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RAPID COMMUNICATION

Benzodiazepine Receptor Mediation of the Anxiolytic-Like Effect of (-)-Nicotine in Mice

ALYSSA B. O'NEILL AND JORGE D. BRIONI[1]

Neuroscience Research, Pharmaceutical Products Division, Abbott Laboratories, Building AP9A-LL (47 W), Abbott Park, IL 60064-3500

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O'NEILL, A. B. AND J. D. BRIONI. *Benzodiazepine receptor mediation of the anxiolytic-like effect of (-)-nicotine in mice.* PHARMACOL BIOCHEM BEHAV **49**(3) 755–757, 1994.—The anxiolytic-like effect of (-)-nicotine (1.9 μmol/kg, IP) on the elevated plus-maze in CD1 mice was blocked by the benzodiazepine receptor antagonist flumazenil (1 and 10 μmol/kg, IP). On the other hand, the cholinergic nicotinic channel blocker mecamylamine (1 to 15 μmol/kg, IP), did not affect the anxiolytic-like properties of diazepam in the same test. These data suggest that the reduction in anxiety induced by (-)-nicotine occurs indirectly via the release of endogenous substances that can activate the benzodiazepine receptor.

Nicotine Diazepam Anxiety

THERE is increasing evidence that the neuronal nicotinic acetylcholine receptor (nAChR) participates in the control of emotional behavior in rodents. The acute administration of (-)-nicotine increased the number of transitions in the light-dark transition test (4), and enhanced the activity of mice in the mirrored chamber test (2). In the elevated plus-maze model of anxiety, intraperitoneal injections of the nAChR agonists (-)-nicotine and (-)-lobeline increased the time spent by the mice in the open arms of the maze, and the effect of (-)-nicotine was blocked by the centrally acting nicotinic channel blockers mecamylamine and chlorisondamine (1).

It is presently unknown which neurotransmitter systems are involved in the induction of the anxiolytic-like effect of (-)-nicotine beyond the activation of the nAChR, although (-)-nicotine is known to influence the release of several neurotransmitters such as acetylcholine, dopamine, norepinephrine, and GABA (6). With regards to the control of emotional behavior, substantial clinical and experimental data support the participation of the benzodiazepine receptor in anxiety as the benzodiazepines are the treatment of choice for generalized anxiety disorders and panic attacks (12). Diazepam induces a significant anticonflict effect in rodents after systemic injections, and this effect can be blocked by flumazenil, a benzodiazepine receptor antagonist (7).

In the present study, the participation of the benzodiazepine receptor in the anxiolytic-like properties of (-)-nicotine was investigated in mice that were pretreated with flumazenil. If (-)-nicotine enhances the release of endogenous benzodiazepines to elicit antiaversive effects, flumazenil should block the anxiolytic action of (-)-nicotine. Conversely, to evaluate if nAChRs participate in the anxiolytic-like actions of diazepam, the interaction between diazepam and the cholinergic channel blocker mecamylamine was studied.

METHODS

Animals

Male CD1 mice from Charles River (Portage, MI) weighing 25–30 g were used. They were housed in groups of 14 in Plexiglas cages and located in a temperature-regulated environment with lights on between 0700 and 1900 h. Food and water were available ad lib. All animals used were naive to the elevated plus-maze.

Elevated Plus-Maze

The elevated plus-maze was made of grey Plexiglas and consisted of two open arms (17 × 8 cm) and two enclosed

[1] To whom requests for reprints should be addressed.

arms (17 × 8 × 15 cm) extending from a central platform (8 × 8 cm) as previously described (1). It was mounted on a Plexiglas base raised 39 cm above the floor. Fluorescent lights located in the ceeling of the room provided the only source of light to the apparatus. Light intensity in the open and enclosed arms was approximately 350 lx. At the beginning of the experiment, mice were placed in the center of the maze and the time spent in the open arms and the total distance traveled were recorded. The behavior of the mice was automatically recorded by a camera mounted above the apparatus and analyzed by computer software (Videomex-V system, Columbus Instruments, Columbus, OH). The Videomex-V system digitizes the live image of the animal, reduces it to a binary image, and then finds the center point of the image. The center point of the image correspond closely to the average center of the animal and is used to determine its position during the test. The test lasted 5 min and the apparatus was cleaned after removal of the mice. In the first experiment, (-)-nicotine was injected 30 min before the test and flumazenil 35 min before the test. In the second experiment, mecamylamine and diazepam were injected 35 and 30 min before the test, respectively.

Drugs

(-)-Nicotine bitartrate and mecamylamine hydrochloride (Sigma, St. Louis, MO) were dissolved in physiological saline solution. Doses are expressed as μmol/kg [0.3 mg/kg (-)-nicotine base = 1.9 μmol/kg]. Diazepam and flumazenil (Roche, Nutley, NJ) were suspended in physiological saline solution and Tween 80. Drugs were injected IP, in a volume of 10 ml/kg.

Statistics

Data were analyzed by a one-way analysis of variance (ANOVA) followed by the Fisher Protected Least Significant Difference test for pair-wise comparisons of group means.

RESULTS

Prior studies demonstrated that flumazenil (1-10 μmol/kg, IP) and mecamylamine (1-15 μmol/kg, IP) did not influence the behavior of mice in the elevated plus-maze test (1). The upper graph in Fig. 1 shows the anxiolytic-like effect of (-)-nicotine in CD1 mice [$F(3, 28) = 6.9, p < 0.01$]. Intraperitoneal injections of (-)-nicotine (1.9 μmol/kg) significantly increased the time spent by the mice in the open arms as compared to the saline-treated group ($p < 0.01$), and the effect of (-)-nicotine was blocked by the pretreatment with flumazenil (1 and 10 μmol/kg, IP; $p < 0.05$). On the contrary, the anxiolytic-like effect of the 3 μmol/kg dose of diazepam [$F(4, 35) = 5.3, p < 0.01$] was not affected by the pretreatment with mecamylamine (1, 5, and 15 μmol/kg, IP). (-)-Nicotine significantly increased the activity of the mice in the plus-maze [$F(3, 28) = 4.4, p < 0.05$], but this effect was not blocked by flumazenil; similarly, diazepam significantly increased locomotion in the plus-maze [$F(4, 35) = 10.0, p < 0.001$], but this effect was not blocked by mecamylamine (data not shown).

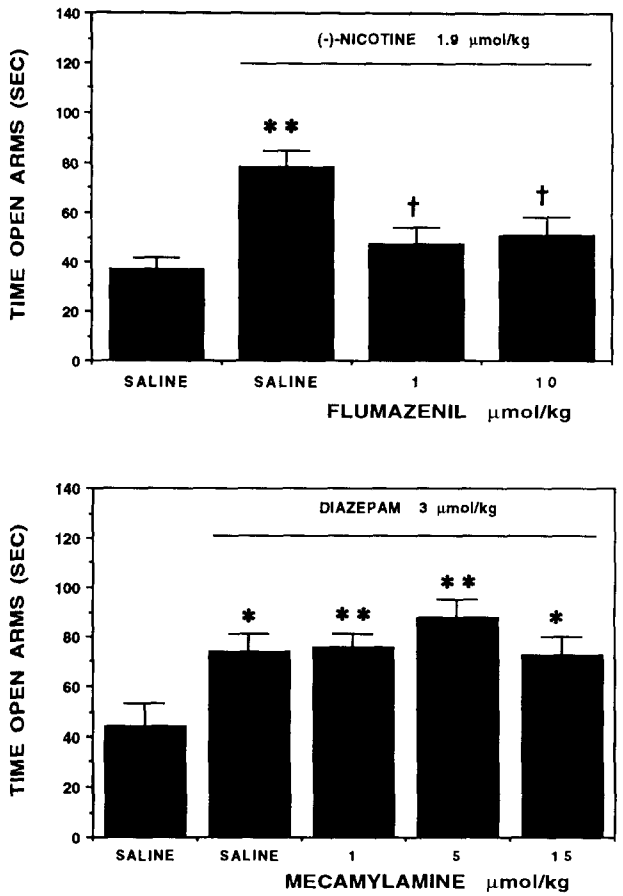

FIG. 1. Blockade of the anxiolyticlike effect of (-)-nicotine by flumazenil (upper graph) and lack of effect of mecamylamine on the anxiolyticlike action of diazepam on the elevated plus-maze (lower graph). (-)-Nicotine or diazepam were injected IP, 30 min before the test. Animals were pretreated with saline, flumazenil, or mecamylamine 5 min. before. Data represent the mean ± SEM of 8 mice per group. *$p < 0.05$; **$p < 0.01$ as compared to controls; †$p < 0.05$ as compared to the saline-nicotine group.

DISCUSSION

This study demonstrates that the nAChR agonist (-)-nicotine increased the time spent by CD1 mice in the open arms of the elevated plus-maze and that flumazenil significantly blocked the anxiolytic-like effect of (-)-nicotine. The antagonism of the effect of (-)-nicotine induced by flumazenil indicate that the activation of the benzodiazepine receptor is involved in the anxiolytic-like action of (-)-nicotine in rodents. These findings are consistent with the suggestion that (-)-nicotine's action occurs indirectly via the release of endogenous substances that can activate the benzodiazepine receptor. However, activation of nAChR is not a necessary step in the expression of the anxiolytic-like effect of diazepam, as the pretreatment with the cholinergic channel blocker mecamylamine did not influence the effect of diazepam in the elevated plus-maze.

In addition to its effect on postsynaptic targets, (-)-nicotine can act directly on presynaptic nAChRs and modulate the release of a large number of neurotransmitters in the absence of a depolarizing stimulus. The widespread distribution of the presynaptic nAChRs in the mammalian brain allows (-)-nicotine to modulate the release of acetylcholine, dopamine, norepinephrine, and GABA among others (6).

The activity of the GABAergic neurons can be allosterically modulated by endozepines, by a diazepam binding inhibitor (DBI), or by its related polypeptides (3,11), substances that have been described as putative endogenous ligands of the

benzodiazepine receptor. As they coexist with GABA in several axon terminals they are likely to be coreleased with GABA after stimulation of the nAChR located at the presynaptic site. (-)-Nicotine does not compete for the binding sites of the GABA-benzodiazepine receptor (5), but electrophysiological and biochemical studies have shown that (-)-nicotine modulates GABAergic neurotransmission by increasing the release of GABA in different brain areas such as the caudate and hippocampus (5,9,13). The extensive evidence demonstrating the participation of the hippocampus on anxiety suggest that the hippocampus could be one of the central areas mediating the antiaversive effect of (-)-nicotine.

Although flumazenil can block the facilitatory effect of (-)-nicotine in memory tests (10), the participation of the benzodiazepine receptor in the behavioral effects of (-)-nicotine is not a general phenomena. In the drug discrimination paradigm [in animals trained to discriminate 1.9 μmol/kg (-)-nicotine from a saline solution], flumazenil was devoid of any intrinsic effect and it did not block the stimulus properties of (-)-nicotine (8). As the ability to induce an interoceptive cue is one of the actions of (-)-nicotine that may lead to its abuse, the lack of effect of flumazenil in the expression of the nicotine cue suggests that a different neurobiological mechanism is regulating the abuse liability of (-)-nicotine. Despite that the exact biochemical mechanisms by which (-)-nicotine exerts the antiaversive actions are not determined, the present findings support the notion that the GABA-benzodiazepine system is involved in the anxiolytic-like actions of (-)-nicotine.

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