films were developed in a 50% Kodak D19 developer solution (Sigma–Aldrich, Poole, UK) and analyzed using MCID image analyzer (Image Research, Ontario, Canada).

2.3. Statistical analysis

All values were expressed as mean ± SEM. For the analysis of regional OTR binding, two-way ANOVA was performed for factors 'treatment (saline/nicotine)' and 'brain region' at different bregma levels. Bonferroni *post-hoc* test was used when ANOVA reached significance (*i.e.*, $p < 0.05$). All statistical analyses were performed using *Statistica 8.0* (Statsoft Inc., France).

3. Results

High levels of OTR binding (0.97–1.15 fmol/mg tissue) were observed within the olfactory nuclei, medium binding levels (0.28–0.68 fmol/mg tissue) were identified within the mediolateral septum, ventral limb of the diagonal band of Broca, amygdala and hypothalamus, while low levels of binding (0.07–0.17 fmol/mg tissue) were observed in striatal regions (*i.e.*, nucleus accumbens, caudate putamen and olfactory tubercle) as well as the thalamus (Fig. 1A–E).

3.1. Olfactory nuclei

Two-way ANOVA showed a significant effect of 'brain region' ($F_{[2,24]} = 7.37$, $p < 0.01$), but no 'treatment' ($F_{[1,24]} = 0.30$, $p > 0.05$) or 'treatment' × 'brain region' interaction effect ($F_{[2,24]} = 0.01$, $p > 0.05$).

3.2. Striatum

Two-way ANOVA revealed a significant effect of 'brain region' ($F_{[2,24]} = 4.48$, $p < 0.05$), but no 'treatment' ($F_{[1,24]} = 0.26$, $p > 0.05$) or 'treatment' × 'brain region' interaction effect ($F_{[2,24]} = 0.06$, $p > 0.05$).

3.3. Septum

Two-way ANOVA revealed a significant effect of 'brain region' ($F_{[2,24]} = 10.06$, $p < 0.001$), but no 'treatment' ($F_{[1,24]} = 0.64$, $p > 0.05$) or 'treatment' × 'brain region' interaction effect ($F_{[2,24]} = 0.09$, $p > 0.05$).

3.4. Forebrain

Two-way ANOVA revealed a significant effect of 'brain region' ($F_{[3,27]} = 53.50$, $p < 0.001$) and 'treatment' × 'brain region' interaction effect ($F_{[3,27]} = 3.40$, $p < 0.05$). Bonferroni's *post-hoc* comparison test showed a significant, 46% increase of OTR binding in the amygdala following nicotine treatment ($p < 0.01$). No effects of nicotine administration on the OTR binding were observed in the hippocampus, thalamus, or hypothalamus ($p > 0.05$).

4. Discussion

The present study demonstrated, for the first time, a region-specific alteration of the OTR binding in the brain of mice treated with a chronic nicotine administration paradigm. This up-regulation of the OTR was specifically localized in the amygdala, a region involved in stress and emotional regulation [27,28]. Therefore, this oxytocinergic system alteration may be involved in