ELSEVIER

Pharmacology, Biochemistry and Behavior 69 (2001) 511–518

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

The anxiolytic-like effect of nicotine undergoes rapid tolerance in a model of contextual fear conditioning in rats

J. Szyndler[a], H. Sienkiewicz-Jarosz[b], P. Maciejak[c], M. Siemiątkowski[c], D. Rokicki[a], A.I. Członkowska[a], A. Płaźnik[a,d,*]

[a]*Department of Experimental and Clinical Pharmacology, Medical University, Krakowskie Przedmieście 26/28, 00-927 Warsaw, Poland*
[b]*Department of Neurology, Institute of Psychiatry and Neurology, Sobieskiego 1/9, 02-957 Warsaw, Poland*
[c]*Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Sobieskiego 1/9, 02-957 Warsaw, Poland*
[d]*Department of Neurochemistry, Institute of Psychiatry and Neurology, Sobieskiego 1/9, 02-957 Warsaw, Poland*

Received 6 December 2000; received in revised form 21 March 2001; accepted 22 March 2001

Abstract

The effects of repeated administration of nicotine on contextual fear conditioning, locomotor activity, and pain threshold, were examined in rats. It was found that a single injection of nicotine prior to the training session (three 0.7-mA footshocks, each 0.5 s long), decreased the freezing reaction during the retest 24 h later. The locomotor activity was moderately enhanced, and the pain threshold remained unchanged. The baseline freezing measured immediately after administration of a single dose of nicotine was not significantly different from the saline-treated group. The anxiolytic-like effect of nicotine was as potent as that of midazolam, a benzodiazepine derivative. After five day-by-day injections, the anxiolytic-like effect of nicotine (0.6 mg/kg, sc) was no longer present, independently whether the last drug injection was given 24 h or 5 min (i.e., the sixth, additional, nicotine injection), prior to the training session. Thus, it appeared that the expression of tolerance to the nicotine-induced anxiolytic-like action did not require a direct stimulation of nicotinic receptors. Simultaneously, in this group of animals, nicotine caused a potent stimulation of locomotor activity in the open field test. The applied dosage and regimen of nicotine administration did not change rat pain threshold (flinch–jump test). Collectively, the present data showed for the first time, that short-term, intermittent, administration of nicotine was sufficient to induce tolerance to the anxiolytic-like effect of this drug, in the model of fear conditioning to context. Importantly, a clear dissociation between the locomotor and anxiolytic-like effects of nicotine was present. This effect appeared independent also of changes in rat pain threshold. The possible mechanisms of this phenomenon are discussed.

Keywords: Nicotine; Contextual fear conditioning; Locomotor activity; Pain threshold; Tolerance; Rats

1. Introduction

It is generally recognized that cigarette smoking brings about, among others, the relief in stress and tension accompanying everyday life events. Anxiolytic effects of nicotine can be modeled in animals, and in some preclinical tests (elevated plus maze, two compartment white/black test, novelty-induced neophobia), the anxiolytic-like potency of nicotine and nicotinic receptor ligands was found to be similar to benzodiazepine derivatives (Brioni et al., 1993, 1994; Costall et al., 1989; Decker et al., 1994; Sienkiewicz-Jarosz et al., 2000). These findings have stimulated studies on the mechanisms of nicotine-induced effects, as well as screening for new nicotinic receptor ligands with more selective profile of action (Brioni et al., 1993; Decker et al., 1994). It is considered that nicotine receptor ligands may constitute a novel class of anxiolytic compounds with advantageous profile of central effects, including pro-cognitive effect (cf. Lloyd and Williams, 2000). However, there are still many unanswered questions regarding nicotine anxiolytic-like action. For example, it is not clear whether the anxiolytic-like effect of nicotine changes over repeated expo-

* Corresponding author. Department of Neurochemistry, Institute of Psychiatry and Neurology, Al. Sobieskiego str 1/9, 02-957 Warsaw, Poland. Tel.: +48-22-8427644; fax: +48-22-8427644.

E-mail address: adaplaz@yahoo.com (A. Płaźnik).

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00548-2

sures to the drug, and how rapidly this process may occur. The review of literature shows the scarcity of related data, and therefore does not allow us to draw any firm conclusion about the duration and permanency of nicotine-induced anxiolytic-like action. For instance, Ericson et al. (2000) and Olausson et al. (1999) reported that repeated nicotine injections, once a day for 15 days, simultaneously enhanced nicotine-induced locomotor stimulation and drug-induced behavioral disinhibition in the elevated plus maze. On the other hand, development of tolerance to the anxiogenic- and anxiolytic-like effects of nicotine was found in the social interaction test (Irvine et al., 1999). It is well established that tolerance develops to several effects of nicotine in rats, including antinociception, hypothermia, and anorexia, but with different dynamics (Horstmann, 1984; Mariathasan and Stolermann, 1993; McCallum et al., 1999, 2000; Yang et al., 1992). The mechanisms of these phenomena are not elucidated and may involve different central substrates. The effects of nicotine on emotional processes have been studied in numerous species and strains with a wide variance in behavioral models, doses, treatment length, and administration routes. It is, thus, not surprising that currently, no consensus exists on nicotine's effect on those processes. For example, in the social interaction test of anxiety, acute nicotine administration was found to produce both anxiolytic- and anxiogenic-like effects in a dose- and time-dependent way (Irvine et al., 1999). The different effects of nicotine suggest a different neurobiological background underlying animal behavior in the applied tests.

The aim of the present study was to investigate adaptive processes that occur over repeated administration of nicotine in another model of anxiety. This could help to better characterize the profile of nicotine-induced central effects, and to predict the potential of nicotinic receptor agonists, now undergoing clinical trials, for the treatment of anxiety disorders. To this end, the effects of repeated injections of nicotine were examined in a model of contextual fear conditioning (the freezing reaction of rats exposed to the environment previously paired with the aversive stimulation). It is noteworthy that the model of conditioned fear bears considerable construct and face similarity to the human emotional behavior, and is frequently used in preclinical studies on the emotional processes (LeDoux, 1998). The conditioned fear has been validated as an experimental model of anxiety in rodents by showing the selective anxiolytic-like effects of benzodiazepine receptor ligands, 5-HT_{1A} receptor agonists, selective serotonin reuptake inhibitors, and monoamine oxidase inhibitors, with verified clinical efficacy, injected either systemically or centrally (Beck and Fibiger, 1995; Harris and Westbrook, 1998; Inoue et al., 1996; Maki et al., 2000; Muraki et al., 1999; Yoshioka et al., 1995). Further, it was found that a benzodiazepine derivative produced dose-related decreases in the frequency of freezing, and in conditioned stress-induced the immediate early gene c-*fos* expression, in the brain limbic structures (Beck and Fibiger, 1995). Simultaneously, the effects of repeated nicotine treatment on rat locomotor activity and pain perception were examined in the presented study.

2. Methods

2.1. Subjects

Adult male Wistar rats (200 ± 20 g) were used in the study. The animals were housed two per cage in standard laboratory conditions under 12 h cycle (lights on at 6:00 a.m.) in a controlled temperature (2 ± 2°C) and 70% humidity. The rats were given free access to food and water. Separate groups of animals were used in each part of the study: contextual fear conditioning, locomotion testing, and the flinch–jump test. All experiments were done between 9:00 a.m. and 3:00 p.m. All experimental procedures using animal subjects were approved by the Committee for Animal Care and Use at the Institute of Psychiatry and Neurology.

2.2. Drugs

The following drugs were used in the experiments: midazolam maleate (Hoffmann-La Roche), and nicotine-di-D-tartrate (RBI, Natick, USA). The drugs were dissolved in saline. Nicotine solutions were adjusted to pH 7.0–7.2 with diluted NaOH.

2.3. Administration regimen

2.3.1. Acute treatment

The animals received a single injection of saline, midazolam maleate (1.5 mg/kg), and nicotine (0.6 and 1.0 mg/kg) either intraperitoneally (saline, midazolam, ip), or subcutaneously (saline, nicotine, sc), 20 (saline, midazolam) or 5 min before test (saline, nicotine). The drugs were given in a volume of 2.0 ml/kg of saline. The control rats received injections of saline.

2.3.2. Repeated treatment

The animals received six repeated, once-a-day, injections of saline (control, 2.0 ml/kg, sc) or nicotine (0.6 mg/kg, 2.0 ml/kg, sc). On the fifth day, injections were given after habituation of the animals to the experimental chamber. On the sixth day, the injections of saline or nicotine were given 5 min before the training session of the contextual fear conditioning.

2.4. Contextual fear conditioning test

The test was done in two boxes (30 × 30 × 60 cm each) made of Plexiglas, with a grid floor made of stainless steel bars wired to shock generator. The boxes

were cleaned after each trial with 95% ethanol. The experiment was performed during three consecutive days in the same testing boxes and experimental chamber. On the first day, the animals were placed separately for 2 min in a training box, for adaptation to the experimental conditions. The following day after the animals were placed in the experimental box, they were observed and videotaped for 5 min, via a short-circuit television, for spontaneously occurring freezing behavior (baseline freezing). Immediately afterwards, the animals received three 0.5-s footshocks (trains of stimuli: 0.7 mA, 150/300 ms, repeated every 60 s). The animals were removed from the testing boxes 3 min after the last shock was delivered. On the following day, the freezing behavior of rats was examined for 10 min. The conditioned response was recorded with the help of a video camera for a later analysis of the freezing reaction. The freezing behavior was defined as the absence of any visible body movements except for those required for respiration. The behavioral observation was performed by an experimenter unaware of group membership.

2.5. Flinch–jump test

The test was performed in the footshock boxes used in the part of the experiment on contextual fear conditioning. The naive rats were placed individually into the box. Shocks were delivered to the grid floor of the test box through a shock generator. After a 3-min period of habituation to the test box, shock titrations were continued upwards and downwards in a stepwise manner (0.05 mA, 0.05–0.85 mA range) depending upon responsiveness of the rat. The flinch threshold was defined as the lowest shock intensity that elicited any detectable response. The jump threshold was defined as the lowest shock intensity that elicited simultaneous removal of at least three paws (both hindpaws) from the grid. To avoid foot damage, the cut-off = 1.0 mA was established. In this way, the flinch and jump thresholds in milliamperes were defined for each rat. The time gap between shocks was 10 s, and each animal was tested only once. The time between drug administration and testing was the same as in the contextual fear conditioning test. Nicotine (0.6 mg/kg, sc) was administered acutely and repeatedly according to the same experimental schedule as in case of contextual fear conditioning.

2.6. Open field test

The test was performed in a soundproof chamber under dim light and continuous white noise (65 dB) without previous habituation. The open field test apparatus used in the experiment consisted of two round arenas (80-cm diameter) with 30-cm high walls, each equipped symmetrically with three photocells 8 cm above the floor. During a 20-min registration period, the general activity, defined as a number of photobeam interruptions, was measured. The animals were observed and videotaped via a short-circuit television for a later analysis of behavior. The time between drug administration and testing was the same as in the contextual fear conditioning test. Nicotine (0.6 mg/kg, sc) was administered acutely and repeatedly according to the same experimental schedule as in case of contextual fear conditioning.

2.7. Statistical analysis

The results are shown as means ± S.E.M. The data involving one control group and one treated group were analyzed using Student's t test for independent samples. The data involving multiple comparisons were analyzed by one-way or two-way ANOVA, followed by Newman–Keuls post hoc test. A probability value ($P < .05$) was considered significant in this study.

3. Results

3.1. Contextual fear conditioning test

Midazolam administered intraperitoneally at the dose 1.5 mg/kg enhanced in a statistically significant way spontaneously occurring freezing reactions, evaluated in rats for 5 min immediately prior to contextual fear conditioning ($t = 2.25$, $P < .05$, Student's t test) (Fig. 1). The same dose of this drug significantly decreased freezing reactions examined 24 h after aversive conditioning ($t = 2.66$, $P < .05$, Student's t test) (Fig. 1).

A single injection of nicotine did not change the duration of a freezing reaction during the preconditioning session [$F(2,27) = 0.22$, $P > .05$] (Fig. 1). However, this drug significantly decreased the time of freezing exhibited by rats next day after aversive training [$F(2,27) = 3.15$, $P = .05$]. Post hoc test revealed a significant antifreezing effect of both examined doses of nicotine (0.6 mg/kg, $P < .04$; 1.0 mg/kg, $P < .03$) (Fig. 1).

Two-way ANOVA showed a significant main effect of pretreatment conditions (repeated saline vs. repeated nicotine) on rat freezing reaction during the preconditioning session [$F(1,29) = 7.66$, $P < .009$] (Fig. 2). No significant effect of treatment conditions (acute saline vs. acute nicotine) [$F(1,29) = 0.37$, $P > .05$], or pretreatment × treatment interaction [$F(1,29) = 1.66$, $P > .05$] was found (Fig. 2). Post hoc test indicated that rats in the repeated nicotine/acute nicotine-treated group were much less inhibited and expressed less freezing during the preconditioned session ($P < .02$).

Two-way ANOVA revealed also an overall significant effect of treatment conditions [$F(1,29) = 9.01$, $P < .005$], as well as of pretreatment × treatment interaction [$F(1,29) = 18.58$, $P < .001$], on rat freezing reaction examined 24 h after fear conditioning (Fig. 2). In the post hoc

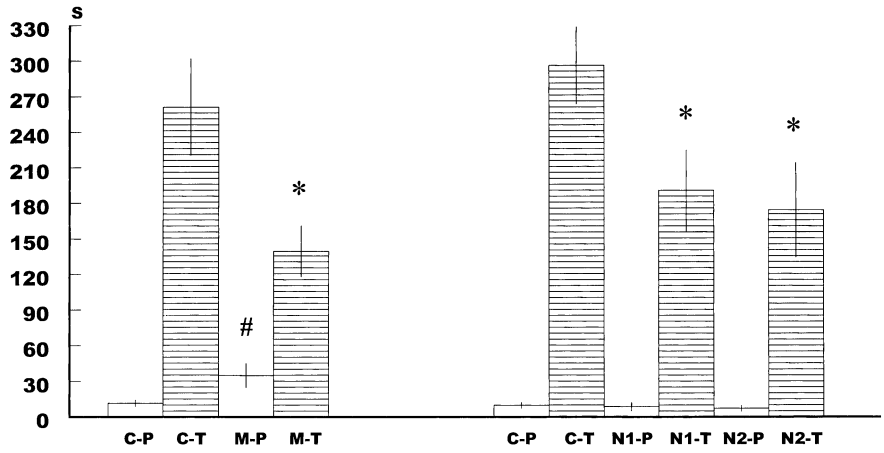

Fig. 1. The effects of a single injection of midazolam (M) and nicotine (N) on rat behavior in the contextual fear conditioning test. The data are shown as mean ± S.E.M. Ordinate: duration of freezing behavior (s), recorded during the preconditioning (P) and postconditioning (test — T) session. C: control; M: midazolam 1.5 mg/kg; N1: nicotine 0.6 mg/kg; N2: nicotine 1.0 mg/kg. Open bars: preconditioning session; striped bars: postconditioning session. The number of rats in each group varied from 8 to 10. # differs from C-P group; * differs from appropriate C-T group. $^{\#}$, * $P < .05$.

analysis of data, only the group of rats given an acute injection of nicotine (0.6 mg/kg) against the background of chronic saline pretreatment showed reduced duration of the freezing behavior ($P < .01$).

3.2. Open field test

Two-way ANOVA showed a significant main effect of pretreatment conditions (repeated vs. acute treatment) [$F(1,16) = 42.45$, $P < .001$] (Fig. 3), and a significant effect of treatment conditions (saline vs. nicotine) [$F(1,16) = 50.10$, $P < .001$], on rat locomotor activity. There was no significant pretreatment × treatment interaction [$F(1,16) = 1.87$, $P > .05$]. Post hoc test revealed that rat locomotor activity was increased after both acute and repeated nicotine administration at the dose of 0.6 mg/kg (acute, $P < .01$; subchronic, $P < .001$). The effect of repeatedly administered nicotine was more potent than that of acutely injected drug ($P < .01$).

3.3. Flinch–jump test

Two-way ANOVA did not reveal significant effects of experimental conditions on the rat's flinch reaction to the painful stimulation [Pretreatment, $F(1,32) = 0.46$, $P > .05$; Treatment, $F(1,32) = 1.21$, $P > .05$] (Fig. 3). Two-way ANOVA did reveal a main effect of treatment conditions on the jump reaction [$F(1,32) = 4.72$, $P < .03$]. Post hoc test

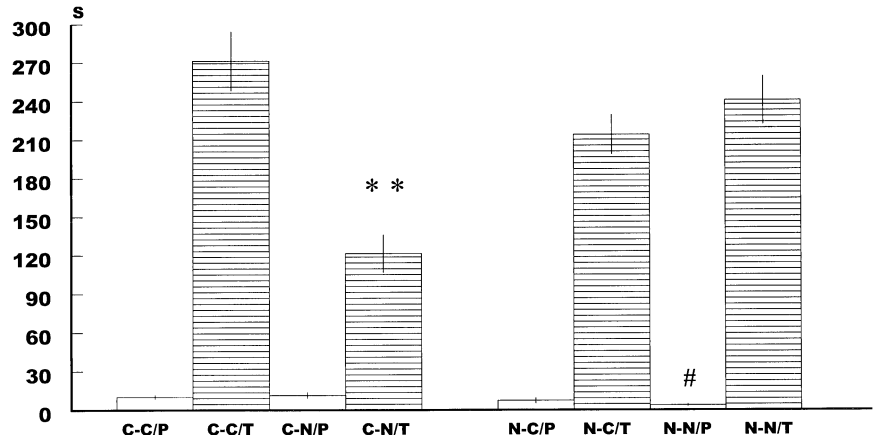

Fig. 2. The effects of repeated administration of nicotine (0.6 mg/kg, sc) on rat freezing behavior in the contextual fear conditioning test. The data are shown as means ± S.E.M. Ordinate: duration of freezing behavior (s), recorded during the preconditioning (P) and postconditioning (test — T) session. C-C: control rats treated repeatedly with saline; C-N: animals treated repeatedly with saline and given an acute injection of nicotine on the last sixth day, 5 min before fear conditioning; N-C: rats treated repeatedly with nicotine, and given an acute injection of saline on the last sixth day, 5 min before fear conditioning; N-N: rats treated repeatedly with nicotine, and given the same drug on the last sixth day, 5 min before fear conditioning. Open bars: preconditioning session; striped bars: postconditioning session. The number of rats in each group varied from 8 to 10. # differs from C-C/P group; * differs from C-C/T group. $^{\#}$ $P < .05$; ** $P < .01$.

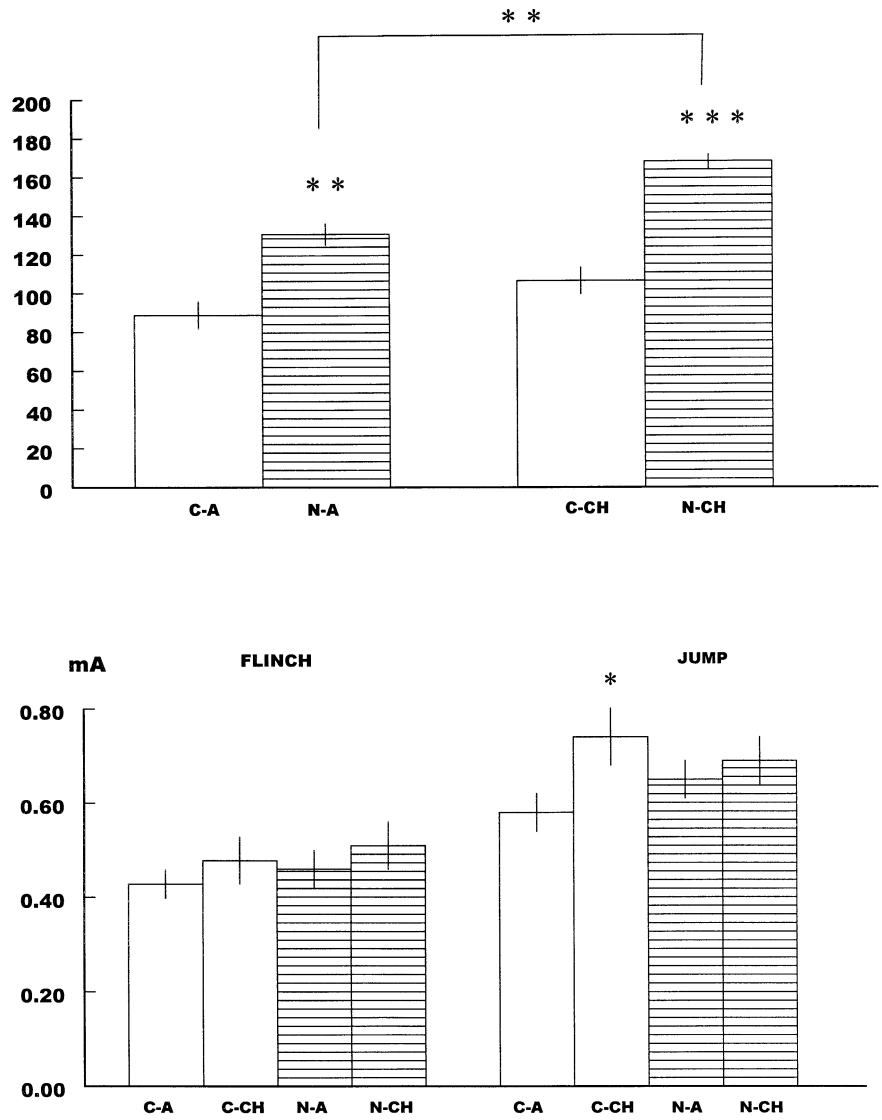

Fig. 3. The effects of acute and repeated nicotine administration (0.6 mg/kg, sc) on rat locomotor behavior (upper part of the figure), and pain perception (lower part of the figure). The data are shown as means ± S.E.M. Ordinate: the number of photobeam interruptions, and the shock threshold in milliamperes (mA). C-A: control rats given a single injection of saline; C-CH: animals treated repeatedly with saline (for 6 days); N-A: animals treated acutely with nicotine; N-CH: animals treated repeatedly with nicotine, and injected with this drug on the last sixth day, 5 min before the test. The number of rats in each group varied from 8 to 10. Open bars: control group; striped bars: nicotine group. * differs from C-A and C-CH group (upper part of the figure), and from C-A (lower part of the figure). * $P < .05$; ** $P < .01$; *** $P < .001$.

showed that in the repeatedly saline-injected rats, the threshold to the nociceptive stimulus was significantly increased in the control group only ($P < .02$) (Fig. 3).

4. Discussion

A major finding of the present study is that the development of tolerance to the anxiolytic-like effect of nicotine occurred already after a few days of drug administration. To our knowledge, this is the first report on attenuation of the anxiolytic-like effect of nicotine in an aversively motivated conditioned task. Limited data on this topic indicate either a development of tolerance to the anxiogenic- and anxiolytic-like effects of nicotine in the social interaction test (Irvine et al., 1999), sensitization to the nicotine-induced locomotor stimulation (Dwoskin et al., 1999; Ericson et al., 2000; Whiteaker et al., 1995), or drug-induced behavioral disinhibition in the elevated plus maze (Ericson et al., 2000).

The effect of acute injection of nicotine on contextual fear conditioning was similar to that of midazolam, a benzodiazepine derivative. Nicotine (0.6 and 1.0 mg/kg, sc) significantly attenuated the aversive influence of a stressful environment on retest, when the drug was given immediately

prior to conditioning trial. The action of nicotine was independent of changes in the pain threshold and animal spontaneous activity, as shown by the unchanged freezing time during the preconditioning session. Moreover, although acutely administered nicotine enhanced rat locomotor activity, the drug-induced moderate changes in animals motility were not likely to contribute directly to fear conditioning, as the much more potent motor stimulation after repeatedly injected nicotine, correlated in a negative way with the animal's freezing behavior. Recently, a single injection of nicotine (1.0 mg/kg) was found to decrease context-associated freezing also in mice (Gould and Wehner, 1999). No effect was found at the lower dose of the drug (0.5 mg/kg). On the other hand, nicotine (0.5 mg/kg) given on both training and testing days enhanced freezing reaction, what was interpreted as an improvement of contextual learning, a phenomenon related to the cognitive enhancing effect of nicotine.

Repeated nicotine administration sensitized the animals to the drug-induced locomotor stimulation (motor sensitization). Behavioral disinhibition was also manifested as a decrease in the duration of freezing during the preconditioning session, in the group of rats chronically pretreated with nicotine. Importantly, this phenomenon, reported also by others (Benwell and Balfour, 1992; Ericson et al., 2000; Ksir et al., 1985; Olausson et al., 1999), did not interfere with the return of a fear-controlled behavior to the control values, in the nicotine-pretreated rats. The pain threshold remained unchanged after acute and repeated nicotine injections (0.6 mg/kg, sc). Likewise, it was previously shown that nicotine administered to rats over a similar range of doses (0.25–1.0 mg/kg), failed to alter the aversive threshold in this particular model of pain reactivity (Rodgers, 1979). Collectively, the data on changes in locomotor activity and pain sensitivity suggest that attenuation of the anxiolytic-like effect of nicotine over repeated drug administration is selectively related to modification of the brain emotional processes. It is possible that this phenomenon reflects the different roles played by the subtypes of nicotinic receptors in the regulation of various central nervous system functions, characterized also by the different dynamics of nicotine-induced adaptive processes (Clementi et al., 2000; Marubio and Changeux, 2000; Picciotto et al., 2000).

It has been shown that contextual fear conditioning is associated with increased acetylcholine (ACh) release in the hippocampus of the rat's brain (Nail-Boucherie et al., 2000). Specifically, reexposure to the conditioning chamber the day after conditioning produced a significantly greater increase in ACh level in the conditioned (that otherwise displayed conditioned freezing behavior to contextual cues), than in the control group (which displayed no freezing). The enhanced hippocampal ACh release was suggested to result from the hippocampal processing of contextual stimuli in conditioned animals. This result supports the view that cholinergic system is specifically involved in processing of the contextual stimuli as a cue, and the hypothesis that enhanced cholinergic activity is an important element of brain circuitry engaged in coping with a stressful event (Nail-Boucherie et al., 2000). If such, an acute injection of nicotine could further add to the increase in activity of the cholinergic system, and to manifest as the anxiolytic-like action in the contextual fear conditioning test. Accordingly, it was found that administration of nicotine (0.4 mg/kg, sc) significantly increased the levels of ACh in hippocampal dialysates in rats (in vivo microdialysis) (Reid et al., 1999). The underlying mechanism may involve stimulation by nicotine of the release of endogenous GABA (Lu et al., 1998; Köfalvi et al., 2000).

It seems that changes in the cognitive processes could not directly account for the discussed phenomenon, as the well-known pro-cognitive influence of nicotine should rather increase rat behavior controlled by fear. Nicotine has been shown to enhance learning and memory performance in a variety of experimental animal and human studies (cf. Levin and Rezvani, 2000). Furthermore, chronic nicotine infusion has been demonstrated in rat studies to improve memory performance (Levin et al., 1993). The fact that, in the present experiment, tolerance was revealed in the group of animals given the last nicotine injection 24 h before conditioning trial, excludes also the possibility of a state-dependent learning as the underlying mechanism, and the direct influence of the drug on the acquisition and retrieval processes. Similar effects of acute administration of both drugs, nicotine and midazolam, with very well recognized and opposite influence on the cognitive functions of the brain, further indicate that changes in memory and learning probably do not play an important role in the tolerance development. Thus, the role of more specific, emotion-related mechanisms, is suggested. Besides, the short half-life time of subcutaneously administered nicotine (about 50 min in the brain) makes also the possibility of a direct involvement of the pharmacokinetic factors (i.e., changes in the tissue drug disposition and metabolism) in the described phenomenon less likely (Ghosheh et al., 1999).

In contrast with the present data, in a recently published paper, Ericson et al. (2000) reported that repeated nicotine injections (0.35 mg/kg, sc, once a day for 15 days), simultaneously sensitized the animals to the nicotine-induced locomotor stimulation, and drug-induced behavioral disinhibition in the elevated plus maze. The nicotine-induced disinhibition was interpreted to reflect increased impulsivity rather than anxiolysis (Ericson et al., 2000; Olausson et al., 1999). Such a conclusion disagrees with the results of the present study. A clear separation of the enhancement of animal motility and increase of the freezing time, almost to the control group level, was found after intermittent administration of nicotine. Importantly, similar changes in the freezing behavior were observed in the group of animals given the last nicotine injection either 5 min or 24 h before fear conditioning. It means that the expression of tolerance to the nicotine-induced anxiolytic-like action does not require a direct stimulation of nicotinic receptors. These findings may be difficult to interpret. It is conceivable that the differences between the results of the present study and of the previously

published papers may be due to an employment of different models of anxiety, involving separate neural substrates, and therefore undergoing adaptive changes in a different time- and dose-dependent way. Apparently, the model of contextual fear conditioning allows for a more direct control of the role of behavioral disinhibition in the examined processes. The presented data indicate a clear dissociation of changes in motor activity and the development of tolerance to the anxiolytic-like effects of nicotine.

The underlying central mechanism may involve adaptive changes in the brain dopaminergic systems. Accordingly, it was recently found using similar experimental procedure (daily injections for 5 days with nicotine, 0.15 mg/kg, sc), that repeated nicotine administration produced differential effects on dopaminergic systems, with mesolimbic projections (nucleus accumbens septi) showing sensitization with enhanced dopamine turnover, and mesocortical projections (medial prefrontal cortex) showing tolerance to acute nicotine effects (George et al., 1998, 2000). Furthermore, it was demonstrated that repeated but not acute nicotine pretreatment selectively reduced the responsivity of these mesoprefrontal projections to acute electrical footshock stress (George et al., 1998, 2000). These biochemical data indicate how changes in locomotion and a freezing response, observed in the present study, may evolve over repeated nicotine administration, and that the anatomical substrates for these adaptive phenomena are separately located.

Collectively, the presented data show that short-term, intermittent administration of a moderate dose of nicotine is sufficient to induce tolerance to the drug-induced anxiolytic-like action, in the model of contextual fear conditioning. This effect appears selective, independent of changes in the locomotor activity and pain threshold. Thus, tolerance to the nicotine's psychotropic effect, reported also in the clinic (Perkins et al., 1994), has been demonstrated in the animal model of a fear-controlled behavior. The available data indicate that selective reduction in responsivity of dopaminergic mesoprefrontal projections to the acute electrical footshock stress may constitute an intrinsic mechanism of tolerance to the anxiolytic-like effect of nicotine.

Acknowledgments

This work was supported by Grant No. 4 P05A 009 18 from the Polish State Committee for Scientific Research. H. Sienkiewicz-Jarosz, A.I. Członkowska and M. Siemiątkowski were supported by Grants from the Foundation for Polish Science.

References

Beck CH, Fibiger HC. Conditioned fear-induced changes in behavior and in the expression of the immediate early gene c-*fos*: with and without diazepam treatment. J Neurosci 1995;15:709–20.

Benwell MEM, Balfour DJK. The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. Br J Pharmacol 1992;105:849–56.

Brioni JD, O'Neill AB, Kim DJB, Decker MW. Nicotinic receptor agonists exhibit anxiolytic-like effects on the elevated plus maze test. Eur J Pharmacol 1993;238:1–8.

Brioni JD, O'Neill AB, Kim DJB, Buckley MJ, Decker MW, Arneric SP. Anxiolytic-like effects of the novel cholinergic channel activator ABT-418. J Pharmacol Exp Ther 1994;271:353–61.

Clementi F, Fornasari D, Gotti C. Neuronal nicotinic receptors, important new players in brain function. Eur J Pharmacol 2000;393:3–10.

Costall B, Kelly ME, Naylor RJ, Onaivi ES. The actions of nicotine and cocaine in a mouse model of anxiety. Pharmacol, Biochem Behav 1989;33:197–203.

Decker MW, Brioni JD, Sullivan JP, Buckley MJ, Radek RJ, Raszkiewicz JL, Kang DJB, Giardina WJ, Wasicak JT, Garvey DS, Williams M, Arneric S. (*S*)-3-Methyl-5(1-methyl-2-pyrrolidynyl)isoxazole (ABT 418): a novel cholinergic ligand with cognition-enhancing and anxiolytic activities: II. In vivo characterisation. J Pharmacol Exp Ther 1994;270(1):319–28.

Dwoskin LP, Crooks PA, Teng LH, Green TA, Bardo M. Acute and chronic effects of nornicotine on locomotor activity in rats: altered response to nicotine. Psychopharmacology 1999;145:442–51.

Ericson M, Olausson P, Engel JA, Söderpalm B. Nicotine induces disinhibitory behavior in the rat after subchronic peripheral nicotinic acetylcholine receptor blockade. Eur J Pharmacol 2000;397:303–11.

George TP, Verrico ChD, Roth RH. Effects of repeated nicotine pre-treatment on mesoprefrontal dopaminergic and behavioral responses to acute footshock stress. Brain Res 1998;801:36–49.

George TP, Verrico ChD, Xu L, Roth RH. Effects of repeated nicotine administration and footshock stress on rat mesoprefrontal dopamine systems: evidence for opioid mechanisms. Neuropsychopharmacology 2000;23:79–88.

Ghosheh O, Dwoskin LP, Li WK, Crooks PA. Residence times and half-lives of nicotine metabolites in rat brain after acute peripheral administration of [2′-(14)C]nicotine. Drug Metab Dispos 1999;27(12):1448–55.

Gould TJ, Wehner JM. Nicotine enhancement of contextual fear conditioning. Behav Brain Res 1999;102:31–9.

Harris JA, Westbrook RF. Benzodiazepine-induced amnesia in rats: reinstatement of conditioned performance by noxious stimulation on test. Behav Neurosci 1998;112:183–92.

Horstmann M. Influence of mecamylamine and atropine on tolerance development to nicotine hypothermia in rats. J Pharm Pharmacol 1984;36(11):770–1.

Inoue T, Tsuchiya K, Koyama T. Serotonergic activation reduces defensive freezing in the conditioned fear paradigm. Pharmacol, Biochem Behav 1996;53:825–31.

Irvine EE, Cheeta S, File SE. Time course of changes in the social interaction test of anxiety following acute and chronic administration of nicotine. Behav Pharmacol 1999;10:691–7.

Köfalvi A, Sperlágh B, Zelles T, Vizi S. Long-lasting facilitation of 4-amino-*n*{2,3-[^{3}H]butyric acid ([^{3}H]GABA) release from rat hippocampal slices by nicotinic receptor activation. J Pharmacol Exp Ther 2000;295(2):453–62.

Ksir C, Hakan R, Hall J, Kellar KJ. Exposure to nicotine enhances the behavioral stimulant effect of nicotine and increases binding of [^{3}H]acetylcholine to nicotinic receptors. Neuropharmacology 1985; 24(6):527–32.

LeDoux J. Fear and the brain: where have we been, and where are we going. Biol Psychiatry 1998;44:1229–38.

Levin ED, Rezvani AH. Development of nicotinic drug therapy for cognitive disorders. Eur J Pharmacol 2000;393:141–6.

Levin ED, Briggs SJ, Christopher NC, Rose JE. Chronic nicotine stimulation and blockade effects on working memory. Behav Pharmacol 1993;4:179–82.

Lloyd GK, Williams M. Neuronal nicotinic acetylcholine receptors as novel drug targets. J Pharmacol Exp Ther 2000;292(2):461–7.

Lu Y, Grady S, Marks MJ, Picciotto M, Changeux JP, Collins AC. Pharmacological characterisation of nicotinic receptor stimulated GABA release from mouse brain synaptosomes. J Pharmacol Exp Ther 1998;287(2):648–57.

Maki T, Inoue T, Izumi T, Maraki I, Ita K, Kitaichi Y, Li X, Koyama T. Monoamine oxidase inhibitors reduce conditioned fear stress-induced freezing behavior in rats. Eur J Pharmacol 2000;406:411–8.

Mariathasan EA, Stolermann IP. Discrimination of agonist–antagonist mixtures: experiments with nicotine plus mecamylamine. Behav Pharmacol 1993;4:555–61.

Marubio LM, Changeux JP. Nicotinic receptor knockout mice as animal models for studying receptor function. Eur J Pharmacol 2000; 393:113–21.

McCallum SE, Caggiula AR, Epstein LH, Saylor S, Ploskina T, Sved AF. Mecamylamine blocks the development of tolerance to nicotine in rats: implications for the mechanisms of tolerance. Psychopharmacology 1999;141(3):332–8.

McCallum SE, Caggiula AR, Breese ChR, Lee MJ, Donny EC, Leonard S, Sved AF. Mecamylamine prevents tolerance but enhances whole brain [^{3}H]epibatidine binding in response to repeated nicotine administration in rats. Psychopharmacology 2000;150:1–8.

Muraki I, Inoue T, Hashimoto S, Izumi T, Ito K, Ohmori T, Koyama T. Effect of subchronic lithium carbonate and MKC-242 in conditioned fear stress in rats. Eur J Pharmacol 1999;383:223–9.

Nail-Boucherie K, Dourmap N, Jaffard R, Costentin J. Contextual fear conditioning is associated with an increase of acetylcholine release in the hippocampus of rats. Cognit Brain Res 2000;9:193–7.

Olausson P, Engel JA, Söderpalm B. Behavioral sensitization to nicotine is associated with behavioral disinhibition: counteraction with citalopram. Psychopharmacology 1999;142:111–9.

Perkins KA, Grobe JE, Fonte C, Goettler J, Caggiula AR, Reynolds WA. Chronic and acute tolerance to subjective, behavioural and cardiovascular effects of nicotine in humans. J Pharmacol Exp Ther 1994; 270(2):628–38.

Picciotto MR, Caldarone BJ, King SL, Zachariou V. Nicotinic receptors in the brain: links between molecular biology and behavior. Neuropsychopharmacology 2000;22(5):451–65.

Reid RT, Lloyd GK, Rao TS. Pharmacological characterization of nicotine-induced acetylcholine release in the rat hippocampus in vivo: evidence for a permissive dopamine synapse. Br J Pharmacol 1999;127:1486–94.

Rodgers RJ. Effects of nicotine, mecamylamine, and hexamethonium on shock induced fighting, pain reactivity, and locomotor behaviour in rats. Psychopharmacology 1979;66:93–8.

Sienkiewicz-Jarosz H, Członkowska AI, Siemiątkowski M, Maciejak P, Szyndler J, Płaźnik A. The effects of physostigmine and cholinergic receptor ligands on novelty-induced neophobia. J Neural Transm 2000;107(12):1403–10.

Whiteaker P, Garcha HS, Wonnacott S, Stolerman IP. Locomotor activation and dopamine release produced by nicotine and isoarecolone in rats. Br J Pharmacol 1995;116:2097–105.

Yang C, Wu W, Zbuzek VK. Antinociceptive effect of chronic nicotine and nociceptive effect of its withdrawal measured by hot-plate and tail-flick in rats. Psychopharmacology 1992;106(3):417–20.

Yoshioka M, Matsumoto M, Togashi H, Saito H. Effects of conditioned fear stress on 5-HT release in the rat prefrontal cortex. Pharmacol, Biochem Behav 1995;51(2–3):515–9.