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(+)-Nantenine isolated from *Nandina domestica* Thunb. inhibits adrenergic pressor responses in pithed rats

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Abstract

The effects of (+)-nantenine on various pressor responses, recently reported exerting competitive antagonistic activity at the α_1-adrenoceptor/5-hydroxytryptamine (5-HT) $_{2A}$ receptor, were examined in vivo. (+)-Nantenine (0.03–3 mg/kg) caused a dose-dependent inhibition of the pressor response to phenylephrine (α_1-adrenoceptor agonist) or 5-HT (5-HT receptor agonist) in both anesthetized and pithed rats. The pressor response to UK 14304 (5-Bromo-*N*-[2-imidazolin-2-yl]-6-quinoxalinamine) (an α_2-adrenoceptor agonist) was inhibited by (+)-nantenine (0.003–3 mg/kg) in pithed rats in a dose-dependent manner without affecting the angiotensin II-induced pressor response in anesthetized rats. The pressor response to sympathetic nerve stimulation was also inhibited by (+)-nantenine (0.3–3 mg/kg) in a dose-dependent manner. (+)-Nantenine (3 mg/kg) facilitated the norepinephrine release induced by sympathetic nerve stimulation in pithed rats. In the guinea pig vas deferens, the initial component of contractions induced by electrical field stimulation was enhanced by (+)-nantenine (1–30 μM) in a concentration-dependent manner, while the later component was inhibited by it. These data suggest that (+)-nantenine has antagonistic activities on α_1-adrenoceptors, α_2-adrenoceptors and 5-HT_{2A} receptors in pithed rats.

Keywords: (+)-Nantenine; Pithed rat; Pressor response; Sympathetic nerve stimulation; α-Adrenoceptor; 5-HT receptor; Sympathetic nerve stimulation

1. Introduction

Natural products able to antagonize the actions of various neurotransmitters would be of great interest both as pharmacological tools and as therapeutic agents for the treatment of various diseases. In the course of our survey on pharmacologically active substances in medical plants including *Nandina domestica* Thunberg, much attention has been given to the occurrence of natural products possessing antagonistic activities on the α-adrenoceptors or the 5-HT receptors (Indra et al., 2002a,b). (+)-Nantenine [(+)-9,10-methylen-dioxy-1,2-dimethoxyaporphine] is an aporphine alkaloid isolated from the fruit of *N. domestica* Thunberg. The fruit of this plant has been used to treat asthma, whooping cough, pharynx tumor and uterine bleeding in Japan for many years (Shoji et al., 1984). A number of aporphine alkaloids and related synthetic compounds have been shown to possess α_1-adrenoceptor antagonistic properties in vascular smooth muscles (Ivorra et al., 1992, 1993; Ko et al., 1993, 1994; Chulia et al., 1994, 1996; Martinez et al., 1999). Recently in our laboratory, it was found that (±)-nantenine competitively inhibited the contraction by phenylephrine and 5-HT in the rat thoracic aorta (Indra et al., 2002a,b). These results suggested that (+)-nantenine exerted competitive antagonistic activity at the α_1-adrenoceptor/5-HT_{2A} receptor. But, the action of (+)-nantenine on blood pressure has not ever been clarified in vivo. In this paper, we present the first report concerning the action of (+)-nantenine on various pressor responses in pithed rats.

2. Materials and methods

2.1. Materials

A lot of (+)-nantenine was isolated from the fruit of *N. domestica* Thunb. as described previously (Shoji et al.,

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1984) for the present in vivo study. Briefly, the dried powdered fruit of *N. domestica* Thunberg. was extracted with methanol and the methanol extract was separated using ethyl acetate and water. The water-soluble portion was extracted with *n*-butyl alcohol and the extract separated by chromatography on a silica gel column, yielding pure (+)-nantenine. (+)-Nantenine was resolved in hydrochloride and then lyophilized to yield (+)-nantenine hydrochloride in order to experiment in vivo.

5-Hydroxytriptamine hydrochloride, phenylephrine hydrochloride, *d*-tubocurarine chloride and UK 14304 (5-Bromo-*N*-[2-imidazolin-2-yl]-6-quinoxalinamine) were purchased from Sigma (St. Louis, MO, USA). All compounds except UK 14304 were dissolved in distilled water. UK 14304 was dissolved in dimethylsulphoxide (DMSO), and diluted 100-fold in distilled water.

2.2. Anesthetized rat preparation

Male Wistar rats (9–11 weeks old, 210–260 g body weight) were maintained on a normal rats chow and tap water freely. Each rat was anesthetized with 50 mg/kg of sodium pentobarbital (i.p.), and a tracheal cannula was inserted for artificial respiration. The right and left jugular veins were cannulated for drug administration and bilateral vagotomy was performed at the midcervical level. The left carotid artery was cannulated for the measurement of arterial blood pressure with a pressure transducer (San-ei, Tokyo, Japan). Diastolic blood pressure was used for evaluating the pressor responses.

2.3. Pithed rat preparation and measurement of arterial plasma norepinephrine concentrations

After the induction of preparation same as anesthetized rats, arterial respiration with room air at 50 strokes/min and a tidal volume of 10 ml/kg was started and then a stainless steel rod was passed into the spinal column for pithing and electrical stimulation of the sympathetic outflow. A second stainless steel rod was inserted subcutaneously from the left shoulder to the left hindlimb and served as an indifferent electrode. Somatic motor effects were blocked with *d*-tubocurarine chloride (1 mg/kg i.v.). Stimulation of the spinal sympathetic outflow was performed with rectangular 1-ms pulses of 3 Hz, at 50 V for 30 s. After completion of the pithing procedure, about 40 min was allowed for stabilization. For arterial blood samplings, the left femoral artery was cannulated with polyethylene tube filled with heparin to prevent blood clotting. Catecholamines were extracted from plasma by the alumina absorption method, and plasma norepinephrine concentration was determined by high performance liquid chromatography (HPLC) with an amperometric detector (ECD-300, EI-COM, Kyoto, Japan), as described previously (Hayashi et al., 1987; Koganei et al., 1995).

2.4. Pressor responses

After completion of the operation, 40–60 min for pithed rats and 10–20 min for anesthetized rats were allowed for stabilization and then the experiments were performed.

The pressor response to phenylephrine or 5-HT was studied in each rat under controlled conditions and 3 min after i.v. administration of (+)-nantenine (anesthetized rats, 0.3 mg/kg; pithed rats, 0.03–3 mg/kg).

(+)-Nantenine (0.003–3 mg/kg) was applied cumulatively 3 min after i.v. administration of UK 14304.

2.5. Vas deferens preparation

Male guinea pigs (6 weeks old, 400 g body weight) were killed by cervical dislocation and exsanguination. The vas deferens was dissected and suspended vertically in a 10-ml organ bath containing Kreb–Henseleit buffer (KH) at 37 °C and aerated with 95% O_2–5% CO_2, between two parallel platinum plate electrodes, with the lower end fixed and the upper end attached to a force-displacement transducer (T-7, Orientec, Tokyo, Japan). The composition of the KH was 120 mM NaCl, 4.8 mM KCl; 25.2 mM $NaHCO_3$; 1.2 mM KH_2PO_4; 1.3 mM $MgSO_4 \cdot 7H_2O$; 1.2 mM $CaCl_2 \cdot 2H_2O$; and 11 mM glucose. After the initial load of 0.5 g was applied, tissues were equilibrated for 45–60 min and then continuously stimulated with block pulses (interval 10 min, 10 Hz, 0.2 ms duration for 20 s, supramaximal voltage).

2.6. Data analysis

Results of the experiments are expressed as means ± S.E.M. Significance was tested with Student's *t*-test, or Dunnett's multiple comparison test when comparisons involved more than two groups.

3. Results

3.1. Effects of (+)-nantenine on pressor response to receptor agonists in anesthetized rats

The effect of (+)-nantenine was tested against the phenylephrine, 5-HT or angiotensin II-induced pressor response in anesthetized rats. Phenylephrine-(10 μg/kg), 5-HT- (100 μg/kg) and angiotensin II (0.3 μg/kg)-induced changes of diastolic blood pressure were 58 ± 4, 54 ± 3 and 47 ± 7 mm Hg ($n = 5$), respectively. (+)-Nantenine (0.3 mg/kg) did not significantly change the baseline values of diastolic blood pressure in the anesthetized rats (control, 102.3 ± 5.3 mm Hg; after (+)-nantenine, 99.4 ± 4.7 mm Hg; $n = 15$). When the dose of (+)-nantenine increased to 3 mg/kg or more, the sustained hypotensive action was observed in anesthetized rats (data not shown). (+)-Nantenine (0.3 mg/kg) decreased the pressor responses to phenylephrine and 5-HT to 45 ± 5% and 32 ± 5% ($n = 5$), respectively, however, did

not affect the angiotensin II -induced pressor response in the anesthetized rats (data not shown).

3.2. Inhibitory effects of (+)-nantenine on pressor responses to phenylephrine or 5-HT in pithed rats

The effects of (+)-nantenine on the phenylephrine- or 5-HT-induced pressor response were examined in pithed rats. The baseline values of diastolic blood pressure were not significantly changed by (+)-nantenine at the high dose (3 mg/kg) in pithed rats (control, 50.7 ± 3.1 mm Hg; after (+)-nantenine, 53.7 ± 3.8 mm Hg; $n = 12$). Phenylephrine- (10 μg/kg) and 5-HT- (10 μg/kg) induced changes of diastolic blood pressure were 61 ± 5 and 72 ± 5 mm Hg ($n = 6$), respectively. Although (+)-nantenine did not affect the basal diastolic blood pressure (data not shown), it inhibited the pressor responses to phenylephrine or 5-HT in a dose-dependent manner (Fig. 1A and B).

3.3. Effects of (+)-nantenine on pressor responses to sympathetic nerve stimulation in pithed rats

The effect of (+)-nantenine on the pressor response to sympathetic nerve stimulation was characterized in pithed rats. The pressor response to sympathetic nerve stimulation was inhibited by (+)-nantenine in a dose-dependent manner. The inhibitory effects of (+)-nantenine on the pressor response were sustained for 10–20 min (0.3 mg/kg), 30–40 min (1 mg/kg) and more than 60 min (3 mg/kg) (Fig. 2).

3.4. Effects of (+)-nantenine on norepinephrine release induced by nerve stimulation in pithed rats

Since (+)-nantenine inhibited the pressor response to sympathetic nerve stimulation, the effect of (+)-nantenine on norepinephrine release from sympathetic nerve endings was tested in pithed rats. (+)-Nantenine did not affect the basal level of plasma norepinephrine in pithed rats (data not shown). (+)-Nantenine (3 mg/kg) increased the release of norepinephrine induced by sympathetic nerve stimulation from 16.47 ± 2.78 to 50.03 ± 9.91 pmol/ml ($n = 15$, $P < 0.01$).

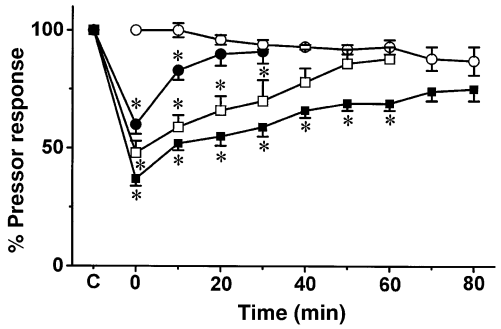

Fig. 2. Time course of the effects of (+)-nantenine (0.3–3 mg/kg) on sympathetic nerve stimulation (3 Hz)-induced pressor response. After completion of the preparation, 40–60 min was allowed for stabilization and then (+)-nantenine was administered intravenously (or not). (+)-Nantenine was administered and after about 3 min, the pressor response was induced by sympathetic nerve stimulation for 30 s every 10 min. Control response was taken as 100%. Data are means ± S.E.M. from five experiments. Control (○); (+)-nantenine (0.3 mg/kg, ●; 1 mg/kg, □; 3 mg/kg, ■). *$P < 0.01$ (test of Dunnett) as compared to control.

3.5. Effects of (+)-nantenine on pressor responses to the α_2-adrenoceptor agonist in pithed rats

Since (+)-nantenine facilitated the norepinephrine release from sympathetic nerve endings, we examined the effect of (+)-nantenine on the pressor response to UK 14304 (an α_2-adrenoceptor agonist) in pithed rats. After the pressor response to UK 14304 reached to a steady level, (+)-nantenine was administered cumulatively within 15 min in pithed rats. There were no statistical differences among each diastolic blood pressure at 0, 10, 20 and 30 min after the administration of UK 14304 (data not shown), suggesting

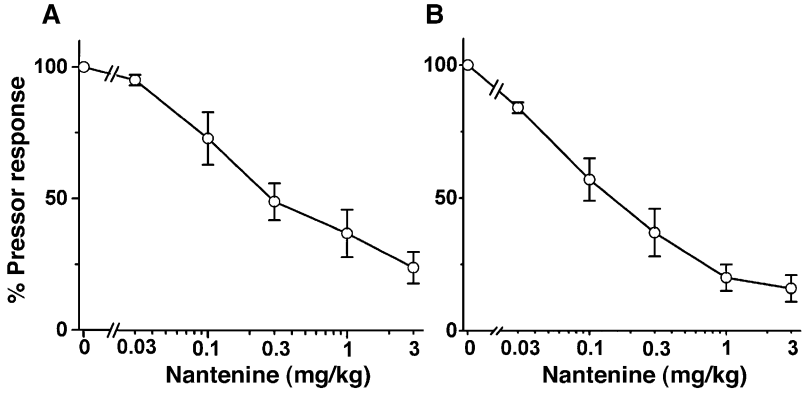

Fig. 1. Effects of (+)-nantenine on phenylephrine (A, 10 μg/kg)- and 5-HT (B, 10 μg/kg)-induced pressor response in pithed rats. After completion of the preparation, 40–60 min was allowed for stabilization and then the experiments were performed. The pressor response to phenylephrine or 5-HT was studied in each rat under controlled conditions and 3 min after i.v. administration of (+)-nantenine. Control response was taken as 100%. Data are means ± S.E.M. from six experiments.

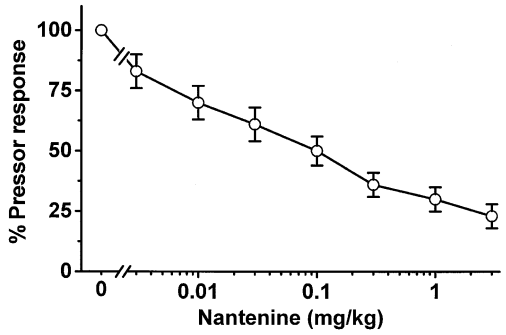

Fig. 3. Effects of (+)-nantenine on UK 14304 (100 μg/kg)-induced pressor response in pithed rats. After the pressor response to UK 14304 reached to a steady level, (+)-nantenine was administered cumulatively. Changes in diastolic blood pressure by UK 14304-induced pressor response was taken as 100%. Data are means ± S.E.M. from seven experiments.

that the pressor response of UK 14304 was long lasting. (+)-Nantenine inhibited the pressor response to UK 14304 in a dose-dependent manner (Fig. 3).

3.6. Effects of (+)-nantenine on contraction induced by electrical field stimulation in guinea pig vas deferens

We examined the effect of (+)-nantenine on the contractile response of the guinea pig vas deferens to electrical field stimulation, which is a useful model to test the effect of drugs on the presynaptic α_2-adrenoceptor (Hammarström and Sjöstrand, 1984; Todorov et al., 1999). As shown in Fig. 4A, the configuration of contraction was drastically changed by the administration of (+)-nantenine. The later component of contraction was inhibited by (+)-nantenine, while the initial component was potentiated by it in a concentration-dependent manner (Fig. 4B).

4. Discussion

In previous report, we have found that (±)-nantenine exerts competitive antagonistic activities at the α_1-adrenoceptor/5-HT_{2A} receptors in rat thoracic aorta (Indra et al., 2002a,b). Thus, to test the effect of (+)-nantenine in vivo, we examined its action on blood pressure in anesthetized or pithed rats. Pithed rats, in which the central and afferent aspects of neural control are effectively eliminated by spinal cord destruction, is a useful model for analysis of interaction of (+)-nantenine with the peripheral tissues including the sympathetic nervous system. In both anesthetized and pithed rats, (+)-nantenine inhibited the pressor response to phenylephrine or 5-HT in a dose-dependent manner, however, did not affect the angiotensin II-induced pressor response. In pithed rats, the intravenous administration of phenylephrine causes a dose-dependent vasocontraction and an increase in blood pressure via the α_1-adrenoceptor (Timmermans et al., 1979). The previous reports showed that 5-HT caused the vasoactive and hypertensive action via the 5-HT_{2A} receptor

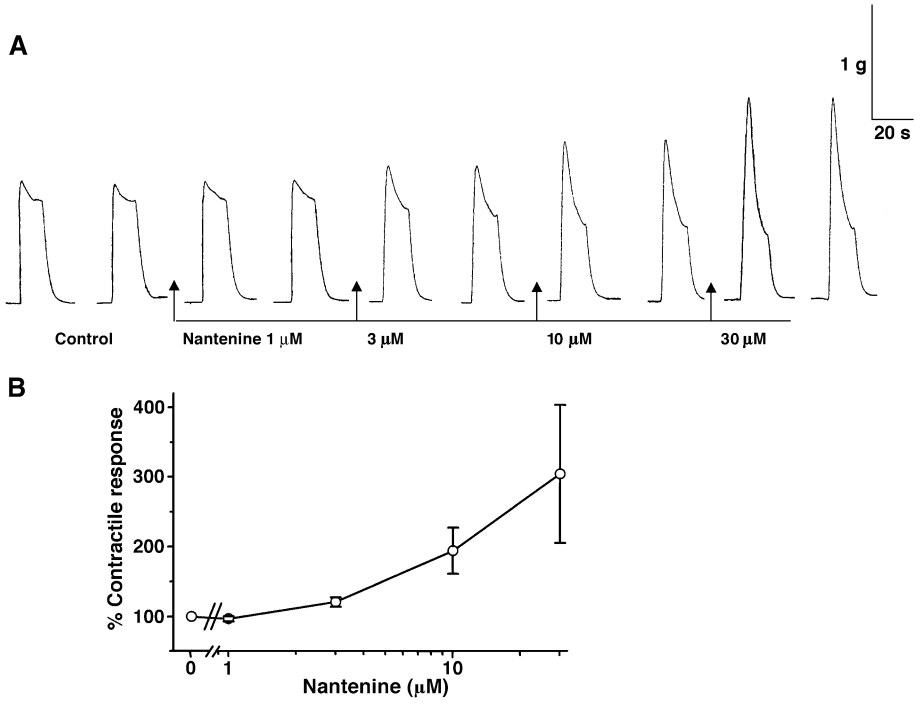

Fig. 4. Effects of (+)-nantenine (1–30 μM) on the contraction induced by electrical field stimulation (interval 10 min, 10 Hz, 0.2 ms duration for 20 s, supramaximal voltage) in the guinea pig vas deferens. (A) Typical traces of the contraction induced by electrical field stimulation before and after application of (+)-nantenine. (B) The maximum initial contractile responses of the vas deferens to electrical field stimulation before and after application of (+)-nantenine was compared. Control response was taken as 100%. Data is mean ± S.E.M. from five experiments.

(Kalkman et al., 1984; Docherty, 1988). Similar to other α_1-adrenoceptor antagonists (Kimura et al., 1983) or 5-HT_{2A} antagonists (Pawlak et al., 1998) (+)-nantenine showed the inhibitory effect on the pressor response to phenylephrine or 5-HT. Taken together with our previous report (Indra et al., 2002a,b), the antagonistic activity of (+)-nantenine on the α_1-adrenoceptor and the 5-HT_{2A} receptor may account for its inhibitory effects on the pressor responses to phenylephrine and 5-HT in vivo. However, we cannot exclude the involvement of other 5-HT receptor subtypes in the antagonistic activity of (+)-nantenine on the 5-HT-induced pressor response.

The effect of (+)-nantenine on norepinephrine release induced by sympathetic nerve stimulation was studied in pithed rats. Sympathetic nerve stimulation induces norepinephrine release from sympathetic nerve endings and increases norepinephrine level in plasma (Yamaguchi and Kopin, 1979, 1980; Zukowska-Grojec et al., 1983; Mizunuma et al., 2000). Interestingly, (+)-nantenine facilitated the norepinephrine release induced by sympathetic nerve stimulation. The inhibition of the presynaptic α_2-adrenoceptor by drugs prevents feedback inhibition to enhance the release of norepinephrine (Langer, 1974; Starke, 1977). Thus, it was suggested that (+)-nantenine had the inhibitory effect on the sympathetic presynaptic α_2-adrenoceptors in pithed rats.

Furthermore, we examined the action of (+)-nantenine on the postsynaptic α_2-adrenoceptors. The postsynaptic α_2-adrenoceptor occurs on vascular smooth muscle and mediates the pressor responses in pithed rats (Drew and Whiting, 1979; Docherty et al., 1979; Docherty and McGrath, 1980). UK 14304 (an α_2-adrenoceptor agonist) was used as vasopressor which arises the pressor response by the action on peripheral postsynaptic α_2-adrenoceptor in pithed rats (Angel et al., 1992). (+)-Nantenine inhibited the pressor response to UK 14304 in a dose-dependent manner in pithed rats, thus suggesting the inhibitory effect on peripheral postsynaptic α_2-adrenoceptors.

The effect of (+)-nantenine on the α_2-adrenoceptors was examined using the isolated guinea pig vas deferens. The latter component of contractions of the vas deferens induced by electrical field stimulation was inhibited by (+)-nantenine, whereas the former component was potentiated by it in a concentration-dependent manner. It has been reported that the initial contractile response of the guinea pig vas deferens to electrical field stimulation is caused by co-transmitted ATP released from sympathetic nerve endings, while the secondary contraction is mediated by norepinephrine (Hammarström and Sjöstrand, 1984). The α_1-adrenoceptor antagonists inhibit the secondary contraction derived from norepinephrine, while the α_2-adrenoceptor antagonists enhance the initial contraction caused by ATP in the guinea pig vas deferens (Todorov et al., 1999). Therefore, the effect of (+)-nantenine can be described as the inhibition of both postsynaptic α_1-adrenoceptors and presynaptic α_2-adrenoceptors, resulting in the potentiation of the initial component and the inhibition of the secondary component. Indeed, (+)-nantenine caused a concentration-dependent inhibition of the binding of [^{3}H]yohimbine to the α_2-adrenoceptors on mouse cerebral cortex membrane (unpublished observation). These results suggest that (+)-nantenine has the α_2-adrenoceptor antagonistic action in vitro.

In conclusion, the data in the present study indicates that (+)-nantenine has an inhibitory action on the pressor responses via the α_1-adrenoceptor, the 5-HT_{2A} receptor and the α_2-adrenoceptor in vivo. The study on (+)-nantenine in comparison with other α_1-adrenoceptor/5-HT_{2A} receptor antagonists is under way.

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