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Depressive Characteristics of FSL Rats: Involvement of Central Nicotinic Receptors

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TIZABI, Y., A. H. REZVANI, L. T. RUSSELL, K. Y. TYLER, AND D. H. OVERSTREET. *Depressive characteristics of FSL rats: Involvement of central nicotinic receptors.* PHARMACOL BIOCHEM BEHAV **66**(1)73–77, 2000.—Antidepressant effects of acute or chronic nicotine treatments in swim test immobility of Flinders sensitive line (FSL) rats, an animal model of depression, were recently demonstrated (Tizabi et al. *Psychopharmacology* 142:193, 1999). In the present study we sought to determine whether the antidepressant effects of nicotine could be blocked by the nicotinic antagonist, mecamylamine (MEC). Moreover, the effects of chronic nicotine treatment on [^{3}H]cytisine binding in discrete brain regions of FSL and their control Flinders resistant line (FRL) rats were also evaluated. Adult male FSL rats were treated with MEC (0.5 mg/kg) 20 min prior to an acute or chronic nicotine administration. MEC by itself did not affect the immobility in swim test. However, it completely blocked the acute or chronic nicotine effects. Daily nicotine injection (0.4 mg/kg/day for 14 days) resulted in an increase in [^{3}H]cytisine binding primarily in the FRL rats. An increase in nicotinic receptor binding following chronic nicotine administration is believed to reflect desensitization of these receptors. These findings, coupled with previous observation of higher basal nicotinic receptors in FSL rats, further support the involvement of central nicotinic receptors in depressive characteristics of these rats. Moreover, the data suggest therapeutic potential for selective nicotinic receptor agonists in depressive disorders. © 2000 Elsevier Science Inc.

Animal model of depression FSL rats Nicotine Nicotinic receptors Mecamylamine Swim test

EPIDEMIOLOGIC studies indicate a high incidence of cigarette smoking among depressed individuals (3,13,14). Moreover, individuals with a history of depression have a much harder time giving up smoking (8,13). It has been postulated that smoking may reflect an attempt at self-medication with nicotine by these individuals (3,8,13). Indeed, several animal and human studies support an antidepressant effect of nicotine (9,12,13,26,27,29,30).

Flinders Sensitive Line (FSL) rats have been proposed as an animal model of depression (23). These rats, selectively bred for their hyper responsiveness to cholinergic stimulation, show an exaggerated immobility in the forced swim test compared to their control Flinders Resistant Line (FRL) rats (23). Immobility in the swim test is a reliable marker of anergia or depressive characteristics of the rats which may be used for evaluating the efficacy of antidepressant drugs (17,22). Indeed, the exaggerated immobility in the forced swim test in the FSL rats can be counteracted by the administration of antidepressant drugs that are used clinically (22,23,25,28). Recently, we demonstrated that acute or chronic (14 days) administration of nicotine (0.4 mg/kg SC) significantly improved the performance of the FSL but not the FRL rats in the swim

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test (30). The effects of nicotine on swim test were dissociable from its effects on locomotor activity (30). Moreover, the FSL rats had significantly higher [^{3}H]cytisine binding (selective for the $\alpha_4\,\beta_2$ nicotinic receptor subtype) but not [^{125}I]alpha-bungarotoxin binding (selective for the α_7 subtype) in several brain regions compared with FRL rats (30). Although these data strongly implicated the central nicotinic receptors in the depressive characteristics of the FSL rats, blockade of the antidepressant effects of nicotine by a centrally effective nicotinic antagonist was not evaluated. Hence, in this study we examined the effects of mecamylamine on immobility of FSL rats in the swim test following acute or chronic nicotine administration. In addition, to further characterize the involvement of alpha4-beta2 nicotinic receptor subtype in depressive characteristics of these rats, we also evaluated the effects of chronic nicotine treatment on [^{3}H]cytisine binding in FSL and FRL rats.

METHOD

Animals and Housing

The FSL and FRL rats were randomly selected from the breeding colonies maintained at the Center for Alcohol Studies at the University of North Carolina. Adult male rats of approximately 70 days old and weighing 250 to 300 g were maintained in groups of 2 to 3 in plastic cages in a temperature (22°C) and humidity (50%) controlled room with free access to food and water. For behavioral testing, the 12 h light:dark cycle was reversed(lights on at 2200 h) to allow convenient testing of the animals during their wakeful hours. The animals were handled daily for one week prior to experiments to reduce the stress of novel handling. The experimental protocols were approved by the Institutional Review Committee for the use of Animal Subjects. The procedures applied were in compliance with the National Institutes of Health Guides for Care and Use of Laboratory Animals (Pub No. 85-23, revised 1985).

Swim Test

The swim test was conducted in a cylindrical tank 40-cm tall and 18-cm diameter, over a single 5-min period as described in detail previously (30). Briefly, the animal was placed in the tank containing enough 25°C water so that it could not touch the bottom with its hindpaws. The amount of time it was immobile was recorded. Moreover, because it was previously observed that FSL rats are very immobile, it was not necessary to have the 15-min pretest session, as is common in standard Porsolt test protocols for other rats (23). The test was carried out during the early part of the dark phase, between 10.00 and 13.00 h. The drugs were administered 10 min prior to the swim test. When two drugs (nicotine and MEC) were administered, MEC was given 20 min prior to nicotine and the swim test was performed 10 min after nicotine administration.

Drug Administration

Mecamylamine HCl (MEC) and -(-)nicotine bitartrate, purchased from Research Biochemicals International (Natick, MA, USA), were dissolved in saline and were administered subcutaneously (1 ml/kg). For behavioral studies, 0.5 mg/kg MEC and 0.2 mg/kg nicotine (base) were used. The nicotine dose was selected on the basis of a previous study where the effectiveness of such a dose in the swim test was demonstrated (30). The MEC dose was chosen on the basis of preliminary tests to assure the selection of a dose that by itself would not affect the immobility in the swim test. It is important to note that MEC at higher doses may act as a peripheral (ganglionic) nicotinic antagonist that can result in immobility in the swim test. For neurochemical studies, we used 0.4 mg/kg nicotine (base), a dose which was used previously in a chronic behavioral paradigm (30). Controls were administered saline (1 ml/kg). To ensure against potential influence of repeated habituation, separate groups of animals and their appropriate controls were used for acute or chronic studies.

Tissue Collection

Twenty-four hours following the last nicotine or saline injection, 16 FSL and 16 FRL rats were decapitated between 10.00 h and 14.00 h. The order of killing was alternated between the groups. Brains were rapidly removed and frozen on dry ice and stored at −80°C until dissected and assayed for receptor density and binding affinity. The 24 h wait following the last injection of nicotine in obtaining the brains is to ensure clearance of nicotine from the body and hence to minimize any interference of nicotine with the receptor measurement. It is also of relevance to note that no withdrawal effects were detected on the swim test at this time period (30).

Brain Dissection and Determination of Nicotinic Receptors

The following brain regions were dissected: frontal cortex (up to the genu of corpus callosum and excluding the olfactory bulb and olfactory tubercle), cerebral cortex (the rest of the cortex, left and right hemisphere), striatum (bilateral), hippocampus (bilateral), midbrain (containing the thalamus), colliculi (superior and inferior), and cerebellum. [^{3}H]Cytisine binding was determined as described in detail previously (30). Briefly, tissue was homogenized in ice-cold 50 mM Tris-HCl buffer (pH 7.0 at room temperature). The tissue homogenate was centrifuged at 38,000 × g for 12 min at 4°C. The pellet was washed twice by suspension in fresh buffer and centrifuged again. Aliquots of homogenate equivalent to approximately 10 to 20 mg tissue were used for determination of [^{3}H]cytisine binding according to Pabreza et al (1991). For total binding approximately 4 nM [^{3}H]cytisine (38.2 Ci/mmol, Dupont/NEN, Boston, MA) was incubated in a final volume of 0.25 ml at 2°C for 75 min. Nonspecific binding was obtained in the presence of 100 μM (-)-nicotine bitartrate. Membrane-bound [^{3}H]cytisine, was separated from free ligand by filtration using Brandel GF/B filter paper (soaked in 0.5% polyethylenimine to reduce nonspecific binding), and a Brandel cell harvester. Samples were run in triplicate for both total and nonspecific binding. For binding affinity measurements in cortex, 8 concentrations of [^{3}H]cytisine (0.3 to 12 nM) were utilized. Scatchard plots (for determination of Bmax and Kd) were generated by Radioligand Binding Analysis Program.

Statistical Analysis

Behavioral data were analyzed by one-way, and the neurochemical data by two-way ANOVA. These were followed by Newman-Keuls post hoc tests when significant main or interaction effects were indicated. All analyses were two-tailed and used an alpha of 0.05 or less to determine significance.

RESULTS

Effects of MEC on Acute Nicotine Administration

Fig. 1 illustrates the effects of acute nicotine, MEC or their combination on swim test immobility in FSL rats. In agree-

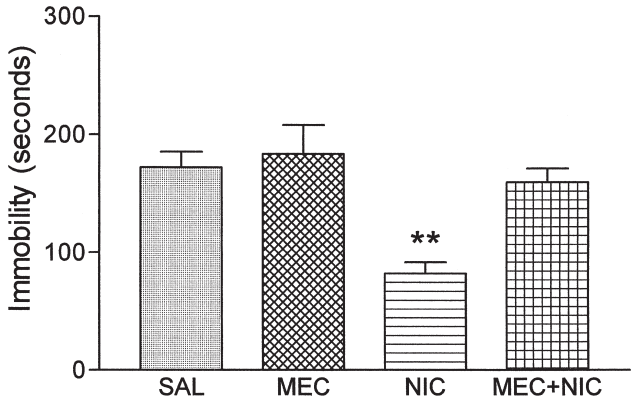

FIG. 1. Effects of acute nicotine (0.2 mg/kg), mecamylamine (MEC 0.5 mg/kg), or their combination on swim test immobility and locomotor activity in FSL rats. In combination studies, MEC preceded nicotine by 20 min. Values are mean ± SEM; ** $p < 0.01$ compared with Saline or other groups; $n = 8$ per group.

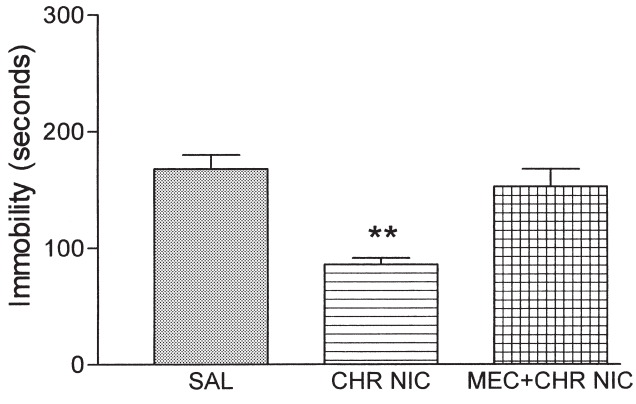

FIG. 2. Effects of acute mecamylamine (MEC) pretreatment on chronic nicotine administration on swim test immobility in FSL rats. Animals were injected daily for 14 consecutive days with 0.2 mg/kg nicotine. MEC (0.5 mg/kg) was given 20 min before nicotine on the last day of injection. Values are mean ± SEM. ** $p < 0.01$ compared with control or chronic nicotine (CHR N) + MEC; $n = 8$ per group.

ment with previous finding (30), nicotine (0.2 mg/kg) significantly reduced the swim test immobility in FSL rats $F(3, 28) = 8.17$, $p < 0.01$. MEC (0.5 mg/kg) did not have any effect on the swim test immobility by itself, however, it completely blocked the antidepressant effects of nicotine.

Effects of MEC on Chronic Nicotine Administration

Fig. 2 illustrates the effects of chronic nicotine administration alone and following a single MEC pretreatment on swim test immobility in FSL rats. Nicotine (0.2 mg/kg) for 14 days resulted in a significant reduction in swim test immobility in FSL rats $F(2, 21) = 9.2$, $p < 0.01$. This effect was completely blocked by pretreatment with MEC (0.5 mg/kg) prior to the last administration of nicotine.

Effects of Chronic Nicotine on [^{3}H]Cytisine Binding

Table 1 depicts the [^{3}H]cytisine binding in discrete brain regions of FSL and FRL rats following two weeks of nicotine or vehicle (saline) administration. A significant drug effect $F(1, 174) = 13.4$, $p < 0.01$ as well as interaction (strain x treatment) was observed $F(1, 12) = 10.1$, $p < 0.01$. Post hoc analysis revealed that in saline treated rats, binding density was significantly higher in frontal cortex (FCX), striatum (STR), midbrain (MB), and colliculi (COL) of the FSL compared with FRL rats. These results are in agreement with previous findings of higher [^{3}H]cytisine binding in the same regions of untreated FSL compared to FRL rats (30). Chronic nicotine administration resulted in an increase in [^{3}H]cytisine binding in the FCX (29%) and hippocampus (78%) of FRL, but not FSL rats. In the colliculi, where [^{3}H]cytisine binding was elevated in both FSL and FRL rats, the percent increase over baseline was significantly higher in FRL (43%) compared with FSL (26%) rats. Scatchard analysis of the ligand binding in the cortex confirmed a significant increase ($p < 0.05$) in the Bmax of the FSL saline (51.8 ± 5.0 fmole/mg protein, mean ± SEM) compared to FRL saline (40 ± 3.8 fmole/mg protein, mean ± SEM). Chronic nicotine treatment did not affect the Bmax in either FRL or FSL rats. Similarly, the binding affinity in the cortex following chronic nicotine treatment was not affected in FRL or FSL rats. The K_d values (nM, mean ± SEM) for respective groups were: FSL saline = 0.94 ± 0.19, FSL nicotine = 0.84 ± 0.12, FRL saline = 0.90 ± 0.11, FRL nicotine = 0.87 ± 2.1.

DISCUSSION

The results of this study confirm and extend our previous findings of antidepressant effects of nicotine in FSL rats (30). Here, we report that the effects of nicotine can be blocked by preadministration of the nicotinic antagonist, mecamylamine. Moreover, chronic nicotine treatment resulted in an increase in [^{3}H]cytisine binding primarily in FRL rats which do not show the depressive characteristics.

Various nicotinic receptor subtypes with distinct structural, physiological and pharmacological characteristics have been identified (1,4,5,7,18,21). The most predominant and most extensively studied subtype in the brain has a high affinity for cytisine, nicotine or acetylcholine and is formed from alpha-4 and beta-2 subunits (6,10,24). The other major class with a low affinity for nicotine and high affinity for alpha-

TABLE 1

[^{3}H]CYTISINE BINDING (fmole/mg Pr) IN VARIOUS BRAIN REGIONS OF FSL AND FRL RATS FOLLOWING 2 WEEKS OF DAILY INJECTION WITH 0.4 mg/kg NICOTINE (NIC) OR SALINE (SAL)

Region	FSL-SAL	FSL-NIC	FRL-SAL	FRL-NIC
Frontal cortex	34.7 ± 2.5*	39.0 ± 3.0	27.1 ± 1.9	35.1 ± 2.1*
Hippocampus	25.8 ± 2.0	30.4 ± 2.4	21.6 ± 2.2	38.6 ± 3.6*
Striatum	54.6 ± 3.8*	64.8 ± 6.2	41.0 ± 3.4	47.6 ± 4.9
Midbrain	39.5 ± 2.6**	39.8 ± 2.9	26.4 ± 1.2	29.1 ± 1.5
Colliculi	30.0 ± 2.5**	38.0 ± 3.0†	19.2 ± 1.6	27.6 ± 3.1**
Cerebellum	8.1 ± 0.6	9.5 ± 1.2	8.4 ± 0.4	9.8 ± 0.8

Values are Mean ± SEM, $n = 6$–8.
* $p < 0.05$ compared to FRL-Saline.
** $p < 0.01$ compared to FRL-Saline.
† $p < 0.05$ compared to FSL-Saline.
Rats were sacrificed 24 h after the last injection.

bungarotoxin is formed from alpha-7 subunits and can be labeled by [^{125}I]alpha-bungarotoxin (2). The FSL and FRL rats do not differ in [^{125}I]alpha-bungarotoxin binding (30). Mecamylamine is primarily an antagonist of the high-affinity nicotinic receptors (11,16). Hence, blockade of nicotine effect by a dose of MEC which by itself did not affect the swim test immobility further supports the involvement of the high affinity central nicotinic receptors in the depressive characteristics of the FSL rats.

An increase in nicotinic receptor binding following chronic nicotine administration is believed to reflect desensitization of these receptors and may underlie tolerance development to nicotine (19,20,33). Curiously, this effect was noted primarily in the FRL and not the FSL rats (Table 1). Previously, we had observed that compared to FRL, the FSL rats have a higher basal [^{3}H]cytisine binding in various discrete brain regions (30). It is likely that this elevated basal binding reflects an increase in functional nicotinic receptors in the FSL rats. This contention is supported by preliminary findings demonstrating that nicotine-induced dopamine release in the striatum of the FSL rats is significantly higher than in FRL rats (15). Hence, it may be suggested that the antidepressant effects of acute nicotine administration in FSL rats is related to the higher basal nicotinic receptor activity, and the continued effectiveness of chronic nicotine in this line of rats is related to the lack of desensitization and continued functioning of the central nicotinic receptors. Moreover, it is likely that specific agonists for the high-affinity nicotinic receptor subtype may have preferential antidepressant effects compared to nicotine or other nonspecific nicotinic receptor agonists.

The majority of central nicotinic receptors are located presynaptically (31,32). Stimulation of these receptors can influence the release or activity of other neurotransmitters such as dopamine and serotonin (31,32). Therefore, it is possible that the antidepressant effects of nicotine are mediated through activation of specific neuronal circuitries.

In summary, the antidepressant effects of nicotine in FSL rats, an animal model of depression were replicated in this study. Moreover, the blockade of nicotine effect by mecamylamine and the differential effects of chronic nicotine treatment on [^{3}H]cytisine binding in FRL vs. FSL rats further support a role for central nicotinic receptors in depressive characteristics of FSL rats and suggest therapeutic potential for selective nicotinic receptor agonists in depressive disorders.

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