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EFFECTS OF COMBINED NICOTINE AND FLUOXETINE TREATMENT ON ADULT HIPPOCAMPAL NEUROGENESIS AND CONDITIONED PLACE PREFERENCE

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Abstract—Adult neurogenesis occurs in mammals within the dentate gyrus, a hippocampal subarea. It is known to be induced by antidepressant treatment and reduced in response to nicotine administration. We checked here whether the antidepressant fluoxetine would inverse the decrease in hippocampal neurogenesis caused by nicotine. It is shown that repeated, but not a single injection of rats with fluoxetine was able to abolish the decrease in adult dentate cell proliferation produced by nicotine treatment. We measured the expression of several biochemical parameters known to be associated with neurogenesis in the dentate gyrus. Both drugs increased the expression of p75 neurotrophin receptor, which promotes proliferation and early maturation of dentate gyrus cells. Using the conditioned place preference (CPP) paradigm, we also gave both drugs in a context in which their rewarding properties could be measured. Fluoxetine produced a significant but less robust CPP than nicotine. A single injection of fluoxetine was found to reduce nicotine-induced CPP. Moreover, the rewarding properties of nicotine were completely abolished in response to repeated fluoxetine injections. Expression of nicotine-induced CPP was accompanied by an increase of phospho-CREB (cyclic AMP-responsive element-binding protein) and HDAC2 (histone deacetylase 2) expression in the nucleus accumbens. The data suggest that fluoxetine reward, as opposed to nicotine reward, depends on dentate gyrus neurogenesis. Since fluoxetine was able to disrupt the association between nicotine and the environment, this antidepressant may be tested as a treatment for nicotine addiction using cue exposure therapy.

Key words: conditioned place preference, fluoxetine, histone deacetylase, neurogenesis, nicotine, phospho-CREB.

INTRODUCTION

The dentate gyrus (DG) in the hippocampal formation is one of the few brain structures in which neurogenesis has been demonstrated in adult mammals (Eriksson et al., 1998). Increased neurogenesis can be produced by a variety of treatments, including enriched environment (Brown et al., 2003), physical activity (van Praag et al., 1999), memory (Denny et al., 2014) or antidepressant drugs, including fluoxetine (Mendez-David et al., 2013). The neurogenic hypothesis of depression postulates that decreased production of new granule cells in the DG is linked to the pathophysiology of depression and that increased hippocampal neurogenesis is required for the behavioral effects of antidepressant treatment (Malberg et al., 2000; Santarelli et al., 2003). In contrast to the effect of antidepressants, decreased neurogenesis can be produced by drugs of abuse (Campbell et al., 2011), stress (Schoenfeld and Gould, 2012) or certain brain diseases (Danzer, 2012). For example, early studies have indicated that repeated exposure to nicotine impaired the long-term survival of adult-born neurons (Berger et al., 1998). These data were confirmed by studies using mutant mice lacking the two major nicotinic receptor subtypes (Campbell et al., 2011).

The neurobiological mechanisms by which antidepressants exert their effect are under reassessment. Recent data suggest that an additional mechanism used by the selective serotonin reuptake inhibitor (SSRI) fluoxetine to alleviate depression is by inhibiting the activity of the cholinergic system (Chau et al., 2011). Further arguments indicate a strong relationship between major depression and acetylcholine nicotinic receptors (Bertrand, 2005). Moreover, prevalence of nicotine dependence is significantly higher among individuals with mood disorders (Grant et al., 2004), probably because nicotine alleviates some negative cognitive features in afflicted individuals (Dani and Harris, 2005). Other data show that smokers present a greater risk of becoming depressed than nonsmokers (John et al., 2004), mostly during withdrawal periods. On the other hand, nicotine administration to freely moving mice was shown to produce

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Abbreviations: BrdU, bromodeoxyuridine; CPP, conditioned place preference; CREB, cyclic AMP responsive element binding protein; DAB, 3,3′-diaminobenzidine; DG, dentate gyrus; GFAP, glial fibrillary acidic protein; HDAC, histone deacetylase; i.p., intraperitoneal; NAc, nucleus accumbens; NeuN, hexaribonucleotide binding protein-3; $p75^{NTR}$, p75 neurotrophin receptor; PBS, phosphate-buffered saline; pCREB, phospho-CREB; PFC, prefrontal cortex; s.c., subcutaneous; VTA, ventral tegmental area.

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long-term potentiation (LTP) *in vivo* in the DG (Tang and Dani, 2009), implying that nicotine acts on the DG circuits. The effect may be direct or may occur through dopaminergic afferents from the ventral tegmental area (VTA), the latter establishing a functional link between reward centers and memory systems (Lisman and Grace, 2005).

Given that nicotine reduces adult hippocampal neurogenesis while fluoxetine increases it, we tested the hypothesis that fluoxetine would be able to reverse the decrease in DG neurogenesis caused by nicotine. **N**eurogenesis in the adult rodent brain was assessed by measuring bromodeoxyuridine (BrdU) labeling as well as expression of the cellular markers hexaribonucleotide binding protein-3 (NeuN) and glial fibrillary acidic protein (GFAP) (Kempermann et al., 1997; Bernabeu et al., 2006). Expression of p75 neurotrophin receptor ($p75^{NTR}$) was carried out as the receptor is expressed by adult dentate progenitor cells and it promotes proliferation and early maturation of DG cells in mice (Bernabeu and Longo, 2010). We also characterized the effects of combining both drugs on conditioned place preference (CPP) to evaluate whether fluoxetine modifies the CPP induced by nicotine. Nicotine-induced CPP has been well characterized (Le Foll and Goldberg, 2005; Pascual et al., 2009; Natarajan et al., 2011; Pastor et al., 2011), while fluoxetine-induced CPP has only been shown in initial studies (Collu et al., 1997).

Expression of proteins that were previously associated with nicotine-related conditioning was also analyzed. We measured the expression of phospho-cyclic AMP responsive element binding protein (pCREB) since enhanced phosphorylation of CREB is required for nicotine-induced CPP and reinstatement in rats (Pascual et al., 2009; Pastor et al., 2011). It is also involved in the regulation of adult neurogenesis in the DG (Nakagawa et al., 2002). Phospho-CREB binds the CREB-binding protein CBP, a transcriptional coactivator which possesses histone acetyltranferase (HAT) activity (Kalkhoven, 2004). In addition, histone deacetylase (HDAC) family members, especially HDAC2, are recognized as playing an important role in cognitive functions, inducing memory impairment when over-expressed (Guan et al., 2009). HDAC2 has been proposed to be involved in promoting synaptic plasticity underlying the preference for nicotine (Pastor et al., 2011). By removing acetyl groups from key histone residues, HDACs promote an inactive chromatin state, resulting in the silencing of downstream genes (Klose and Bird, 2006).

In the present study, we found that repeated, but not a single injection of rats with fluoxetine was able to abolish the decrease in adult dentate cell proliferation produced by nicotine treatment. A single fluoxetine injection was also found to reduce nicotine-induced CPP and the rewarding properties of nicotine were completely abolished in response to repeated fluoxetine injections.

EXPERIMENTAL PROCEDURES

Animals

Sixty-six adolescent male Sprague–Dawley rats (School of Pharmacy and Biochemistry, University of Buenos Aires), weighing 80–100 g (PN 25–26) at their arrival at the laboratory were housed in groups of four on a 12-h light/dark cycle with *ad libitum* access to food and water. Animals were handled for 5 min twice a day for 4 days prior to behavioral experiments. Adolescent rats were used since we and others previously found that they establish much stronger nicotine-induced CPP than adult rats (Natarajan et al., 2011; Pastor et al., 2011; Ahsan et al., 2014). All procedures involving animal care were conducted in compliance with national laws and policies, with the approval of the Ethics committee of the University of Buenos Aires.

Pharmacological treatment

Rats were injected intraperitoneally (i.p.) either acutely or repeatedly for 10 days (one injection per day, 'repeated treatment') with 10 mg/kg fluoxetine hydrochloride (Eli Lilly, Indianapolis, IN, USA) or an equivalent volume of phosphate-buffered saline (PBS). Nicotine tartrate (Sigma, St. Louis, MO, USA) was dissolved in PBS and administered subcutaneously (s.c.) at a dosage of 0.4 mg/kg in a volume of 1 ml/kg. This dosage is known to induce strong CPP in adolescent rats (Natarajan et al., 2011; Pastor et al., 2011; Ahsan et al., 2014). An equal volume of PBS was injected for the control condition. Indicated doses are based on the molecular weight of the free base. Bromodeoxyuridine (BrdU; Sigma, St. Louis, MO, USA) was dissolved in sterile 0.9% NaCl, filtered and injected i.p. at the dosage of 50 mg/kg. The various compounds were injected alone or in combination, according to the diagram shown in Fig. 1a. Animals were killed 1 day after the last injection (Pascual et al., 2009).

Conditioned-place preference (CPP)

Place conditioning was essentially performed in a three compartment box, as described previously (Pascual et al., 2009; Pastor et al., 2011). Briefly, home-made acrylic boxes were divided into two equally sized compartments (30 × 25 × 30 cm) separated by a smaller central chamber (12 × 21 × 21 cm) that had gray walls and a smooth plastic floor, with doors allowing access to the two lateral compartments. These two compartments had different visual, tactile and olfactory cues: one compartment had vertically striped black and white walls and a wire mesh floor above pine shavings; the other compartment had horizontal striped black and white walls and a bar-grid floor above cedar shavings. The apparatus was designed so that rats did not present any consistent bias for a particular chamber. During the habituation period, animals were handled twice a day for 5 days and were injected s.c. or i.p. with PBS to habituate them to the injections. A camera connected to a computer was placed approximately 1.2 m above the CPP boxes. During pretest and CPP test, rat behavior was recorded and videos were analyzed, first by direct observation and then using the Noldus Ethovision XT7 software (Noldus Information Technology, The Netherlands).

Pre-conditioning phase. Following the habituation period, animals were injected with PBS and placed in