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Structure–activity relationship studies with (±)-nantenine derivatives for α_1-adrenoceptor antagonist activity

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Abstract

A series of (±)-nantenine derivatives of the natural aporphine alkaloids was synthesized and examined for a blocking action on α_1-adrenoceptors in rat aorta and A10-cells. The potency of these derivatives was compared with that of an aporphine-related compounds (+)-boldine, an α_1-adrenoceptor antagonist. Among nine (±)-nantenine derivatives having different substituents at N-6, C-1, or C-4 of the aporphine skeleton, (±)-domesticine had the most powerful α_1-adrenoceptor-blocking action. The order of pA_2 values was (±)-domesticine (8.06 ± 0.06) > (±)-nordomesticine (7.34 ± 0.03) > (±)-nantenine (7.03 ± 0.03) > (+)-boldine (6.91 ± 0.02) > other derivatives. Study of the structure–activity relationships showed that the replacement of a methoxy moiety at C-1 position of (±)-nantenine with a hydroxyl group increased affinity for the receptor. In contrast, replacement of a methyl group with a hydrogen atom or an ethyl group at N-6 position in the (±)-nantenine structure decreased affinity for the receptor. These results suggest that a hydroxyl group at the C-1 position and a methyl group at the N-6 position in the (±)-nantenine structure are essential for the enhancement of affinity for the α_1-adrenoceptor.

Keywords: α_1-Adrenoceptor; (±)-Nantenine derivatives; Aorta, rat

1. Introduction

Historically, most drugs have been derived from natural products, but there has been a shift away from their use with the increasing predominance of molecular approaches to drug discovery. Nevertheless, their structural diversity makes them a valuable source of novel lead compounds against newly discovered therapeutic targets (Harvey, 1999). α_1-Adrenoceptors are a heterogeneous population of G-protein coupled receptors that mediate the central and peripheral actions of the natural adrenergic amines, epinephrine and norepinephrine (Hieble et al., 1995). A number of aporphine alkaloids and related synthetic compounds have been shown to possess α_1-adrenoceptor antagonistic properties in vascular smooth muscle. (Ivorra et al., 1992, 1993; Ko et al., 1993, 1994; Chulia et al., 1994, 1996; Martinez et al., 1999). (+)-Nantenine [(+)-9,10-methylendioxy-1, 2 dimethoxyaporphine] is an aporphine alkaloid isolated from the fruit of *Nandina domestica* Thunberg (Berberidaceae). 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). Recently, we found that (±)-nantenine (Fig. 1) inhibited the contraction of the rat thoracic aorta induced by phenylephrine (α_1-adrenoceptor). However, neither the contractile response of the vas deferens to transmural simulation (α_2-adrenoceptor) nor the positive inotropic response of left atria to isoproterenol in guinea pig (β_1-adrenoceptor) was affected by (±)-nantenine at doses up to 10 μM (unpublished data). Therefore, in order to characterize the nature of (±)-nantenine, we examined the effects of (±)-nantenine derivatives on α_1-adrenoceptors in the rat aorta. Among (±)-nantenine derivatives, we found that (±)-domesticine was the most powerful to inhibit the concentration–response curves for phenylephrine in rat aorta. In this paper, we present the first report on the structure–activity relation-

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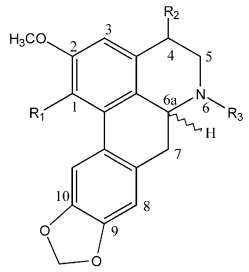

(±)-Nantenine derivatives	R_1	R_2	R_3
(±)-Nantenine	OCH_3	H	CH_3
(±)-Nornantenine	OCH_3	H	H
(±)-Ethylnornantenine	OCH_3	H	C_2H_5
(±)-*N*-Trifluoroacetylnornantenine	OCH_3	H	$COCF_3$
(±)-4α-Hydroxynantenine	OCH_3	α-OH	CH_3
(±)-4β-Hydroxynantenine	OCH_3	β-OH	CH_3
(±)-Domesticine	OH	H	CH_3
(±)-Nordomesticine	OH	H	H
(±)-*N*-Trifluoroacetyldomesticine	OH	H	$COCF_3$

Fig. 1. Chemical structure of (±)-nantenine derivatives.

ship of (±)-nantenine derivatives for causing an inhibitory effect on the α_1-adrenoceptors in the rat aorta.

2. Methods

2.1. Material

[^{3}H]*myo*-inositol (*myo*-[2-^{3}H]inositol 22.2 Ci/mmol) was obtained from DuPont/NEN Research Products (Wilmington, MA, USA). (+)-Boldine was obtained from Aldrich Chemical (USA). Phenylephrine hydrochloride and norepinephrine hydrochloride and carbachol were purchased from Sigma (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade and were obtained from commercial sources. (±)-Nantenine derivatives and (+)-boldine were dissolved in dimethylsulphoxide (DMSO). Other drugs were dissolved in distilled water.

The melting point of the compounds was measured on a Buchi melting point measuring apparatus. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a JEOL JNM-FX 100 (100 MHz) or JEOL-JNM-EX-270 instrument in $CDCl_3$ solution using tetramethyl silane as internal standard. Abbreviations used are s (singlet), t (triplet), q (quartet), dd (doublet doublet), m (multiplet). Infrared (IR) spectra were recorded on a Hitachi 260-10 spectrophotometer in KBr disc. Mass spectra (MS) were run with a Hitachi RMU-7M or M-80 instrument (70 eV).

2.2. Synthesis

(±)-Nantenine, (±)-*N*-trifluoroacetylnordomesticine, (±)-4α-hydroxynantenine, (±)-4β-hydroxynantenine and (±)-domesticine were prepared according to the procedures reported in the literature (Hoshino et al., 1975, 1987; Ogasawara et al., 1998). A suspension of (±)-*N*-trifluoroacetylnordomesticine (64 mg, 0.12 mmol), methyl iodide (1.2 ml, 19 mmol) and NaH (188 mg, 7.8 mmol) [prepared by washing NaH (60% dispersion in mineral oil) with hexane and tetrahydrofuran (20 ml) stirred at room temperature for 1 h. Water (5 ml) was added to the residue obtained and the product was taken up in dichloromethane. Usual work-up of the dichloromethane extract gave a residue which was chromatographed over silica gel with dichloromethane to yield (±)-*N*-trifluoroacetylnornantenine as crystals (62 mg, 94%), melting point (mp) 221–222 °C (dichloromethane–hexane). Analysis $C_{21}H_{18}F_3NO_5$ calculated: C, 59.86; H, 4.31; N, 3.32; F, 13.53. Found: C, 60.00; H, 5.79; N, 3.56; F, 13.56. MS *m/z* 421 (M^+); IR 1680 cm^{-1}, ^{1}H NMR (100 MHz) δ 3.67 (3H, s), 3.88 (3H, s), 4.94 (1H, dd), J=5.7, 12.9 Hz), 5.96 (2H, s), 6.59 (1H, s), 6.72 (1H, s), 7.95 (1H, s).

(±)-Nornantenine was prepared in 89% yield from (±)-*N*-trifluoroacetyl nornantenine (50 mg, 0.12 mmol) by hydrolysis as described for (±)-nordomesticine, mp 163–165 °C (AcOEt); MS *m/z* 325 (M^+). HRMS *m/z* (M^+) calculated for $C_{19}H_{19}NO_4$: 325.1318 Found: 325.1320. IR 1680 cm^{-1}, ^{1}H NMR (100 MHz) δ 3.66 (3H, s), 3.86 (3H, s), 5.94 (2H, s), 6.56 (1H, s), 6.69 (1H, s), 7.91 (1H, s).

A mixture of (±)-nornantenine (50 mg, 0.16 mmol), Ac_2O (24 mg, 0.24 mmol), and K_2CO_3 (43 mg, 0.3 mmol) in dichloromethane (2 ml) was stirred at room temperature for 2 h. To the mixture was added dichloromethane (30 ml). Usual work-up of the dichloromethane layer gave a solid (53 mg) which used in the next step without purification. A mixture of amide (53 mg, 0.14 mmol) and $LiAlH_4$ (19 mg, 0.5 mmol) in THF (5 ml) was stirred at room temperature overnight. Ether (30 ml) and saturated Na_2SO_4 solution (0.5 ml) were added to the mixture and a precipitate was filtered off. The filtrate was dried over anhydrous K_2CO_3. Removal of the solvent under reduced pressure yielded an oily residue (54 mg) which was chromatographed over silica gel with chloroform to leave an oily products (±)-ethylnornantenine (22 mg). Trituration of the oily product in hexane gave crystals (12.8 mg, 27%), mp 122–125 °C. MS *m/z* 353 (M^+); HRMS *m/z* (M^+) calculated: $C_{21}H_{23}NO_4$: 353.1644 Found: 353.1618. ^{1}H NMR (270 MHz) δ 1.15 (3H, t, J=7 Hz), 3.65 (3H, s), 3.87 (3H, s), 5.96, 5.97 (2H, each s), 6.59 (1H, s), 6.75 (1H, s), 7.91 (1H, s).

A mixture of (±)-*N*-trifluoroacetylnordomesticine (50 mg, 0.12 mmol) and 5% K_2CO_3 (1 ml) in methanol (10 ml) was refluxed for 5 h. The residue obtained on removal of the solvent under reduced pressure was taken up in dichloromethane extract to yield a solid, which was re-crystallized from dichloromethane–hexane to give (±)-nordomesticine (34 mg, 89%, mp 221–222 °C). Analysis $C_{18}H_{17}NO_4$ calculated: C, 69.44; H, 5.50; N, 4.50. Found: C, 69.33; H, 5.79; N, 4.46. MS *m/z* 311; IR 3420, 3190 cm^{-1}, ^{1}H NMR (100 MHz) δ 3.88 (3H, s), 5.91 (2H, s), 6.51 (1H, s), 6.68 (1H, s), 7.91 (1H, s).

2.3. Contraction studies in the rat aorta

All of the animals used in this study were treated in accordance with the principles and guidelines on Animal Care of Tohoku University. Male Wistar rats weighing 220–250 g were killed by cervical dislocation. The thoracic aorta was dissected and connective tissues were carefully removed. The endothelium was removed by gently rubbing the endothelial surface with cotton pellets. The lack of endothelium was checked by abolition of the carbachol (1 μM)-induced relaxation. The aorta was cut into helical strips approximately 1.5–2 mm in width and 10 mm in length. One end was anchored in the organ chamber and the other was connected to a force-displacement transducer (Minebea Transducer UL-100GR, Japan). The strip was suspended in a 6-ml organ bath containing Kreb–Henseleit buffer (KH) at 37 °C and aerated with 95% O_2–5% CO_2. The composition of the KH was 119 mM NaCl, 5.4 mM KCl; 25.2 mM $NaHCO_3$; 1.2 mM KH_2PO_4; 1.3 mM $MgSO_4 \cdot 7H_2O$; 2.5 mM $CaCl_2 \cdot 2H_2O$; and 11 mM glucose. An initial load of 1 g was applied to each preparation and was maintained throughout the 75- to 90-min equilibration period. During the basal tension adjustment period, the helical strips were exposed to KCl (60 mM) three times to verify tissue contractility.

The strips were contracted with phenylephrine (10 μM) and washed for 30 min. Isometric contractions were obtained in response to cumulative additions of agonist in the absence and presence of antagonist (incubated for 30 min). Dose-dependent contraction curves for the agonist (0.003–100 μM) in the presence of antagonist (0.3–3 μM) were related to the control dose–response curve, of which the maximum response was taken as 100%. pA_2 values were obtained according to Arunlakshana and Schild (1959). Antagonist was taken to be competitive when the Schild regression slope did not differ significantly ($P > 0.05$) from unity.

2.4. Inositol phosphate accumulation

Total inositol phosphates was monitored by measuring [^{3}H]inositol phosphates as previously described by Nakahata et al. (1989). A10 cells were grown on 12-well plates for 7 days at the density of 1×10^{-5} cells/ml/well and were labeled with 5 μCi/ml [^{3}H]*myo*-inositol for 48 h. After being washed twice with Dulbecco's modified Eagle's medium (DMEM) buffer (pH 7.35), the cells were preincubated in DMEM-HEPES containing drugs or DMSO 0.1% and 10 mM LiCl for 15 min, and then they were incubated with 10 μM norepinephrine for an additional 15 min. The reaction was terminated by the addition of 1 ml of 5% tricarboxylic acid after aspiration of the medium. The tricarboxylic acid extracts were washed three times with diethyl ether to remove tricarboxylic acid. Diethyl ether in the sample was

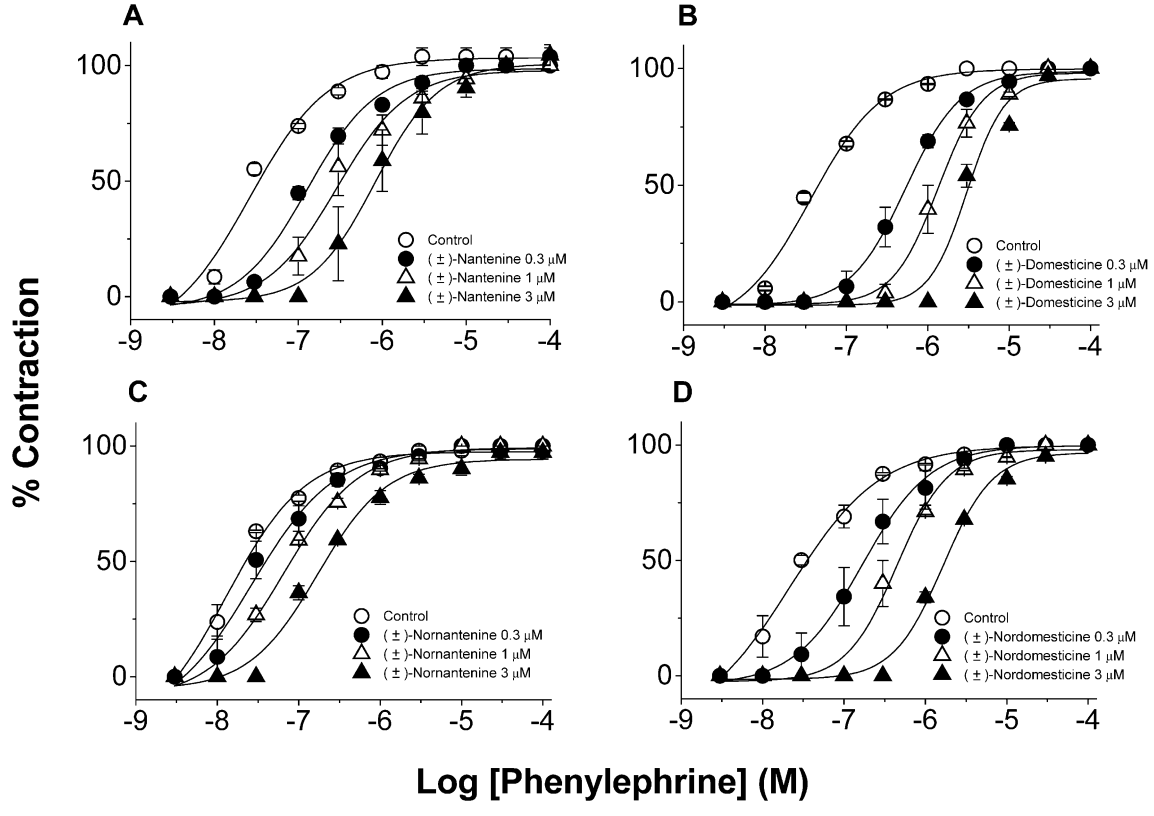

Fig. 2. Effects of (±)-nantenine (A), (±)-domesticine (B), (±)-nornantenine (C) and (±)-nordomesticine (D) on phenylephrine-induced contraction in the rat aorta. Contractile responses are expressed as percentages of the contraction induced by 10 μM of phenylephrine. Data are means ± S.E.M. from four to six experiments.

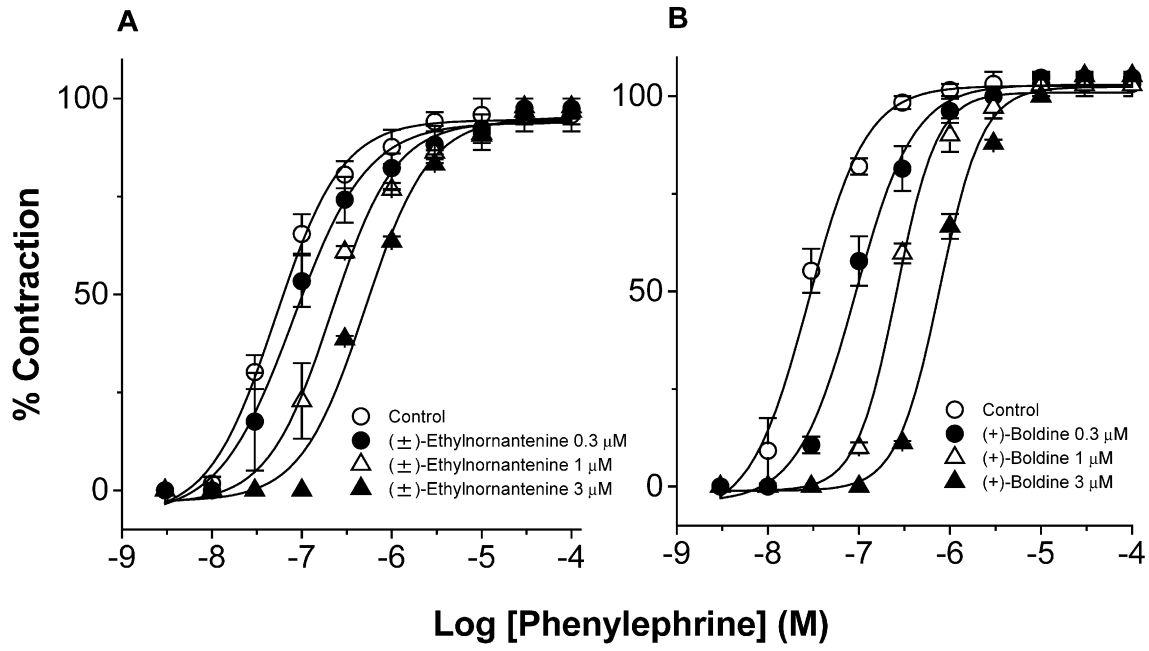

Fig. 3. Effects of (±)-ethylnornantenine (A) and (+)-boldine (B) on phenylephrine-induced contraction in the rat aorta. Contractile responses are expressed as percentages of the contraction induced by 10 μM of phenylephrine. Data are means ± S.E.M. from four to six experiments.

removed by keeping the sample at 47 °C for 20 min. Total [^{3}H]inositol phosphates were separated on an anion-exchange column (AG 1X-8).

2.5. Statistical analysis

All values in the text are expressed as the means ± stan-standard error mean (S.E.M.) [confidence interval 95%] where *n* is the number of assays. Test of significance was performed by using analysis of variance (ANOVA).

3. Results

3.1. Effects of (±)-nantenine derivatives and (+)-boldine on the contractile response to phenylephrine of the rat aorta.

(±)-Nantenine derivatives and (+)-boldine were tested for α_1-adrenoceptor antagonist activity against phenylephrine-induced contractions in the rat aorta. (±)-Nantenine and its derivatives (0.3–3 μM) produced a parallel rightward shift of the concentration–contractile response curves for phenylephrine in a concentration-dependent manner (Fig. 2) without producing inhibitory effects on contractions induced by potassium chloride or histamine in concentrations up to 10 μM (data not shown). These results indicated a α_1-adrenoceptor blocking action. (+)-Boldine also produced competitive α_1-adrenoceptor antagonistic activity, with an affinity approximately 11-fold lower than that of (±)-domesticine (Fig. 3B).

Replacement of the methoxy moiety at C-1 position with a hydroxyl group in (±)-nantenine and (±)-nornantenine ((±)-domesticine and (±)-nordomesticine, respectively) increased the affinity for the receptor by approximately 10-fold (Fig. 2; Table 1). In contrast, replacement of a methyl

Table 1
Effects of structural changes of (±)-nantenine and its derivatives

Compounds	(±)-Nantenine derivatives	Affinity ratio *
(±)-Nantenine	(±)-Nornantenine	0.38
	(±)-Ethylnornantenine	0.24
	(±)-4α-Hydroxynantenine	0.08
	(±) 4β-Hydroxynantenine	0.05
	(±)-Domesticine	10
(±)-Nornantenine	(±)-Nordomesticine	10
(±)-Domesticine	(±)-Nordomesticine	0.19

* Affinity ratio calculated from $K_{b\ compounds}/K_{b\ derivatives}$.

Table 2
Summary of pA_2 values of (±)-nantenine derivatives and (+)-boldine

Compound	pA_2
(±)-Nantenine	7.03 ± 0.03
(±)-Nornantenine	6.61 ± 0.05
(±)-Ethylnornantenine	6.42 ± 0.06
(±)-*N*-Trifluoroacetylnornantenine	i.a.
(±)-4α-Hydroxynantenine	5.96 ± 0.04
(±)-4β-Hydroxynantenine	5.76 ± 0.08
(±)-Domesticine	8.06 ± 0.06
(±)-Nordomesticine	7.34 ± 0.03
(±)-*N*-Trifluoroacetyldomesticine	i.a.
(+)-Boldine	6.91 ± 0.02

i.a. = insurmountable antagonist.

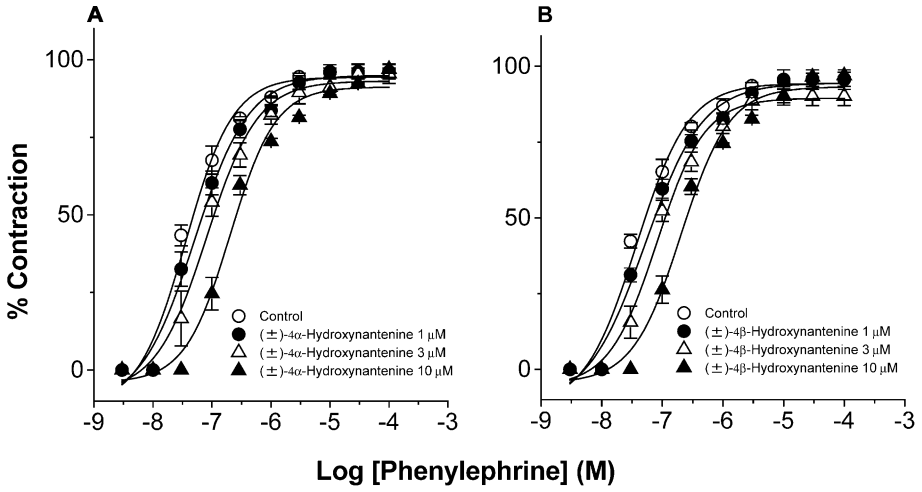

Fig. 4. Effects of (±)-4-α-hydroxylnantenine (A) and (±)-4-β-hydroxylnantenine (B) on phenylephrine-induced contraction in the rat aorta. Contractile responses are expressed as percentages of the contraction induced by 10 μM of phenylephrine. Data are means ± S.E.M. from four to six experiments.

group with a hydrogen atom at N-6 of (±)-nantenine and (±)-domesticine ((±)-nornantenine and (±)-nordomesticine, respectively) decreased the affinity by approximately 3- and 5-fold (Fig. 2; Table 1). Replacement of a methyl group with an ethyl group in (±)-nantenine to (±)-ethylnornantenine (Figs. 2A and 3A) also decreased the affinity by approximately 4-fold (Table 1). Moreover, the introduction of an α/β hydroxyl group at C-4 of (±)-nantenine to yield (±)-4α-hydroxyl nantenine and (±)-4β-hydroxylnantenine decreased the affinity by approximately 12- and 18-fold, respectively (Fig. 4; Table 1). However, (±)-*N*-trifluoroacetylnornantenine or (±)-*N*-trifluoroacetyl nordomesticine showed insurmountable blockade of the contractile response (Table 2). The relative order of pA_2 values was (±)-domesticine ($pA_2 = 8.06 \pm 0.06$) > (±)-nordomesticine ($pA_2 = 7.34 \pm 0.03$) > (±)-nantenine ($pA_2 = 7.03 \pm 0.03$) > (+)-boldine 6.91 ± 0.02 > (±)-nornantenine ($pA_2 = 6.61 \pm 0.05$) > (±)-ethylnornantenine ($pA_2 = 6.42 \pm 0.06$) > (±)-4α-hydroxylnantenine (5.96 ± 0.04) > (±)-4β-hydroxylnantenine ($pA_2 = 5.76 \pm 0.08$), respectively (Table 2).

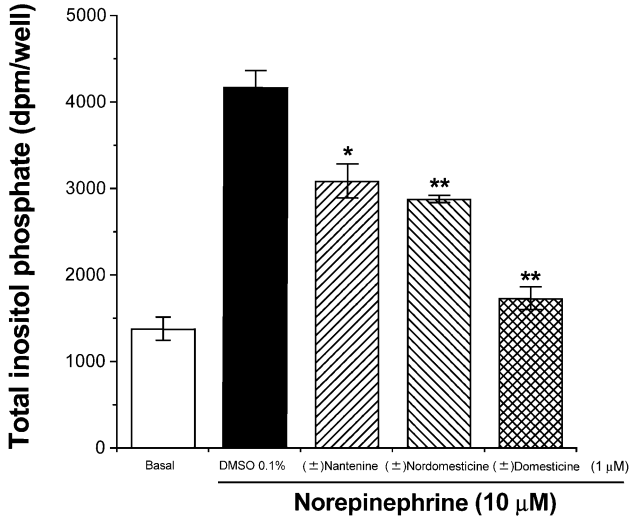

Fig. 5. Effects of (±)-nantenine, (±)-nordomesticine and (±)-domesticine on norepinephrine-stimulated inositol phosphate accumulation in A10 cells. Data are means ± S.E.M. from four experiments. * $P < 0.05$, ** $P < 0.01$ significantly different from control.

3.2. Effects of (±)-nordomesticine, (±)-nantenine and (±)-domesticine on [^{3}H]inositol phosphate accumulation in A10 cells

Norepinephrine (10 μM) markedly stimulated [^{3}H]inositol phosphate accumulation in the A10 cells. The levels of basal inositol accumulation and inositol incorporation were 1382 ± 91 and 10532 ± 198 (dpm/well), respectively. Pretreatment of cells with (±)-nantenine, (±)-nordomesticine and (±)-domesticine (1 μM) markedly blocked the norepinephrine-induced formation of inositol phosphates. The relative order of blocking activity was (±)-domesticine > (±)-nordomesticine > (±)-nantenine (Fig. 5).

4. Discussion

(±)-Nantenine derivatives of the natural aporphine alkaloids exerted competitive antagonistic activity at the α_1-adrenoceptor of the rat aorta in a concentration-dependent manner. Among nine (±)-nantenine derivatives having different substituents at N-6, C-1, or C-4 of the aporphine skeleton, we found two potent derivatives ((±)-domesticine and (±)-domesticine) which inhibited the concentration–response curve induced by phenylephrine in the rat aorta more than (±)-nantenine did.

A10 cells derived from the rat aorta are reported to have α_1-adrenoceptors (Ko et al., 1994) and are extensively used as a model of vascular smooth muscle cells (Rao et al., 1997). The experiment involving inositol phosphate accumulation was done to confirm the α_1-adrenoceptor blocking

action of these compounds in A10 cells. In the present study, (±)-domesticine, (±)-nordomesticine and (±)-nantenine suppressed the norepinephrine-induced accumulation of [^{3}H]inositol phosphates in A10 cells. These results suggest that these compounds inhibit the accumulation of inositol phosphates mediated through α_1-adrenoceptors.

Replacement of the methoxy moiety at C-1 position with a hydroxyl group in (±)-nantenine and (±)-nornantenine ((±)-domesticine and (±)-nordomesticine, respectively) increased affinity for the receptor. In contrast, replacement of a methyl group with a hydrogen atom at N-6 of (±)-nantenine and (±)-domesticine ((±)-nornantenine and (±)-nordomesticine) decreased affinity. Moreover, introduction of a hydroxyl group at C-4 position with α/β in the (±)-nantenine structure reduced affinity, and thus it seems likely that the lack of affinity may relate to a steric effect that involves a ''lock and key'' system (Boustie et al., 1998). Taken together, these results revealed that a hydroxyl group at the C-1 position and a methyl group at the N-6 position of the aporphine skeleton of (±)-nantenine are important for increasing affinity for the α_1-adrenoceptor.

It has been reported that a small change in aporphine structure may lead to dramatic changes in the pharmacological profile. For example, the hydroxyl group at the C-2 position of (+)-boldine has been reported to be a critical factor for discrimination between α_1-adrenoceptor subtypes (Madrero et al., 1996). Furthermore, Martinez et al. (1999) reported that halogen substitution at the C-3 position of (+)-boldine increased selectivity for the α_{1A}-adrenoceptor rather than for the α_{1B}-adrenoceptor. We found that (±)-nordomesticine was selective for the α_{1D}-adrenoceptor rather than for the α_{1A}- and α_{1B}-adrenoceptor and that it was more selective than (±)-nantenine (unpublished data). The study of the selectivity of other (±)-nantenine derivatives for α_1-adrenoceptor subtypes is still in progress.

In conclusion, among the derivatives tested, (±)-domesticine had the most powerful α_1-adrenoceptor blocking action in rat aorta. The relative order of affinity was (±)-domesticine > (±)-nordomesticine > (±)-nantenine > (+)-boldine > other derivatives. This study of the structure–activity relationship has clarified the essential role of a hydroxyl group at the C-1 position and a methyl group at the N-6 position in the aporphine skeleton of (±)-nantenine for the development of α_1-adrenoceptor blocking activity.

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