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Nicotine-induced changes in cerebrocortical neuroactive steroids and plasma corticosterone concentrations in the rat

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Abstract

Nicotine, one of the most widely used psychotropic substances, is able to induce both anxiolytic and anxiogenic effects. The effect of this drug on the brain and plasma concentrations of neuroactive steroids was examined in the rat. Anxiolytic doses of nicotine (0.03–0.3 mg/kg) had no significant effect, whereas administration of anxiogenic doses (0.5 to 2 mg/kg) produced a dose- and time-dependent increase in the cerebrocortical concentrations of pregnenolone, progesterone, and allopregnanolone, with the greatest observed effects (+180%, +223%, and +124%, respectively) apparent at the dose of 2 mg/kg. In contrast, nicotine (1–2 mg/kg) decrease by 31% and 38%, respectively, the concentration of 3α,21-dihydroxy-5α-pregnan-20-one (allotetrahydrodeoxycorticosterone, or THDOC) in the cerebral cortex. Nicotine also increased the plasma concentrations of pregnenolone and progesterone, whereas failed to affect significantly those of allopregnanolone or THDOC. Nicotine induced a dose- and time-dependent increase in the plasma concentration of corticosterone, indicating that this drug activates the hypothalamic–pituitary–adrenal (HPA) axis. These results suggest that the changes in emotional behavior elicited by nicotine, similar to those induced by stressful stimuli or other anxiogenic drugs, are associated with an increase in neuroactive steroids content of the brain.

Keywords: Nicotine; Allopregnanolone; THDOC; Neuroactive steroids; Corticosterone; Brain; Plasma; Rat

1. Introduction

Nicotine, the major psychoactive constituent of tobacco, affects many physiological functions of the central nervous system by modulating the activity of populations of neurons that contribute to emotional behavior, learning and memory, reward, and sexual receptivity (Cordero-Erausquin et al., 2000). Through its interaction with nicotinic acetylcholine receptors in the brain, which are located predominantly on presynaptic terminals, nicotine thus modulates the release of many neurotransmitters, including serotonin (5-HT), dopamine, noradrenaline, and γ-aminobutyric acid (GABA) (Imperato et al., 1986; Brazell et al., 1991; Ribeiro et al., 1993; Lena and Changeux, 1997). Like heroin, cocaine, and alcohol, nicotine induces both a sense of well-being and physical dependence (Jones, 1987). At doses commonly inhaled by smokers, this alkaloid improves physical and intellectual performance (Pritchard et al., 1992), increases moodiness, and affects anxiety and depression (Breslau et al., 1991). In particular, it reduces stress-related anxiety in humans (Wesnes and Warburton, 1983) and may have an antidepressant action in nonsmokers with major depression (Salìn-Pascual et al., 1996). At higher doses, nicotine induces such unpleasant effects as bodily discomfort, nausea, vomiting, and seizures (Taylor, 2001).

Although the effects of nicotine on emotional behavior have been studied extensively in both humans and animals, the results appear equivocal given that both anxiolytic and anxiogenic actions have been described (Costall et al., 1989; Vale and Green, 1996; File et al., 1998, 2000a, 2001; Netter et al., 1998). For instance, in the social interaction test of anxiety, systemic administration of low doses of nicotine elicits anxiolytic effects similar to those induced by drugs

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usually used in the treatment of anxiety; in contrast, anxiogenic effects are induced by administration of high doses of this drug (File et al., 1998). These two actions are mediated by distinct brain regions, with the dorsal raphé nucleus mediating the anxiolytic effects and the dorsal hippocampus and lateral septum mediating the anxiogenic effects (Cheeta et al., 2000, 2001; File et al., 2000b).

Dopamine appears to be the main neurotransmitter responsible for mediating the behavioral effects of nicotine (Di Chiara, 2000). Indeed, like several other drugs of abuse, nicotine activates the mesolimbic dopamine system, and this effect appears to be critical for the reinforcing properties of this drug. However, 5-HT also may play an important role in mediating specifically the acute effects of nicotine on anxiety. The local administration in specific brain regions of the 5-HT_{1A} receptor antagonist WAY100635, thus, completely reversed both the anxiolytic and anxiogenic effects of nicotine (Cheeta et al., 2000, 2001).

The neuroactive steroids 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) and 3α,21-dihydroxy-5α-pregnan-20-one (allotetrahydrodeoxycorticosterone, or THDOC) induce pharmacological (anxiolytic, sedative, hypnotic, anticonvulsant) effects similar to those elicited by anxiolytic drugs (Biggio and Purdy, 2001). The concentrations of these neurosteroids are increased in the brain and plasma of animals or humans both in response to treatment with anxiogenic, antidepressant, or antipsychotic drugs (Barbaccia et al., 1997, 2001; Uzunova et al., 1998; Concas et al., 2000; Marx et al., 2000) as well as in physiological or pathological conditions (such as depression, stress, the luteal phase of the menstrual cycle, and pregnancy) that affect mood and emotional state (Purdy et al., 1991; Barbaccia et al., 1996a,b, 1997; Wang et al., 1996; Concas et al., 1998; Uzunova et al., 1998). These observations have suggested that changes in the synthesis of neuroactive steroids might contribute to both the physiopathology and pharmacology of a variety of neurological and psychiatric disorders. Indeed, a specific decrease in the plasma and cerebrospinal fluid concentrations of allopregnanolone, and the normalization of these concentrations after treatment with antidepressant drugs, have been described in individuals with major depression (Uzunova et al., 1998). Moreover, the plasma concentration of allopregnanolone appears to correlate with a feeling of well being in women with premenstrual syndrome (Wang et al., 1996). Finally, the recent observations that olanzapine and clozapine, two atypical antipsychotic drugs, increase the brain and plasma concentrations of neuroactive steroids in the rat (Marx et al., 2000; Barbaccia et al., 2001) are consistent with data implicating endogenous allopregnanolone as a physiological regulator of both basal and stress-induced dopamine release in rat brain (Motzo et al., 1996; Dazzi et al., 2002).

We have now investigated whether the administration of nicotine, a drug that induces behavioral changes similar to those triggered by stressful events and which modulates the function of neuronal populations that contribute to emotion and reward, affects the concentrations of allopregnanolone and THDOC, or those of their precursors progesterone and pregnenolone, in the brain or plasma of rats.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley CD rats (Charles River, Como, Italy) with body masses of 200–250 g were studied. After arrival at the animal facility, rats were acclimatized to the new housing conditions for at least 1 week. They were housed six per cage under an artificial 12-h-light–12-h-dark cycle (light on from 0800 to 2000 h) and at a constant temperature of 22 ± 2 °C and a relative humidity of 65%. They had free access to water and standard laboratory food at all times. Animal care and handling throughout the experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Chemicals

(−)Nicotine hydrogen tartrate, pregnenolone, progesterone, allopregnanolone, THDOC, and corticosterone were obtained from Sigma (Milan, Italy). FG 7142 (β-carboline-3-carboxylic acid ethylestermethylamide) was obtained from Tocris (Bristol, UK). Rabbit or sheep antiserum to allopregnanolone or to THDOC was generated and characterized as previously described (Purdy et al., 1991). Antisera to pregnenolone, to progesterone, or to corticosterone were obtained from ICN (Costa Mesa, CA). All other reagents and organic solvents (HPLC grade) were of the best quality available from commercial sources.

2.3. Drug treatment

To eliminate the stress of handling and injection, which might influence the cerebrocortical concentrations of neuroactive steroids (Barbaccia et al., 1997), animals were habituated to an intraperitoneal injection of physiological saline for 5 days prior to experimentation. Nicotine was dissolved in distilled water, buffered to pH 7–7.4 with NaOH, and administered by a single intraperitoneal injection (0.03–2 mg/kg of body mass) in a volume of 3 ml/kg either 10, 20, 40, or 120 min before sacrifice. All doses are given as free base. FG 7142 was dissolved in distilled water with one drop of Tween 80 per 5 ml. Control rats received an equal volume of vehicle.

2.4. Steroid extraction and assay

Animals were sacrificed and the cerebral cortices were rapidly (< 1 min) dissected from the brain, frozen in dry ice and stored at −20 °C until steroid extraction. Blood was

collected from the trunk into heparinized tubes and centrifuged at 900 × *g* for 20 min, after which the plasma was frozen until assayed for steroids.

Steroids were extracted and purified as previously described (Barbaccia et al., 1996b). In brief, steroids present in brain tissue homogenates (400 mg of tissue in 4 ml of phosphate-buffered saline) were extracted four times with an equal volume of ethyl acetate. The combined organic phases were dried under vacuum, the resulting residue was dissolved in 5 ml of *n*-hexane and applied to Seppak-silica cartridges (Waters), and residue components were eluted with a mixture of *n*-hexane and 2-propanol (7:3, v/v). Steroids were further purified by high-performance liquid chromatography on a 5-μm Lichrosorb-diol column (250 by 4 mm) (Merck) with a gradient of 2-propanol in *n*-hexane. Given that we previously observed that cholesterol, which coelutes from the Lichrosorb-diol column with progesterone, reduces the sensitivity of the radioimmunoassay (RIA) for progesterone, this latter steroid was separated from cholesterol by washing the corresponding dried column fractions twice with 200 μl of dimethyl sulfoxide and 400 μl of water. Progesterone was extracted from the aqueous phase twice with 1.5 ml of *n*-hexane. The removal of cholesterol increased the sensitivity of the progesterone RIA by a factor of 5–10 relative to that previously described (Barbaccia et al., 1996b). The recovery of each steroid through the extraction–purification procedures (70–80%) was monitored by adding trace amounts (4000–6000 cpm) of ^{3}H-labeled standards to the brain tissue homogenate. Steroids were quantified by RIA as previously described (Barbaccia et al., 1996b). Plasma steroid concentrations were measured in 1 ml of plasma after extraction four times with 1.5 ml of ethyl acetate.

2.5. Statistical analysis

Data are presented as means ± S.E.M. The statistical significance of differences was assessed by analysis of variance followed by Newman–Keuls test. A *P*-value less than .05 was considered statistically significant.

3. Results

3.1. Effects of nicotine on neuroactive steroid concentrations in the cerebral cortex

The cerebrocortical concentrations of neuroactive steroids 40 min after systemic administration of nicotine (0.03–2 mg/kg ip) are shown in Fig. 1. The injection of

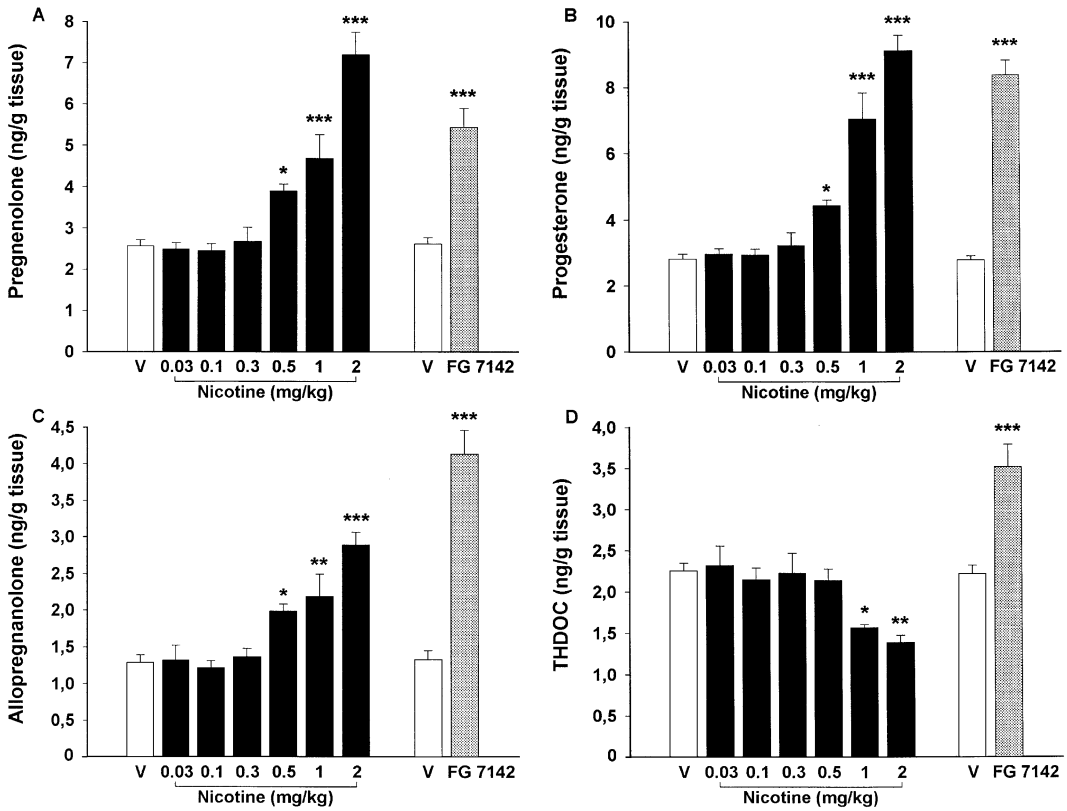

Fig. 1. Effect of nicotine and FG 7142 on the concentrations of neuroactive steroids in the cerebral cortex of rats. Rats were injected with nicotine (0.03–2 mg/kg ip), FG 7142 (15 mg/kg ip), or vehicle (V) and sacrificed 40 min later. The concentrations of pregnenolone (A), progesterone (B), allopregnanolone (C), and THDOC (D) in cerebrocortical homogenates were determined. Control animals (V) received the same amount of vehicle. Data are expressed as nanograms of steroid per gram of homogenate tissue and are means ± S.E.M. of values from to 10 rats; * $P < .05$, * * $P < .01$, * * * $P < .001$ vs. the respective vehicle-treated rats.

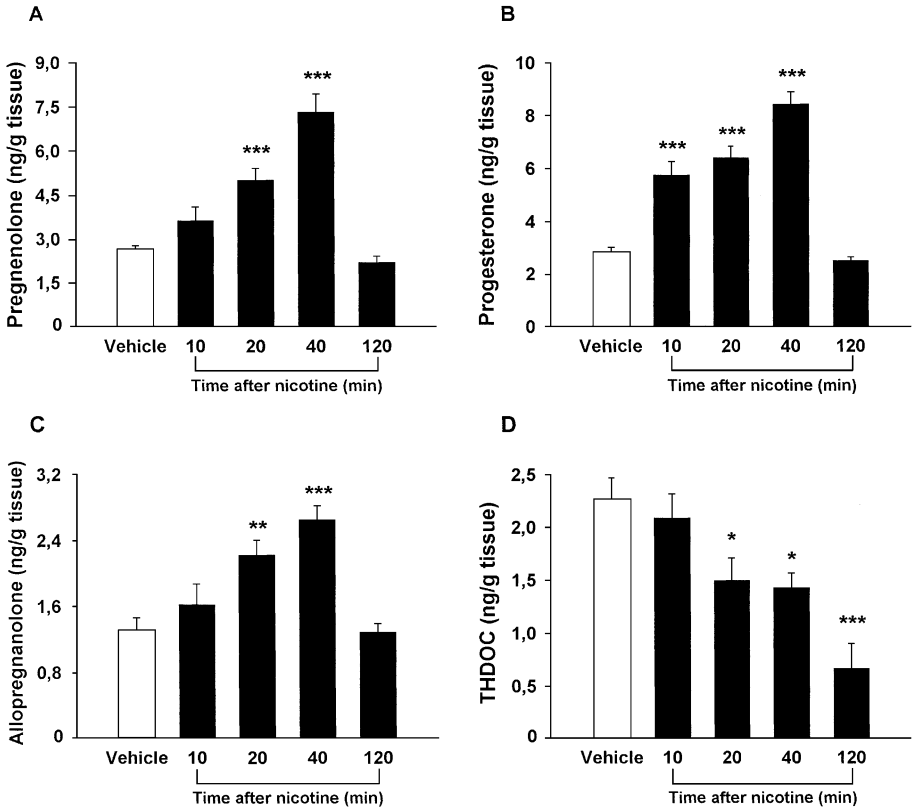

Fig. 2. Time course of the cerebrocortical concentrations of neuroactive steroids after the injection of nicotine. Rats were injected with nicotine (2 mg/kg ip) at the indicated times before sacrifice, after which the concentrations of pregnenolone (A), progesterone (B), allopregnanolone (C), and THDOC (D) were measured in cerebrocortical homogenates. Control animals received the same volume of vehicle. Data are means ± S.E.M. of values from 10 rats; * $P < .05$, ** $P < .01$, *** $P < .001$ vs. vehicle-treated rats.

low doses of nicotine (0.03–0.3 mg/kg), which induce anxiolytic effects in rats (Costall et al., 1989; Vale and Green, 1996; File et al., 1998), had no significant effect on the concentrations of pregnenolone, progesterone, allopregnanolone, or THDOC. The administration of higher doses of nicotine (0.5–2 mg/kg), which induce anxiogenic effects in rats (File et al., 1998; Ouagazzal et al., 1999) produced a dose-dependent increase in the cerebrocortical concentrations of pregnenolone, progesterone, and allopregnanolone, with the greatest observed effects (+180%, $P < .001$; +223%, $P < .001$; and +124%, $P < .001$, respectively) apparent at the dose of 2 mg/kg. In contrast, nicotine at the higher doses tested (1–2 mg/kg) decreased by 31% ($P < .05$) and 38% ($P < .01$), respectively, the concentration of THDOC in the cerebral cortex (Fig. 1). As previously described (Barbaccia et al., 1996a, 1997), the administration

Table 1
Effect of nicotine and FG 7142 on the plasma concentrations of neuroactive steroids

Treatment	Dose (mg/kg)	Steroid (ng/ml)			
		Pregnenolone	Progesterone	Allopregnanolone	THDOC
Vehicle		2.43 ± 0.08	2.66 ± 0.37	2.48 ± 0.15	2.78 ± 0.18
Nicotine	0.03	2.59 ± 0.17	2.58 ± 0.20	2.51 ± 0.24	2.71 ± 0.24
Nicotine	0.1	2.66 ± 0.32	2.42 ± 0.38	2.59 ± 0.27	2.87 ± 0.21
Nicotine	0.3	2.56 ± 0.22	2.58 ± 0.28	2.62 ± 0.39	2.67 ± 0.26
Nicotine	0.5	2.63 ± 0.43	2.71 ± 0.36	2.29 ± 0.10	2.48 ± 0.21
Nicotine	1	4.05 ± 0.26[a]	4.88 ± 0.19[a]	3.34 ± 0.28	2.42 ± 0.26
Nicotine	2	3.48 ± 0.05[b]	4.02 ± 0.22[b]	2.85 ± 0.24	2.46 ± 0.31
Vehicle		2.50 ± 0.13	2.60 ± 0.34	2.41 ± 0.16	2.67 ± 0.28
FG 7142	15	4.82 ± 0.34[c]	6.37 ± 0.35[c]	4.57 ± 0.37[c]	6.73 ± 0.34[c]

Nicotine, FG 7142 or vehicle were administered by intraperitoneal injection 40 min before sacrifice. Data are expressed as nanograms of steroid per milliliter of plasma and are means ± S.E.M. of values from 10 rats; Control animals (vehicle) received the same amount of vehicle.

[a] $P < .001$ vs. the respective vehicle-treated rats.

[b] $P < .05$ vs. the respective vehicle-treated rats.

[c] $P < .001$ vs. the respective vehicle-treated rats.

of the anxiogenic β-carboline derivative FG 7142 (15 mg/kg ip) increased the cerebrocortical concentrations of pregnenolone, progesterone, allopregnanolone and THDOC by 108%, 200%, 211%, and 60%, respectively (Fig. 1).

The effects of nicotine at the dose of 2 mg/kg on the cerebrocortical concentrations of pregnenolone, progesterone, allopregnanolone, and THDOC were also time dependent (Fig. 2). The brain concentration of pregnenolone, progesterone, and allopregnanolone were maximal (+178%, $P<.001$; 194%, $P<.001$; and 102%, $P<.001$, respectively) at 40 min and had returned to control values by 120 min. The concentration of THDOC was significantly decreased (−34%, $P<.05$) 20 min after administration and was minimal (−71%, $P<.001$) at 120 min.

3.2. Effects of nicotine on neuroactive steroid concentrations in plasma

Similar to its effects on the brain concentrations of pregnenolone and progesterone, intraperitoneal administration of nicotine increases the plasma concentrations of these steroids [$F(6,63)=5.88$, $P<.001$; $F(6,63)=10.20$, $P<.001$, respectively] that were apparent after 40 min (Table 1). The effects of nicotine on the plasma concentrations of pregnenolone and progesterone were maximal (+67%, $P<.001$ and +84%, $P<.001$) at the dose of 1 mg/kg, while the dose of 2 mg/kg failed to increase further the plasma concentrations of these steroids. In contrast to its action in the cerebral cortex, nicotine (1–2 mg/kg) slightly increased the plasma concentration of allopregnanolone, but the effect failed to reach significance. At variance with its effects on the brain concentrations of these steroids, intraperitoneal administration of nicotine (0.03–2 mg/kg ip) had no significant effect on the concentrations of THDOC in the plasma of animals sacrificed 40 min after drug injection (Table 1). Consistent with previous results (Barbaccia et al., 1996a, 1997), the concentrations of neuroactive steroids were also increased in plasma 40 min after the administration of FG 7142 (15 mg/kg) (Table 1).

3.3. Effects of nicotine on the plasma corticosterone concentration

Consistent with previous observations (Balfour, 1980; Cam and Bassett, 1983), the injection of nicotine (0.03–2 mg/kg ip) resulted in a dose-dependent increase in the plasma concentration of corticosterone [$F(6,63)=10.02$, $P<.001$]. Post hoc analysis revealed that the effect of nicotine was significant (+53%, $P<.01$) at the dose of 0.1 mg/kg, greatest (+73%, $P<.001$) at the dose of 0.3 mg/kg, while higher doses (0.5–2 mg/kg) failed to produce any further elevation in plasma corticosterone (Fig. 3A). The effect of nicotine (0.3 mg/kg) on the plasma concentration of corticosterone was also time dependent [$F(4,45)=7.80$, $P<.001$] (Fig. 3B). The plasma concentration of this hormone was significantly increased (+61%, $P<.01$) 10 min after drug administration, was maximal (+72%, $P<.001$) at 20 min, and had returned to control values by 120 min.

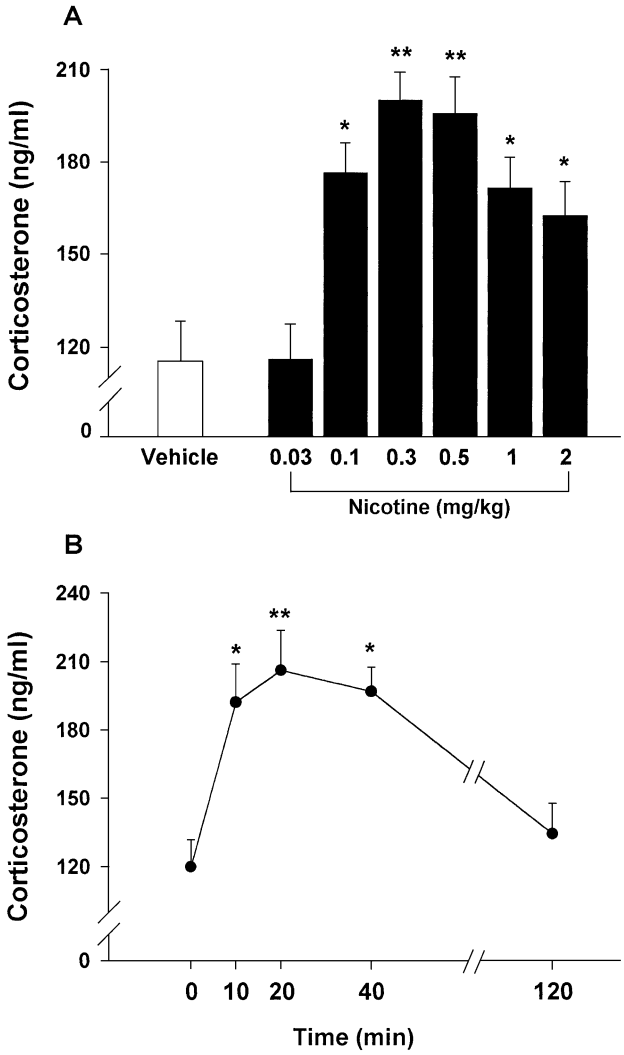

Fig. 3. Dose (A) and time (B) dependence of the nicotine-induced increase in the plasma concentration of corticosterone. Rats were injected either with nicotine at the indicated doses 40 min before sacrifice (A) or with nicotine at a dose of 0.3 mg/kg at the indicated times before sacrifice (B). Control animals (V) received the same volume of vehicle. Data are expressed as nanograms of steroid per milliliter of plasma and are means ± S.E.M. of values from 10 rats; $*P<.01$, $**P<.001$ vs. vehicle-treated rats.

4. Discussion

We have shown that the intraperitoneal administration of a single dose of nicotine, a drug that modulates emotional behavior in various animal tests (File et al., 2000a), resulted in dose- and time-dependent increases in the cerebrocortical concentrations of allopregnanolone as well as in those of its precursors pregnenolone and progesterone. The doses of nicotine required for these effects (0.5–2 mg/kg) are similar to those that induce experimental anxiety in the elevated plus-maze test (Ouagazzal et al., 1999) as well as reduce the

level of social interaction among rats (File et al., 1998). At variance, lower doses of nicotine (0.03–0.1 mg/kg) that induces anxiolytic effects (Costall et al., 1989; Vale and Green, 1996; File et al., 1998) had no effect on the cerebrocortical concentrations of these neuroactive steroids.

These results appear consistent with the notion that stressful stimuli and anxiogenic drugs increase the plasma and brain concentrations of neuroactive steroids. The administration of β-carbolines, which induce proconflict effects in rats and experimental anxiety in humans and other primates (Biggio et al., 1990), thus results in marked increases in the concentrations of allopregnanolone, progesterone, and pregnenolone in rat brain and plasma (Barbaccia et al., 1997; Fig. 1 and Table 1). Moreover, caffeine, a naturally occurring stimulant of the central nervous system that at high doses induces an anxiogenic action (Udhe, 1990), also markedly increases the production of neuroactive steroids (Concas et al., 2000). The effects of these anxiogenic compounds are similar to those induced by various acute stress paradigms. Thus, exposure of rats to mild foot shock, CO_2 inhalation, forced swimming, or handling maneuvers that precede sacrifice induces rapid and marked increases in both brain and plasma concentrations of neuroactive steroids (Purdy et al., 1991; Barbaccia et al., 1996a,b, 1997). The failure of nicotine to affect the concentrations of neuroactive steroids at doses that induced anxiolytic effects is also consistent with the evidence that abecarnil, a β-carboline derivative know to elicit anxiolytic actions in different behavioural tests (Stephens et al., 1990), failed to affect the concentrations of these steroids in the rat cerebral cortex (Barbaccia et al., 1996a, 1997). Our present data together with the results of previous studies (Purdy et al., 1991; Barbaccia et al., 1996a,b, 1997) thus show that both pharmacological treatments and experimental conditions that induce anxiety-like behavior also induce increases in the brain concentrations of neuroactive steroids. Given that allopregnanolone enhances $GABA_A$ receptor function and plays an important role in the regulation of anxiety and mood disorders (Biggio and Purdy, 2001), the transient increase in the brain concentration of this neuroactive steroid triggered by nicotine may represent a homeostatic mechanism to reduce or counteract the neuronal excitability and anxiogenic-like action elicited by nicotine.

It is worth noting that the effects of nicotine on the plasma concentrations of neuroactive steroids differed from those in brain. In fact, while the maximum increases in plasma pregnenolone and progesterone are achieved at a lower dose of nicotine (1 mg/kg) the increase in brain pregnenolone and progesterone is dose-related being greatest at 2 mg/kg. Moreover, at variance with its effect in the cerebrocortical concentration of allopregnanolone, nicotine failed to significantly increase the concentration of this steroid in plasma. These data might indicate that in some pharmacological conditions in the rat brain, the synthesis of allopregnanolone is strictly related to the brain progesterone concentration and does not follow the plasma level of allopregnanolone. This conclusion might suggest that, under the effect of nicotine, the synthesis of neuroactive steroids in the cerebral cortex is greater than in plasma. Accordingly, also, the surge of pregnenolone and progesterone in the cerebral cortex was higher than in plasma. The present results suggest that in the brain, the synthesis/accumulation of neuroactive steroids is not simply a function of their plasma concentrations. Accordingly, a different regulation in the concentrations of neuroactive steroids between plasma and brain has been already suggested using different pharmacological treatments (Concas et al., 2000; Barbaccia et al., 2001; Follesa et al., 2002) and physiological conditions (Concas et al., 1998). On the other hand, the possibility that nicotine may influence directly and/or differentially in brain and plasma the activity of the enzymes involved in steroidogenesis, therefore selectively changing rates of these compounds synthesis, cannot be ruled out. This conclusion is consistent with evidence that nicotine affects peripheral steroidogenesis in both rats and humans. Cigarette alkaloids thus inhibited both progesterone synthesis in cultured human granulosa cells (Gocze et al., 1999) as well as specific P450 enzymes in rat adrenal and testicular cells (Kasson and Hsueh, 1985; Barbieri et al., 1987). The failure of nicotine to increase allopregnanolone concentrations in plasma might thus reflect complex neuroendocrine effects that result in concomitant activation of the hypothalamic–pituitary–adrenal (HPA) axis and direct inhibition of peripheral steroidogenesis.

The observations that stress, anxiogenic β-carbolines, and caffeine fail to affect the concentrations of allopregnanolone, THDOC, pregnenolone, and progesterone in adrenalectomized–orchiectomized rats (Barbaccia et al., 1997; Concas et al., 2000) suggest that the HPA axis plays an important role in determining the peripheral and central concentrations of neuroactive steroids both under stressful conditions and after the administration of anxiogenic drugs. Our present data show that nicotine, at doses able to elicit anxiety (0.5–2 mg/kg) and to increase the plasma concentration of corticosterone (the main corticosteroid hormone produced by the adrenals in stressed rats), increased the brain concentrations of pregnenolone, progesterone, and allopregnanolone. However, the increase in the brain concentrations of these steroids seems not to be strictly related with the changes in plasma corticosterone concentrations. In fact, low doses of nicotine (0.1–0.3 mg/kg), although producing an increase of plasma corticosterone levels, fails to enhance the brain concentrations of pregnenolone, progesterone, and allopregnanolone. These results are consistent with the different sensitivity of peripheral and central steroidogenesis to the effect of nicotine. It is worth noting that the low doses of nicotine that enhances the plasma levels of corticosterone, an index of an altered emotional state, are anxiolytic (Costall et al., 1989; Vale and Green, 1996; File et al., 1998). This result suggests that the effect of nicotine on plasma corticosterone concentrations is probably mediated by an involvement of $5\text{-}HT_{1A}$ receptors. In fact,

the activation of 5-HT_{1A} receptors by specific agonists results in an anxiolytic action in different animal tests (File and Gonzales, 1996; Koek et al., 1998) associated to an increase of plasma corticosterone concentrations (Koek et al., 1998; Li et al., 1999). This hyphothesis is supported by the recent evidence that the administration of low doses of nicotine into the dorsal raphe nucleus induced anxiolytic effects by an indirect stimulation of 5-HT_{1A} receptors (Cheeta et al., 2001).

It is not clear why nicotine decreases the brain concentrations of THDOC, while inducing an opposite effect on allopregnanolone. However, this observation is consistent with previous data showing that the brain concentration of this neuroactive steroid in rats is differentially affected by other pharmacological treatments (Strohle et al., 2000; Concas et al., 2000) and by various stressor (Barbaccia et al., 1996b). Moreover, the synthesis of THDOC in the brain requires the production of deoxycorticosterone (DOC) by the adrenal cortex, whereas the formation of allopregnanolone in the brain may occur independently from peripheral organs (Purdy et al., 1991). Thus, differences in neurotransmitter systems or molecular endocrinologic mechanism that underlies the action of nicotine may contribute to the observed differences in the effect of this drug on the concentrations of neuroactive steroids. Consistent with this notion, the psychopharmacological effects of nicotine are multiple and often opposite at the same time. Through its interaction with nicotinic receptors, nicotine induces a cascade of physiological effects that are dependent on the release of neurotransmitters, such as 5-HT and GABA (Ribeiro et al., 1993; Lena and Changeux, 1997), that regulate anxiety by acting in different cerebral regions in opposite manners (Pesold and Treit, 1996; Cheeta et al., 2000, 2001; File et al., 2000b).

Nicotine selectively activates dopaminergic neurons of the ventral tegmental nucleus that project to the limbic areas and induces a physical dependence more intense and persistent than that apparent with other drugs of abuse (Di Chiara, 2000). Given that allopregnanolone is among the most potent positive modulators of $GABA_A$ receptors (Biggio and Purdy, 2001), which contribute to inhibitory regulation of mesocortical and mesolimbic dopaminergic neurons (Tam and Roth, 1990), the nicotine-induced increase in the brain content of these hormones may facilitate the inhibition of these dopaminergic pathways induced by GABA. Consistent with this notion, we have shown that pretreatment with finasteride, which reduces the brain concentrations of allopregnanolone and THDOC (Concas et al., 1998), markedly potentiates the effect of stress or anxiogenic drugs on cortical dopamine output (Dazzi et al., 2002). Moreover, pharmacological doses of allopregnanolone inhibit both the basal and stress-induced release of dopamine in the rat prefrontal cortex (Motzo et al., 1996). Given that dopaminergic systems play a major role in regulation of reward (Di Chiara, 2000), the increase in allopregnanolone concentration in rat brain might be relevant to the modulation of the reinforcing effects of this drug during the acquisition phase of addiction. Accordingly, systemic administration or intracerebral microinfusion of GABAergic transmission agonists reduced nicotine-induced overflow of dopamine in the nucleus accumbens as well as nicotine self-administration (Dewey et al., 1999; Corrigall et al., 2000).

In conclusion, our data provide the first biochemical evidence that, similar to stressful stimuli and other anxiogenic drugs, anxiogenic doses of nicotine increase the cerebrocortical concentrations of neuroactive steroids. These results further suggest a possible role of brain neuroactive steroids in counteracting the overexcitation of the central nervous system.

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