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European Journal of Pharmacology 473 (2003) 217–223
www.elsevier.com/locate/ejphar

Extrahypothalamic corticotropin-releasing hormone mediates (−)-nicotine-induced elevation of plasma corticosterone in rats

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Received 17 February 2003; received in revised form 26 May 2003; accepted 6 June 2003

Abstract

(−)-Nicotine activates the hypothalamic–pituitary–adrenal axis via an activation of the brainstem catecholaminergic neurons in rats. The present study was undertaken to clarify the mechanisms involved in the (−)-nicotine-induced activation of brainstem catecholaminergic neurons in anesthetized rats. Physostigmine (a cholinesterase inhibitor) (0.31 and 0.77 μmol/animal, i.p.) dose-dependently elevated plasma corticosterone in the presence of scopolamine (a muscarinic receptor antagonist) (2.3 μmol/animal, i.p.). (−)-Nicotine (250 and 500 nmol/animal, i.c.v.) dose-dependently elevated plasma corticosterone with concomitant noradrenaline release in the hypothalamic paraventricular nucleus. The (−)-nicotine (500 nmol/animal, i.c.v.)-induced elevation of corticosterone was abolished by phentolamine (an α-adrenoceptor antagonist) (0.66 μmol/animal, i.c.v.), and attenuated by (±)-sotalol (a β-adrenoceptor antagonist) (0.97 μmol/animal, i.c.v.). The (−)-nicotine-induced increases of plasma corticosterone and hypothalamic noradrenaline release were abolished either by hexamethonium (a nicotinic acetylcholine receptor antagonist) (1.8 μmol/animal, i.c.v.), CP-154,526 (butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]amine) (a selective CRF-1 receptor antagonist) (1.3 μmol/animal, i.c.v.) or indomethacin (a cyclooxygenase inhibitor) (1.2 μmol/animal, i.c.v.). These results suggest that (−)-nicotine elevates plasma corticosterone by CRF-1 receptor- and prostaglandin-mediated noradrenaline release in the paraventricular nucleus in rats.

Keywords: CRH (corticotropin-releasing hormone); Noradrenaline; Prostaglandin; Brain; (−)-Nicotine; Brain nicotinic receptor; Corticosterone, plasma

1. Introduction

It is well documented that peripherally administered (−)-nicotine is a potent stimulant for excitation of the hypothalamic–pituitary–adrenal axis (Conte-Devolx et al., 1981; Andersson et al., 1983; Sharp and Beyer, 1986). Matta and his coworkers have already demonstrated that (−)-nicotine activates adrenocorticotropin secretion from the anterior pituitary by stimulating the release of noradrenaline in the hypothalamic paraventricular nucleus in rats (Matta et al., 1987; 1990a,b, 1993a,b, 1995; Fu et al., 1997). The paraventricular nucleus is one of the essential sites for the central regulation of the hypothalamic–pituitary–adrenal axis (Herman and Cullinan, 1997). The nucleus receives rich innervation of the catecholaminergic neurons from the brainstem, in which three cell groups, the A_1 and C_1 cells in the ventrolateral medulla (lateral reticular nucleus), the A_2 and C_2 cells in the dorsomedial medulla (inside and around the nucleus of the solitary tract) and cells in the locus coeruleus, provide almost all of the noradrenergic inputs and the major part of the adrenergic inputs (Swanson and Sawchenko, 1983; Palkovits, 1987; Cunningham and Sawchenko, 1988). Catecholaminergic fibers synapse with corticotropin-releasing hormone (CRH)- and vasopressin-containing cells in the paraventricular nucleus (Tanaka et al., 1985; Cummings and Seybold, 1988), and these peptides released into the pituitary portal system stimulate the release of adrenocorticotropin from the anterior pituitary gland.

Recently, we reported that the activation of noradrenergic neurons innervating the hypothalamic paraventricular nucleus was involved in the centrally administered 3-morpholino-sydnonimine (a nitric oxide donor)-induced elevation of plasma corticosterone in rats (Okada et al., 2002). This elevation of plasma corticosterone was abolished by centrally administered hexamethonium, a nicotinic acetylcholine receptor antagonist (unpublished data). In the present experiments, therefore, we attempted to re-examine the

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doi:10.1016/S0014-2999(03)01966-6

hypothesis for the (−)-nicotine-induced activation of the hypothalamic–pituitary–adrenal axis described above and to further clarify the mechanisms involved in the (−)-nicotine-induced excitation of brainstem catecholaminergic neurons in rats.

2. Materials and methods

2.1. Experimental procedures

Male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing about 400 g were maintained in a room at 22–24 °C under a constant day–night rhythm for more than two weeks, and given food and water ad libitum. Rats were anesthetized with urethane (600 mg/kg, i.p.) and α−chloralose [(60 mg/kg, i.p.) followed by continuous intravenous administration (50 mg/kg/h)] (Ueta et al., 2000; Okada et al., 2002). The femoral vein and artery were cannulated for infusion of saline (1.4 ml/h) and collecting blood samples, respectively. After these procedures, the animal was placed in a stereotaxic apparatus, as shown in our previous paper (Okada et al., 2002).

Three hours were allowed to elapse for stabilization of the basal plasma levels of corticosterone. A stainless steel cannula (0.35 mm in outer diameter) was inserted into the left cerebral ventricle according to the rat brain atlas (Paxinos and Watson, 1986). The stereotaxic coordinates of the tip of the cannula were as follows (in mm): AP −0.8, L 1.5, V −4.0 (AP, anterior from the bregma; L, lateral from the midline; V, below the surface of the brain). (−)-Nicotine was dissolved in sterile saline and injected into the lateral ventricle in a volume of 5 μl using 10-μl Hamilton syringe. Phentolamine, (±)-sotalol, hexamethonium and water-soluble indomethacin-Na were dissolved in sterile saline and injected into the lateral ventricle in a volume of 10 μl 30 min before the application of (−)-nicotine. CP-154,526 (butyl-ethyl-[2,5-dimethyl-7-(2,4,6 trimethylphenyl)-7*H*-pyrrolo [2,3-*d*]pyrimidin-4-yl]amine) was dissolved in 100% dimethylformamide and injected into the lateral ventricle in a volume of 2.5 μl 30 min before the application of (−)-nicotine. In some experiments, physostigmine and scopolamine dissolved in sterile saline were intraperitoneally administered.

In experiment with microdialysis, a stainless steel guide cannula held on the tip of L-shaped stainless steel was implanted stereotaxically just above the right hypothalamic paraventricular nucleus, as previously reported (Okada et al., 2000, 2002). The stereotaxic coordinates of the sites of implantation were as follows (in mm): AP −1.7, L 0.3, V −7.0. The microdialysis probe (220 μm in outer diameter, 1 mm of membrane length; Eicom, Kyoto, Japan) was inserted into the guide cannula and extended to the paraventricular nucleus. Then, perfusion of paraventricular nucleus with Ringer's solution (147 mM NaCl, 4 mM KCl and 2.3 mM $CaCl_2$) through the microdialysis probe was performed at a flow rate of 2 μl/min using a microinfusion pump (EP-60, Eicom). Three hours were allowed to elapse for stabilization of the basal release of noradrenaline, and dialysate was collected every 20 min in a collection tube containing 20 μl of 0.1 N perchloric acid and 5 pg of 3,4-dihydroxybenzylamine as an internal standard. Three consecutive dialysates were collected to measure the basal release of noradrenaline. Released noradrenaline in the samples was electrochemically measured using high performance liquid chromatography (HPLC) as described below. At the termination of experiment, rat was sacrificed with deep anesthesia, and the brain was removed and fixed with 10% formalin. Serial coronal sections sliced at 20 μm were stained with cresyl violet to verify the location of the tip of dialysis probe. A photomicrograph of the implantation was shown in our previous paper (Okada et al., 2000).

All experiments were conducted in compliance with the guiding principles for the care and use of laboratory animals approved by the Kochi Medical School.

2.2. Measurement of noradrenaline in the microdialysate

Noradrenaline released into the dialysate was electrochemically determined using reverse-phase HPLC system with an Eicompack CA-5ODS column (2.1×150 mm, Eicom), as shown in a previous paper (Okada et al., 2002). The graphite electrode was held at +450 mV against an Ag/AgCl reference electrode. The composition of the mobile phase was 0.1 M phosphate buffer (pH 6.0) containing 15% methanol, 750 mg/l sodium 1-octanesulfonate (Nacalai Tesque, Kyoto, Japan) and 50 mg/l EDTA dihydrate. Dialysate (40 μl) was injected onto the HPLC by a sample injector (Model 231-XL; Guilson, Villiers-le-Bel, France). The amount of noradrenaline in each sample was calculated using the peak height ratio relative to that of 3,4-dihydroxybenzylamine, an internal standard. Using this assay method, 0.5 pg of noradrenaline and adrenaline could be accurately determined. Microdialysis probe has approximately a 25% recovery for noradrenaline and adrenaline.

2.3. Measurement of plasma corticosterone

Blood samples (300 μl) were collected through an arterial catheter. Corticosterone in the plasma was extracted by the method of Hofreiter et al. (1982) with a slight modification and was assayed by HPLC with UV-spectrometric detection (Hay and Mormede, 1997). Briefly, the plasma (100 μl) was transferred into a microcentrifuge tube containing 25 ng of cortexolone as an internal standard and added five volumes of dichloromethane. The tube was then shaken vigorously for 10 min and spun down. After removal of the upper aqueous phase by aspiration, the lower organic phase was washed twice with 100 μl of 0.05 N NaOH. The organic phase was then evaporated in a centrifugal-concentrator (Model VC-36S; Taitec, Saitama, Japan). After dissolving dried residue with 500 μl of 50% of methanol, 100 μl of

which were injected into the HPLC system. A pump (Model 880-PU; Japan Spectroscopic, Tokyo, Japan), a sample injector (Model 851-AS; Japan Spectroscopic) and a UV detector (Model UV-1575; Japan Spectroscopic) were used with HPLC. Analytical conditions were as follows: UV detector, 240 nm; column, Cosmosil $_5C_{18}$, 4.6 mm×150 mm (Nacalai Tesque); mobile phase, 50% methanol at a flow rate of 0.8 ml/min. The amount of corticosterone in each sample was calculated using the peak height ratio relative to that of cortexolone, an internal standard. Using this assay method, 10 ng of corticosterone could be accurately determined.

2.4. Treatment of data and statistics

Released noradrenaline in the hypothalamic paraventricular nucleus region is expressed as a percentage of the basal release. Plasma corticosterone is expressed as the net change above the basal level. These values are expressed as the mean±S.E.M. All data were analyzed by repeated-measure analysis of variance (ANOVA), followed by post hoc analysis with the Bonferroni method for comparing a control to all other means (Figs. 1 and 2). When only two means were compared, an unpaired Student's *t*-test was used (Figs. 2–4). *P* values less than 0.05 were taken to indicate significance.

2.5. Compounds

The following drugs were used: hexamethonium chloride, (−)-nicotine, phentolamine methylsulfate, physostigmine hemisulfate, (−)-scopolamine hydrobromide, (±)-sotalol hydrochloride (Sigma, St. Louis, MO, USA); cortexolone (Aldrich Chem., Milwaukee, WI, USA); CP-154,526 (butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]amine) (a kind gift of Pfizer, Groton, CT, USA); water-soluble indomethacin sodium trihydrate (a kind gift of Merck, Rahway, NJ, USA). All other reagents were of the highest grade available (Nacalai Tesque).

3. Results

3.1. Effect of peripherally administered physostigmine or centrally administered (−)-nicotine on the plasma corticosterone

Intraperitoneal administration of vehicle (0.2 ml of saline/animal) under the presence of scopolamine (a muscarinic receptor antagonist that crosses the blood–brain barrier) [2.3 μmol (1000 μg)/animal, i.p.] and blood sampling for seven times over 120-min period had no effect on the basal plasma levels of corticosterone (Fig. 1A). Administration of physostigmine (a cholinesterase inhibitor that crosses the blood–brain barrier) [0.31 μmol (100 μg) and 0.77 μmol (250 μg)/animal, i.p.] dose-dependently elevated plasma levels of corticosterone (Fig. 1A). These results suggest the involvement of brain nicotinic acetylcholine receptors in the physostigmine-induced elevation of plasma corticosterone levels. Then, we examined the effect of centrally administered (−)-nicotine on the plasma levels of corticosterone.

Intracerebroventricularly (i.c.v.) administered (−)-nicotine (500 nmol/animal) elevated plasma levels of corticosterone, while the treatments with vehicle-1 and vehicle-2 (10 and 5 μl of saline/animal, i.c.v.) had no effect on plasma levels

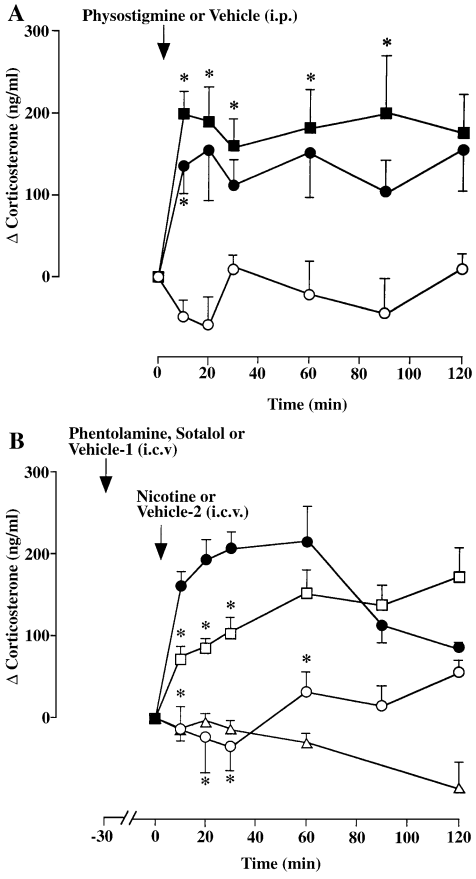

Fig. 1. Effect of physostigmine or (−)-nicotine on plasma corticosterone. Δ Corticosterone, net changes in plasma corticosterone levels above the basal. (A) Scopolamine [2.3 μmol (1000 μg)/animal] was intraperitoneally (i.p.) administered 30 min before administration of physostigmine. Arrow indicates i.p. administration of physostigmine [0.31 μmol (100 μg) and 0.77 μmol (250 μg)/animal]. ○, vehicle (0.2 ml saline/animal, *n*=5); ●, 100 μg physostigmine/animal, *n*=7); ■, 250 μg physostigmine/animal, *n*=7). (B) Phentolamine [0.66 μmol (250 μg)/animal), (±)-sotalol [0.97 μmol (300 μg) /animal] or vehicle-1 (10 μl of saline/animal) was intracerebroventricularly (i.c.v.) applied 30 min before administration of (−)-nicotine (500 nmol/animal, i.c.v.) or vehicle-2 (5 μl saline/animal, i.c.v.). △, vehicle-1 plus vehicle-2 (*n*=5); ●, vehicle-1 plus (−)-nicotine (*n*=10); ○, phentolamine plus (−)-nicotine (*n*=9); □, (±)-sotalol plus (−)-nicotine (*n*=9). *Significantly different (*P*<0.05) from the group treated with vehicle in A and from the group treated with vehicle-1 and (−)-nicotine in B. Basal levels of corticosterone at 0 time were 238.8±32.4 ng/ml (*n*=19) in A, 261.6±29.2 ng/ml in vehicle-1-pretreated group (*n*=15), 199.8±18.2 ng/ml in phentolamine-pretreated group (*n*=9), and 107.2±19.5 ng/ml in (±)-sotalol-pretreated group (*n*=9) in B.

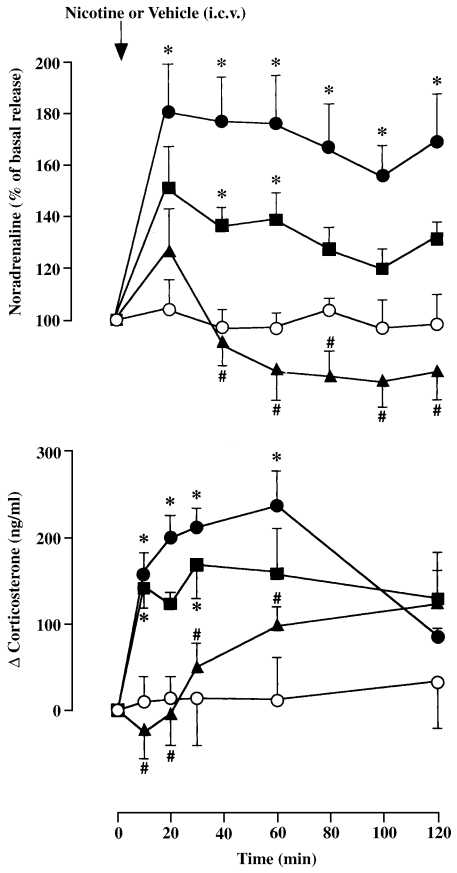

Fig. 2. Effects of (−)-nicotine on noradrenaline release in the hypothalamic paraventricular nucleus and plasma corticosterone. Upper panel, noradrenaline release in the paraventricular nucleus is expressed as percentage of the basal release; lower panel, plasma levels of corticosterone above the basal. Vehicle-1 (10 μl of saline/animal, i.c.v.) or hexamethonium [1.8 μmol (500 μg)/animal, i.c.v.) was applied 30 min before i.c.v. administration of (−)-nicotine or vehicle-2 (5 μl of saline/animal). Other conditions were the same as those in Fig. 1. ○, vehicle-1 plus vehicle-2 (n=7); ■, vehicle-1 plus (−)-nicotine (250 nmol/animal) (n=8); ●, vehicle-1 plus (−)-nicotine (500 nmol/animal) (n=8); ▲, hexamethonium plus (−)-nicotine (500 nmol/animal) (n=7). *Significantly different (P<0.05) from the group treated with vehicle-1 plus vehicle-2. #Significantly different (P<0.05) from the group treated with vehicle-1 plus (−)-nicotine (500 nmol/animal). Basal levels of corticosterone at 0 time were 256.4±27.7 ng/ml in the vehicle-1-pretreated group (n=23) and 275.2±66.0 ng/ml in the hexamethonium-pretreated group (n=7).

of corticosterone (Fig. 1B). Phentolamine [0.66 μmol (250 μg)/animal, i.c.v.], an α-adrenoceptor antagonist, abolished the (−)-nicotine-induced elevation of plasma corticosterone, and (±)-sotalol [0.97 μmol (300 μg)/animal, i.c.v.], a β-adrenoceptor antagonist, slightly, but significantly, attenuated the evoked elevation of plasma corticosterone (Fig. 1B).

3.2. Effects of centrally administered (−)-nicotine on noradrenaline release in the hypothalamic paraventricular nucleus and plasma corticosterone

Treatments with vehicle-1 and vehicle-2 (10 and 5 μl of saline/animal, i.c.v.) had no effect on the release of noradrenaline in the paraventricular nucleus and plasma corticosterone (Fig. 2, upper and lower panels). I.c.v. administered (−)-nicotine increased the release of noradrenaline in the paraventricular nucleus and elevated plasma corticosterone in a dose-dependent manner (250 and 500 nmol/animal) (Fig. 2, upper and lower panels). The maximal response of noradrenaline release in the paraventricular nucleus was observed at 20 min and that of plasma corticosterone was observed during 30–60 min, after the application of (−)-nicotine, respectively.

The (−)-nicotine (500 nmol/animal, i.c.v.)-induced increase of the noradrenaline release in the paraventricular nucleus and plasma corticosterone were attenuated by hexamethonium [1.8 μmol (500 μg)/animal, i.c.v.] (Fig. 2, upper and lower panels), while hexamethonium alone had no effect on noradrenaline release in the paraventricular nucleus and plasma corticosterone (data not shown).

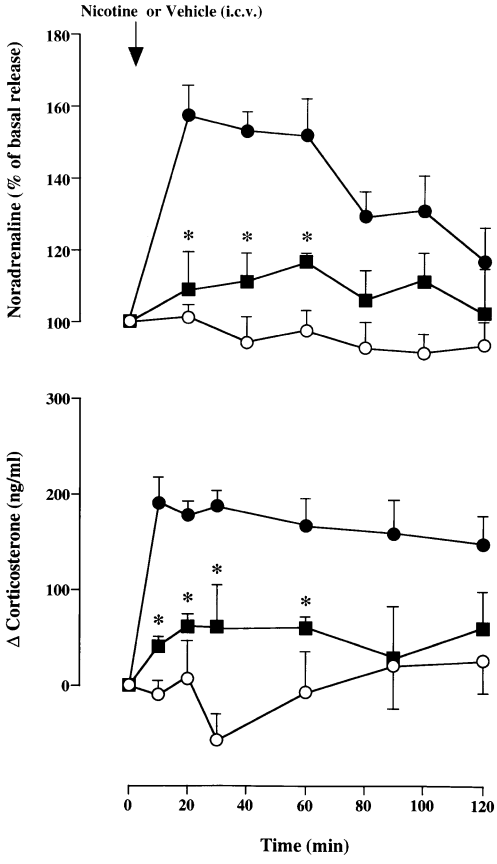

Fig. 3. Effects of CP-154,526, a selective CRF-1 receptor antagonist, on the (−)-nicotine-induced increases of noradrenaline release in the PVN and plasma corticosterone. CP-154,526 [1.3 μmol (500 μg)/animal, i.c.v.) or vehicle-1 [2.5 μl of 100% dimethylformamide/animal, i.c.v.] was administered 30 min before i.c.v. administration of (−)-nicotine (500 nmol/animal) or vehicle-2 (5 μl saline/animal). ○, CP-154,526 plus vehicle-2 (n=5); ●, vehicle-1 plus (−)-nicotine (n=5); ■, CP-154,526 plus (−)-nicotine (n=5). *Significantly different (P<0.05) from the group treated with vehicle-1 plus (−)-nicotine. Other conditions were the same as those in Figs. 1 and 2. Basal levels of corticosterone at 0 time were 235.6±20.2 ng/ml in the vehicle-1-pretreated group (n=5) and 247.1±34.6 ng/ml in the CP-154,526-pretreated group (n=10).

3.3. Effects of centrally administered CP-154,526 on the (−)-nicotine-induced increases of noradrenaline release in the hypothalamic paraventricular nucleus and plasma corticosterone

Treatments with vehicle-1 and vehicle-2 (10 and 5 μl of saline/animal, i.c.v.) had no effect on noradrenaline release in the paraventricular nucleus and basal plasma corticosterone (Fig. 2, upper and lower panels).

The pretreatment with CP-154,526 [1.3 μmol (500 μg)/animal, i.c.v.] had no effect on noradrenaline releasein the paraventricular nucleus and plasma corticosterone (Fig. 3, upper and lower panels). The (−)-nicotine (500 nmol/animal, i.c.v.)-induced elevations of noradrenaline release in the paraventricular nucleus and plasma corticosterone were reduced by CP-154,526 (Fig. 3, upper and lower panels).

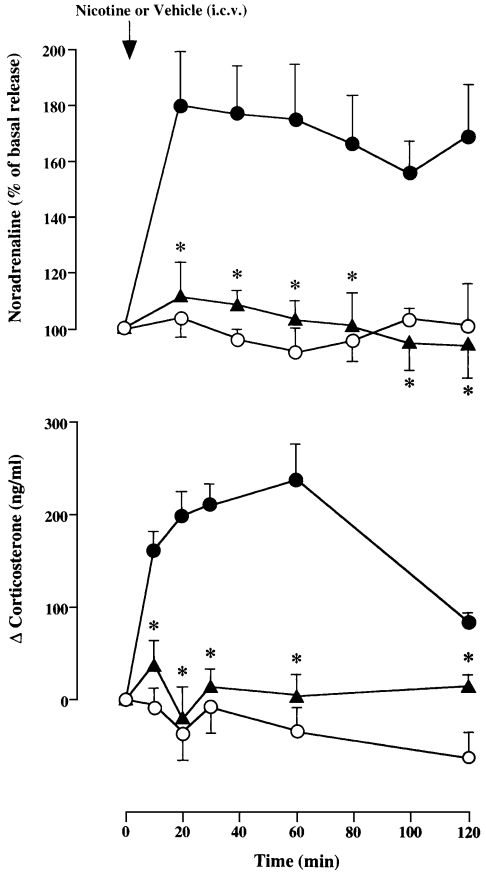

Fig. 4. Effects of indomethacin on the (−)-nicotine-induced increases of noradrenaline release in the PVN and plasma corticosterone. Indomethacin [1.2 μmol (500 μg)/animal, i.c.v.] or vehicle-1 (10 μl saline/animal, i.c.v.) was administered 30 min before i.c.v. administration of (−)-nicotine (500 nmol/animal) or vehicle-2 (5 μl saline/animal). ○, indomethacin plus vehicle-2 (n=5); ●, vehicle-1 plus (−)-nicotine (n=8); ▲, indomethacin plus (−)-nicotine (n=5). *Significantly different (P<0.05) from the group treated with vehicle-1 plus (−)-nicotine. Other conditions were the same as those in Figs. 1–3. Basal levels of corticosterone at 0 time were 257.8±39.1 ng/ml in vehicle-1-pretreated group (n=8) and 299.6±27.0 ng/ml in indomethacin-pretreated group (n=10).

3.4. Effects of centrally administered indomethacin on the (−)-nicotine-induced increases of noradrenaline release in the hypothalamic paraventricular nucleus and plasma corticosterone

The pretreatment with indomethacin [1.2 μmol (500 μg)/animal, i.c.v.] had no effect on noradrenaline release in the paraventricular nucleus and plasma corticosterone (Fig. 4, upper and lower panels). The (−)-nicotine (500 nmol/animal, i.c.v.)-induced increases of noradrenaline release in the paraventricular nucleus and plasma corticosterone were abolished by indomethacin (Fig. 4, upper and lower panels).

4. Discussion

We used anesthetized rats to clarify the mechanisms of centrally administered (−)-nicotine-induced elevation of plasma corticosterone, since several stressors (such as anxiety, fear and pain)-induced inputs from the descending limbic system and ascending brainstem cell groups to the hypothalamus secondarily modulate the activity of the hypothalamic–pituitary–adrenal axis in awake rats (Herman et al., 2002).

In the present experiment, peripherally administered physostigmine (a cholinesterase inhibitor that acrosses the blood–brain barrier) elevated plasma corticosterone in the presence of scopolamine. Since the brain muscarinic receptors were already blocked by scopolamine that acrosses the blood–brain barrier, the brain nicotinic acetylcholine receptors seem to be involved in the physostigmine-induced elevation of plasma corticosterone, as shown by Rhodes et al. (2001). Then, we examined the effect of centrally administered (−)-nicotine on plasma corticosterone. Intracerebroventricularly administered (−)-nicotine also elevated plasma corticosterone and the effect was abolished by intracerebroventricularly administered phentolamine, an α-adrenoceptor antagonist, and also attenuated by (±)-sotalol, a β-adrenoceptor antagonist. These results suggest the involvement of the brain catecholaminergic neurons in the central nicotinic acetylcholine receptor-mediated elevation of plasma corticosterone.

The paraventricular nucleus of the hypothalamus, an essential site for the central regulation of the hypothalamic–pituitary–adrenal axis (Herman and Cullinan, 1997), receives rich innervation of the catecholaminergic neurons from the brainstem. Administration of noradrenaline or isoproterenol into the paraventricular nucleus can stimulate CRH secretion and elevate plasma corticosterone (Daniels et al., 1993; Itoi et al., 1994). In the next experiment, therefore, we measured the release of catecholamines in the paraventricular nucleus using microdialysis technique as shown in our previous paper (Okada et al., 2002). Intracerebroventricularly administered (−)-nicotine increased the release of noradrenaline, but not adrenaline, in the paraventricular

nucleus in concomitant elevation of plasma corticosterone. Both responses were abolished by intracerebroventricularly administered hexamethonium, a blocker of nicotinic acetylcholine receptors. These results suggest that noradrenergic neurons innervating the paraventricular nucleus are involved in centrally administered (−)-nicotine-induced elevation of plasma corticosterone. The present result is consistent with previous papers, in which (−)-nicotine injected into the rat fourth ventricle or catecholaminergic regions of the brainstem stimulates noradrenaline release in the paraventricular nucleus and/or adrenocorticotropin secretion in conscious rats (Sharp and Matta, 1993; Matta et al., 1993a, 1995; Fu et al., 1997). Intravenously administered (−)-nicotine-induced cFos expression in the paraventricular nucleus has also been shown to be dependent on the excitation of catecholaminergic neurons of the brainstem (Valentine et al., 1996).

Recently, CP-154,526, a highly selective nonpeptide antagonist of CRF-1 receptors, has been developed (Schulz et al., 1996). The reagent has been shown to reverse the CRH-elicited increase in adrenocorticotropin, prevent the CRH-induced elevation in locus coeruleus cell firing, and block the CRH-enhanced startle response in rat (Lundkvist et al., 1996; Schulz et al., 1996). In the present study, intracerebroventricularly administered CP-154,526 reduced the (−)-nicotine-induced noradrenaline release in the paraventricular nucleus and elevation of plasma corticosterone. These results suggest the involvement of the brain CRH neurons in the (−)-nicotine-induced activation of noradrenergic neurons in the paraventricular nucleus. The extrahypothalamic CRH neurons in the brain contribute to the coordination of the overall stress responses (Nemeroff, 1992; Makino et al., 1999). Intravenous administration of (−)-nicotine activates the CRH neurons in extrahypothalamic regions such as the Barrington's nucleus (Matta et al., 1997), and acute immobilization stress or footshock stress enhances the expression of CRH mRNA in the Barrington's nucleus (Imaki et al., 1991; Imaki and Vale, 1993). CRH neurons in the Barrington's nucleus seem to have a putative influence on the noradrenergic neurons of the locus coeruleus, since tyrosine hydroxylase-positive dendrites from the locus coeruleus extend into the Barrington's nucleus (Valentino et al., 1994). However, the locus coeruleus is relatively unresponsive than the A_2 and C_2 cells in the nucleus of the solitary tract as measured by cFos expression in response to intravenously administered (−)-nicotine (Valentine et al., 1996) or by adrenocorticotropin secretion evoked by reginonally microinjected (−)-nicotine (Matta et al., 1993a). Since nerve fibers immunoreactive for CRH are also observed in the nucleus of the solitary tract (Sakanaka et al., 1987; Herbert and Saper, 1990), it is likely that CRH neurons adjacent to the A_2 cells in the nucleus of the solitary tract also play a role in the (−)-nicotine-induced elevation of plasma corticosterone, as suggested by Matta et al. (1993a).

Peripherally administered indomethacin, an inhibitor of cycooxygenase, has been shown to inhibit the hypothalamic–pituitary–adrenal axis activated by interleukin-1β (Buller et al., 1998; Parsadaniantz et al., 2000). In addition, an increase in plasma adrenocorticotropin has also been shown to be produced by administration of prostaglandin E_2 directly into the preoptic anterior hypothalamus (Watanabe et al., 1990), the organum vasculosum of lamina terminalis (Katsuura et al., 1990), and the median eminence (McCoy et al., 1994). These results suggest the involvement of brain prostaglandins in activation of the hypothalamic–pituitary–adrenal axis. In the present experiment, intracerebroventricularly administered indomethacin also attenuated the (−)-nicotine-induced elevation of noradrenaline release in the paraventricular nucleus and plasma corticosterone. These results suggest that the brain prostaglandins are also involved in the (−)-nicotine-induced increase of noradrenaline release in the paraventricular nucleus. However, the acting sites of prostaglandins remain to be elucidated.

In summary, we demonstrated here that the centrally administered (−)-nicotine-induced elevation of plasma corticosterone is mediated by CRF-1 receptor- and prostaglandin-mediated release of noradrenaline in the hypothalamic paraventricular nucleus in rats.

Acknowledgements

This work was supported in part by a grant from the Smoking Research Foundation in Japan and by a grant from The President Research Fund of Kochi Medical School Hospital.

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