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Tonic function of nicotinic receptors in stress-induced release of L-DOPA from the nucleus accumbens in freely moving rats

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Abstract

We investigated whether stress induces the release of L-3,4-dihydroxyphenylalanine (DOPA) and dopamine from the nucleus accumbens in conscious rats and characterized the stress-induced response. Electrical foot-shock stress induced both DOPA and dopamine release, measured by microdialysis, from the nucleus accumbens in freely moving rats. Pretreatment of rats with mecamylamine completely blocked stress-induced DOPA release, but only partially blocked dopamine release. Diazepam did not affect the foot-shock-induced release of DOPA, while the same dose of diazepam partially blocked the stress-induced release of dopamine. These findings suggest a tonic function of central nicotinic receptors in stress-induced DOPA release from the nucleus accumbens in conscious rats.

Keywords: Nicotine; L-DOPA (L-3,4-dihydroxyphenylalanine); Dopamine release; Electrical foot-shock; Stress; Behavior; Nucleus accumbens; Microdialysis

1. Introduction

We have obtained several lines of evidence that L-3,4-dihydroxyphenylalanine (DOPA) is a neurotransmitter and/or neuromodulator in the central nervous system (Misu and Goshima, 1993; Misu et al., 1996). Endogenous DOPA is released in a transmitter-like manner in vivo and in vitro from some areas of the brain (Misu et al., 1996; Nakamura et al., 1992). DOPA produces in vivo postsynaptic responses and in vitro presynaptic responses (Misu et al., 1996). These effects appear to be mediated via recognition sites specific for DOPA, since these effects are stereoselective in nature, are antagonized by DOPA ester compounds in a competitive fashion (Goshima et al., 1991), and are not mimicked by dopamine (Misu et al., 1996). Accordingly, neurons containing DOPA as an end product have been demonstrated in the brains of rats, cats and humans using specific antisera against DOPA conjugated with bovine serum albumin (Misu et al., 1996). Although all of these findings suggest a neurotransmitter and/or neuromodulator role for DOPA, the existence of a specific receptor for DOPA as well as the existence of DOPA containing vesicles remains to be determined.

Nicotine is known to affect various types of behavioral parameters, including locomotor activity (Clarke et al., 1988; Imperato et al., 1986; Nakamura et al., 1993). The behavioral effects of nicotine have been attributed in part to its ability to induce neurotransmitter and/or neuromodulator release via nicotinic receptors on nerve terminals and/or cell bodies in the central nervous system. We have previously shown that nicotine increases the turnover of catecholamines in various parts of the rat brain via nicotinic acetylcholine receptors (Amano et al., 1989). In addition to dopamine release, nicotine induces Ca^{2+}-dependent DOPA release in the rat striatum, in vitro (Misu et al., 1996) and in vivo (Nakamura et al., 1992). In in vivo striatum, mecamylamine alone inhibits the release of DOPA but not that of dopamine, thereby suggesting that DOPA release is tonically regulated via nicotinic receptors in the striatum (Nakamura et al., 1992). Several lines of evidence have suggested an important role for the mesolimbic dopaminergic system in the action of nicotine. In the rat for example, nicotine preferentially stimulates dopamine release from the nucleus accumbens compared to the stria-

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tum (Imperato et al., 1986). In addition, we previously demonstrated that DOPA and dopamine release induced by peripherally applied nicotine is greater in the nucleus accumbens than in the striatum, and that this release appears to occur mainly via the activation of nicotinic acetylcholine receptors located in the ventral tegmental area (Goshima et al., 1996). With regard to this, it is noteworthy that nicotine-induced dopamine release in the nucleus accumbens can be attenuated by local administration into the ventral tegmental area of antagonists of *N*-methyl-D-aspartate as well as nicotinic receptors (Schilstrom et al., 2000).

Based upon its anatomy, and a variety of physiological and behavioral data, the nucleus accumbens is thought to have an important role in translating motivationally relevant environmental stimuli into adaptive motor responses (Kalivas and Duffy, 1995). Extracellular dopamine levels in the nucleus accumbens have actually been shown to be elevated after discontinuation of foot-shock (Doherty and Gratton, 1996; Kalivas and Duffy, 1995) and restraint stress (Cabib and Puglisi-Allegra, 1996).

In the present study, using an in vivo microdialysis system, we investigated whether electrical foot-shock stress induces the release of DOPA and dopamine in the nucleus accumbens in freely moving rats. To characterize these stress-induced release responses, we further studied the effects of the in vivo administration of mecamylamine and diazepam on the release of DOPA and dopamine.

2. Materials and methods

All procedures were carried out according to the guidelines outlined in the Institutional Animal Care and Use Committee of the Yokohama City University School of Medicine. All experimental procedures were carried out with maximum effort to minimize the number of animals used and their suffering. Male Sprague–Dawley rats weighing 250–350 g were anesthetized with sodium pentobarbital (50 mg/kg i.p.). Animals were placed in a stereotaxic apparatus (Narishige, SR-6). A guide cannula (CMA/12, BAS) for microdialysis was stereotaxically implanted into the left nucleus accumbens (A 1.7, L1.0 and V 6.5 from the bregma), according to a stereotaxic atlas (Paxinos and Watson, 1986). This compartment is termed the shell of the nucleus accumbens (Goshima et al., 1996). After recovery from surgery (at least 1 days), rats were individually placed in a plastic shock box ($30 \times 30 \times 25$ cm) with a grid floor and habituated to their environment overnight.

The guide cannula was replaced with a dialysis probe (membrane length 1 mm: CMA/12), and perfusion was started with Ringer solution (Na^+ 147 mM, K^+ 4 mM, Ca^{2+} 2.3 mM, Cl^- 155.6 mM, pH 5.8–6.0) at a rate of 1.6 μl/min. Dialysates were collected every 20 min from 2.5 h after the start of perfusion. DOPA and dopamine in each 32-μl sample were measured by high-performance liquid chromatography with electrical detection. The chromatographic conditions used were as described elsewhere (Goshima et al., 1996). The mean absolute values for DOPA and dopamine release in three samples taken 2.5 h after the start of perfusion are given as the percentage control for each group.

One hour after the start of sample collection, rats were subjected to an electric footshock (0.4 mA, 200 ms, 1 Hz) for 20 min in a chamber with a grid floor during microdialysis of the nucleus accumbens. Mecamylamine was dissolved in 10 mM phosphate-buffered saline (pH 7.4). Diazepam was suspended in 0.5% carboxymethyl cellulose sodium salt. Mecamylamine (2.0 mg/kg, s.c.), diazepam (2.5 mg/kg, i.p.) or control vehicle was injected systemically. Statistical analyses were performed by using Analysis of Variance (ANOVA)–Dunn's Multiple Comparison test.

3. Results

During microdialysis of the shell of the left nucleus accumbens in freely moving rats, basal DOPA and dopamine release was consistently detectable and became stable 2.5 h after the start of perfusion. The basal release of DOPA and dopamine from the nucleus accumbens in freely moving rats has been shown to be Ca^{2+} dependent and tetrodotoxin sensitive (Goshima et al., 1996).

An electrical foot-shock of 0.4 mA elicited the release of both DOPA and dopamine from the nucleus accumbens. DOPA release reached peak levels 20 min after the foot-shock and returned to basal levels after a further 80 min. Dopamine release reached peak levels 60 min after the foot-shock. This evoked release of dopamine appeared sustained throughout the time period studied. The i.p. injection of mecamylamine tended to decrease the basal levels of DOPA, but this decrease was not statistically significant. Mecamylamine completely blocked the foot-shock-elicited DOPA release, but only partially blocked dopamine release (Fig. 1A). Diazepam, a classical benzodiazepine full agonist, decreased the basal release of DOPA, but did not have any effect on the foot-shock-induced release of DOPA. However, the same dose of diazepam attenuated the release of dopamine during the period after the onset of the electrical foot-shock (Fig. 1B).

Electrical foot-shock elicited a number of behavioral changes in conscious rats. Jumping, running, vocalization, crouching and flinching were observed during foot-shock stress. Rearing was observed during and after foot-shock. These behavioral changes associated with foot-shock stress were not modified by pretreatment with mecamylamine (data not shown). Administration of diazepam before stress inhibited the basal locomotor activity of animals. Diazepam attenuated some types of behavior, such as sniffing

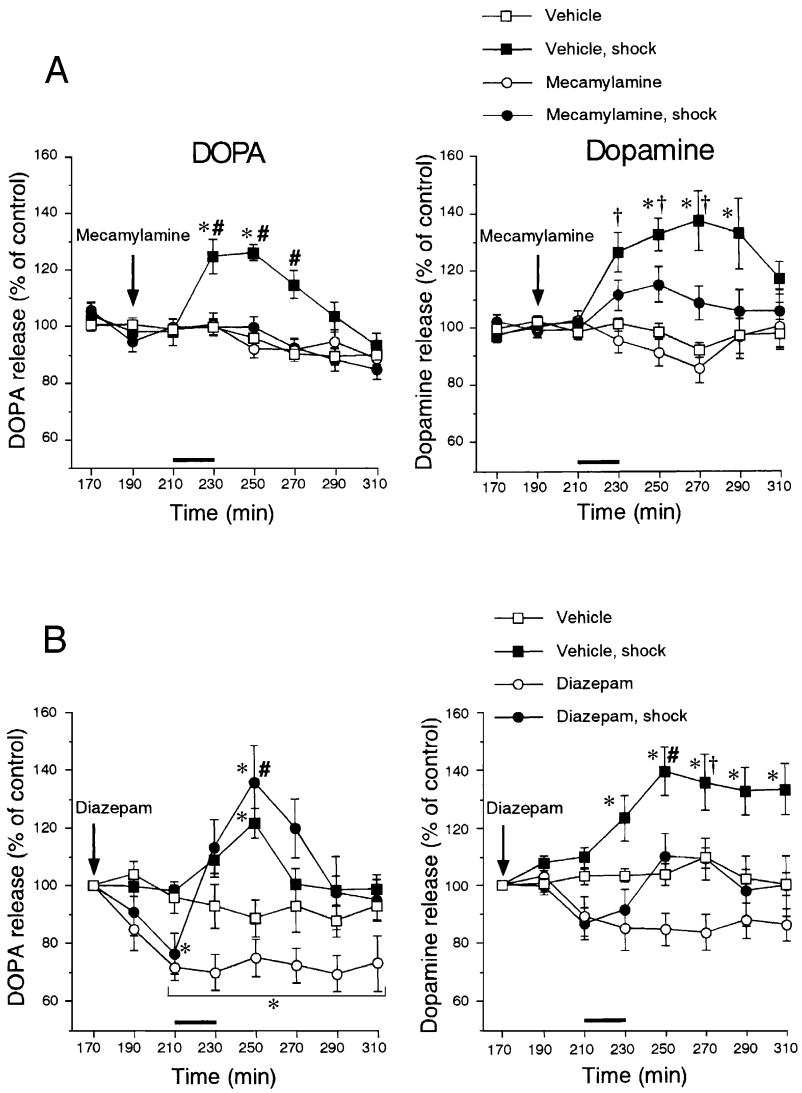

Fig. 1. Electrical foot-shock (0.4 mA) induced the release of L-DOPA (DOPA) and dopamine from the nucleus accumbens in freely moving rats. Electrical foot-shock was administered at horizontal bars. Time after the start of perfusion is indicated on the abscissa. (A) Pretreatment with mecamylamine (2.0 mg/kg, s.c.) completely blocked the foot shock-induced release of DOPA, while the same dose of mecamylamine partially blocked the release of dopamine. Control basal absolute value for DOPA and dopamine release (fmol) was 14.6 ± 1.6 and 59.1 ± 9.7 for saline alone, 17.6 ± 2.2 and 49.4 ± 9.3 for shock with saline, respectively. The basal absolute value for DOPA and dopamine was 19.0 ± 2.7 and 61.1 ± 16.5 for mecamylamine alone, 16.1 ± 1.5 and 57.6 ± 8.3 for shock with mecamylamine, respectively. $^{*}P < 0.05$ vs. the basal value immediately before shock in each group (Repeated Measures ANOVA–Dunn's Multiple Comparison test). $\#P < 0.05$ vs. other three groups, $\dagger P < 0.05$ vs. vehicle alone and mecamylamine alone (ANOVA–Dunn's Multiple Comparison test). (B) Pretreatment with diazepam (2.5 mg/kg, i.p.) did not block the foot-shock-induced release of DOPA, while the same dose of diazepam partially blocked the release of dopamine. Control basal absolute value for DOPA and dopamine release (fmol) was 17.0 ± 1.9 and 38.6 ± 5.5 for vehicle alone, 13.5 ± 1.1 and 30.6 ± 6.4 for shock with vehicle, respectively. The basal absolute value for DOPA and dopamine release was 18.0 ± 2.3 and 52.2 ± 11.6 for diazepam alone, 13.4 ± 0.9 and 46.7 ± 9.9 for shock with diazepam, respectively. $^{*}P < 0.05$ vs. the basal value immediately before treatment with diazepam or vehicle in each group (Repeated Measures ANOVA–Dunn's Multiple Comparison test). $\#P < 0.05$ vs. vehicle alone and diazepam alone, $\dagger P < 0.05$ vs. diazepam alone (ANOVA–Dunn's Multiple Comparison test). Each value represents the mean $\pm$ S.E.M. of 5–8 experiments.

and grooming, that were often seen after foot-shock stress in saline-treated rats.

4. Discussion

Our present experiments demonstrate for the first time that electrical foot-shock stress releases DOPA as well as dopamine from the nucleus accumbens in conscious rats. Moreover, treatment with mecamylamine, a central nicotinic receptor antagonist, inhibited the release of DOPA and dopamine from the nucleus accumbens. This suggests that both DOPA and dopamine release are elicited at least in part via central nicotinic receptors in response to foot-shock stress.

Mecamylamine only partially blocked the stress-induced release of dopamine, while it completely blocked the release of DOPA. Although the reasons for these differential stress-induced responses regarding the in vivo release of DOPA and dopamine from the nucleus accumbens are unknown, these results suggest the relative importance of the tonic function of nicotinic receptors in the release mechanisms for DOPA, compared with those for dopamine. Mecamylamine alone decreases the basal release of DOPA but not that of dopamine from the striatum of conscious rats (Nakamura et al., 1992). Whether tonic nicotinic regulation of DOPA release has some relevance to behavioral changes in response to electrical foot-shock stress remains to be clarified.

Diazepam partially blocked the release of dopamine in rats elicited by electrical foot-shock. This suggests that anxiety is at least partly involved in the foot-shock-induced release of dopamine from the nucleus accumbens in freely moving rats. This observation is consistent with a previous finding that a comparable dose of diazepam blocks the foot-shock induced-release of dopamine from the cortex in freely moving rats (Dazzi et al., 1995). In contrast, however, our study clearly showed that diazepam, at a dose that affected the foot-shock-induced release of dopamine, had no effect on the foot-shock-induced release of DOPA. At present, the reasons for these differential effects of diazepam on the stress-induced release of DOPA and dopamine are unknown. The observation that diazepam attenuated some types of behavior, such as sniffing and grooming, suggests that these behavioral changes seen after electrical foot-shock stress seem to be related to dopamine release rather than DOPA release.

In conclusion, central nicotinic receptors appear to be involved in the electrical foot-shock stress-induced release of DOPA and dopamine from the nucleus accumbens in conscious rats. Our results provide an example of the existence of a tonic function for central nicotinic receptors in some stress-induced responses in rats.

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