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Pharmacology, Biochemistry and Behavior 82 (2005) 559 – 568
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Effect of prolonged nicotine infusion on response of rat catecholamine biosynthetic enzymes to restraint and cold stress

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Received 2 June 2005; received in revised form 17 October 2005; accepted 20 October 2005
Available online 1 December 2005

Abstract

There is a paradoxical relationship between nicotine and stress. To help elucidate their relationship on catecholamine biosynthesis, rats were infused with nicotine for 7–14 days before exposure to cold or restraint stress. Nicotine (5 mg/kg/day, 14 days) did not alter basal plasma corticosterone or its elevation with 24 h cold stress, but prevented corticosterone elevation following 2 h restraint stress. In adrenal medulla (AM), response of dopamine β-hydroxylase (DBH), but not tyrosine hydroxylase (TH) mRNA, to both stressors was attenuated in nicotine-infused rats. In locus coeruleus (LC), restraint stress elevated TH and DBH mRNA in saline-, but not in nicotine-infused rats. Cold stress triggered a similar response of TH and DBH mRNAs in LC with and without nicotine infusion. With shorter nicotine infusion (8 mg/kg/day, 7 days), TH mRNA in AM was not induced by restraint stress on one (1×) or two (2×) consecutive days nor was DBH mRNA in AM or LC by 2×. The findings demonstrate that constant release of nicotine can modulate, or even prevent, some stress responses at the level of the HPA axis and gene expression of catecholamine biosynthetic enzymes in LC and AM.

Keywords: Corticosterone; mRNA; Adrenal medulla; Locus coeruleus; Tyrosine hydroxylase; Dopamine β-hydroxylase; Nicotine; Restraint; Cold stress

1. Introduction

There is a paradoxical relationship between nicotine and stress. On one hand cigarette smoking is reported to be calming, and on the other hand it can trigger many of the same physiological responses seen with stress. The reported calming effect of smoking is more evident during stressful situations (Schachter et al., 1977; Rose et al., 1983). Chronic smokers are more resistant to stress when allowed to smoke than when prevented from smoking. Nicotine is likely responsible for this effect since this calming effect was not observed in people smoking low- or non-nicotine containing cigarettes (Silverstein, 1982). Moreover, stress is an important factor contributing to relapse among smokers attempting to quit (Shiffman, 1982; Abrams et al., 1987).

Despite this calming effect, nicotine was shown to activate several stress reactive systems. Administration of nicotine leads to activation of the hypothalamic–pituitary–adrenal (HPA) axis resulting in the higher plasma levels of the stress hormones, adrenocorticotropin hormone (ACTH) and cortisol (Balfour, 1980; Seyler et al., 1984). Nicotine also stimulates release of catecholamines (CA) from the adrenal medulla, sympathetic nerve endings and brain CA neurons (Haass and Kubler, 1997). Administration of nicotine, like stress, increases heart rate, systolic and diastolic blood pressures and enhances electroencephalogram (EEG) measured potentials in humans and experimental animals (USDHHS, 1998). These cardiovascular effects are largely attributed to the large increases in circulating epinephrine and norepinephrine (NE) in humans and animals (Cryer et al., 1976; Haass and Kubler, 1997). Nicotine stimulation of the ascending NE neurons from the nucleus of the solitary tract was shown to be essential for induction and release of ACTH (Matta et al., 1993). Nicotine also acts centrally to stimulate norepinephrine release from locus coeruleus neurons which is considered a crucial site in CNS stress response (Mitchell, 1993; Dani and De Biasi, 2001).

Nicotine also promotes catecholamine biosynthesis by activating tyrosine hydroxylase (TH), the first and major rate-limiting enzyme (Fossom et al., 1991; Smith et al., 1991; Hiremagalur and Sabban, 1995). In addition, nicotine not only

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doi:10.1016/j.pbb.2005.10.011

increases gene expression of TH, but also other subsequent catecholamine biosynthetic enzymes [dopamine β hydroxylase (DBH) and phenylethanolamine *N*-methyltransferase (PNMT)] in the periphery and in many catecholaminergic regions of the CNS (Fossom et al., 1991; Hofle et al., 1991; Mitchell et al., 1993; Hiremagalur and Sabban, 1995; Serova et al., 1999).

Recent studies show a relationship between genetic polymorphisms in the TH and DBH genes and smoking, indicating that these genes may be involved in nicotine addiction. (McKinney et al., 2000; Anney et al., 2004).

Several hypotheses were advanced to explain the nicotine and stress paradox. It was suggested that the basis could be psychological and not necessarily due to biological explanations. For example it has been suggested that abstinence from nicotine smoking results in increased stress in smokers, and smoking only appears to reduce stress by contrast (Schachter et al., 1977; Schachter, 1978). It was considered that smoking might provide an alternative source of attention thus they are less aware of negative somatic experiences (Silverstein, 1982). However, a considerable amount of recent research points to a biological basis for the nicotine and stress paradox. Chronic (11 days) nicotine administration by osmotic minipumps at high concentrations (12 mg/kg/day nicotine dihydrochloride, expressed as free base) prevented the stress triggered increase in acoustic startle amplitude and pre-pulse amplitude (Acri, 1994). Repeated nicotine injections led to a reduction in footshock stress induced dopamine utilization in the prefrontal cortex and immobility responses (George et al., 1998). Previous studies from our laboratory revealed that prolonged infusion of rats with nicotine attenuated several of the responses to immobilization stress, including activation of gene expression for catecholamine biosynthetic enzymes, TH and DBH (Serova et al., 1999). The attenuation of gene expression for catecholamine biosynthetic enzymes in response to immobilization stress in rats with nicotine infusion was tissue specific (Serova et al., 1999). In adrenal medulla, the immobilization induced elevation of TH mRNA levels was significantly less in nicotine-infused rats compared to saline-infused rats. The response of DBH mRNA to immobilization stress was abolished in both adrenal medulla and locus coeruleus, the site of cell bodies for the major noradrenergic system in the brain. However, it is unknown how nicotine infusion would affect different types of stress and the optimal conditions required to modulate the stress responses in different CA locations.

In this study we examine the effects of infusion of nicotine on the response of TH and DBH gene expression to cold stress and also to restraint, a milder form of immobilization stress, more likely to be encountered by humans. Prior to exposure to stress, rats were pretreated with nicotine by two different treatment paradigms. In the first paradigm, animals were infused 5 mg/kg/day of nicotine for 14 days and then subjected to either 2 h restraint stress or to 24 h cold stress. In the second paradigm, nicotine was infused at 8 mg/kg/day for 7 days prior to exposure to single or twice repeated restraint stress. Levels of TH and DBH mRNA in the adrenal medulla and locus coeruleus as well as plasma nicotine and corticosterone concentrations were compared to control saline treated animals. The findings will help to understand the interactions between nicotine and stress, and how individuals addicted to nicotine respond to stress, and can be helpful in preventing relapse.

2. Materials and methods

2.1. Animal manipulations

All animal experiments were approved by the Institutional Animal Care and Use Committee and were in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Male Sprague–Dawley rats (250–300 g) from Taconic Farms (Germantown, NY) were used for this study. They were housed four per cage in a barrier area to minimize stress on a 12-h light/dark cycle at 23±2 °C with free access to food and water.

Three experimental procedures in which animals pretreated with nicotine were exposed to stress were used in this study and are shown in Table 1. Nicotine-di-D-tartrate (Sigma, St. Louis, MO) dissolved in saline was administrated by continual infusion with an osmotic pump (model 2002; Alzet, Palo Alto, CA). Control groups received an equal volume of saline. Alzet pumps were implanted subcutaneously in the nape of the neck under pentobarbital (50 mg/kg) anesthesia to deliver 5 mg/kg/day nicotine (calculated as free base) for 14 days, 8 mg/kg/day nicotine for 7 days or an equal volume of saline. Animals were then divided into groups of 8 animals each which were either subjected to the restraint or cold stress, or unstressed.

2.1.1. Restraint stress

Rats were placed in a small metal cylinder (diameter: 6 cm) for 2 h once (1×) or 2 h daily on two consecutive days (2×) and euthanized 3 h after stress, a time found to be maximal for elevation of TH mRNA in adrenal medulla with immobilization stress (Nankova et al., 1994).

2.1.2. Cold stress

Rats were kept at 4 °C, two rats per metal cage without bedding for 24 h and euthanized immediately afterwards, based on the time course previously observed for cold triggered changes in TH mRNA levels in the rat adrenal medulla (Baruchin et al., 1990).

Rats were euthanized by decapitation. The adrenals were removed, and the left and right adrenal medulla dissected and frozen separately from each animal in liquid nitrogen. The brain was dissected using a tissue slicer with digital micrometer. The LC was punched from frontal sections, 9.2–10.4 mm from Bregma, and frozen in liquid nitrogen. Blood was collected into EDTA-containing tubes on ice, plasma separated and kept at −70 °C.

Table 1
Summary of experimental procedures

	Nicotine pretreatment	Stress
Experiment 1	5 mg/kg/day, 14 days	Restraint 1× or cold 24 h
Experiment 2	5 mg/kg/day, 14 days	Cold 24 h
Experiment 3	8 mg/kg/day, 7 days	Restraint 1×, 2× or cold 3 h