J. Pharm. Pharmacol. 1996, 48: 629–634
Received October 2, 1995
Accepted February 2, 1996

(±)-Govadine and (±)-THP, Two Tetrahydroprotoberberine Alkaloids, as Selective α_1-Adrenoceptor Antagonists in Vascular Smooth Muscle Cells

FENG-NIEN KO, YA-LING CHANG, CHI-MING CHEN* AND CHE-MING TENG

*Pharmacological Institute, College of Medicine, National Taiwan University, and *School of Pharmacy, Taipei Medical College, Taipei, Taiwan*

Abstract

(±)-Govadine and (±)-THP ((±)-2,3,10,11-tetrahydroxytetrahydroprotoberberine HBr) have been shown to inhibit noradrenaline-induced contraction of rat thoracic aortae. The pharmacological activity of the compounds was determined in thoracic aortae and cardiac tissue isolated from the rat and in trachea isolated from the guinea-pig to determine the selectivity of the compounds towards different types of receptor.

(±)-Govadine and (±)-THP were found to be α_1-adrenoceptor blocking agents in rat thoracic aorta as revealed by their competitive antagonism of vasoconstriction induced by noradrenaline ($pA_2 = 6{\cdot}57 \pm 0{\cdot}07$ and $5{\cdot}93 \pm 0{\cdot}06$, respectively) or phenylephrine ($pA_2 = 6{\cdot}74 \pm 0{\cdot}08$ and $6{\cdot}06 \pm 0{\cdot}10$, respectively). Removal of endothelium did not affect the antagonistic potencies of (±)-govadine ($pA_2 = 6{\cdot}83 \pm 0{\cdot}09$) and (±)-THP ($pA_2 = 6{\cdot}25 \pm 0{\cdot}06$) on phenylephrine-induced vasoconstriction. They were more potent than yohimbine ($pA_2 = 6{\cdot}05 \pm 0{\cdot}05$), but less so than phentolamine ($pA_2 = 7{\cdot}54 \pm 0{\cdot}11$) and prazosin ($pA_2 = 9{\cdot}27 \pm 0{\cdot}12$). (±)-Govadine and (±)-THP, furthermore, inhibited [^{3}H]inositol monophosphate formation caused by noradrenaline (3 μM) in rat thoracic aorta. (±)-Govadine and (±)-THP were also α_2-adrenoceptor blocking agents with pA_2 values $5{\cdot}50 \pm 0{\cdot}13$ and $5{\cdot}41 \pm 0{\cdot}11$, respectively. A high concentration of (±)-govadine (30 μM) or (±)-THP (30 μM) did not, however, affect the contraction induced by the thromboxane receptor agonist U46619, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 5-hydroxytryptamine (5-HT), angiotensin II, endothelin or high K^+ in rat aorta denuded of endothelium. Neither the cyclic AMP nor cyclic GMP content of rat thoracic aorta was, furthermore, changed by (±)-govadine or (±)-THP. Contraction of guinea-pig trachea caused by carbachol, histamine, leukotriene C_4 or neurokinin A was not affected by (±)-govadine or (±)-THP. (±)-Govadine or (±)-THP also did not block β_1- or β_2-adrenoceptor-mediated responses induced by isoprenaline in rat right atria and guinea-pig trachea.

It is concluded that (±)-govadine and (±)-THP are selective α_1-adrenoceptor antagonists in vascular smooth muscle.

α-Adrenoceptors play important roles in a variety of physiological processes, including in the control of blood pressure, myocardial contractile rate and force, airway reactivity and metabolic functions. Thus, they have been of major interest for many years as targets for drug action. Although the role played by α-adrenoceptors in hypertensive disease remains unclear, the blockade of α-adrenoceptors by appropriate antagonistic drugs, particularly selective α_1-antagonists such as prazosin, is effective at lowering blood pressure (Cavero & Roach 1980; Stanaszek et al 1983; Titmarsch & Monk 1987). Although the chemical structures of α-adrenoceptor antagonists may be of pharmaceutical interest or useful for pharmacological study, these structures are strikingly unrelated to their pharmacological activity. Phentolamine is an imidazoline derivative. Phenoxybenzamine is a phenylethylamine derivative which is chemically related to endogenous catecholamines. Prazosin is a piperazinyl quinazoline derivative which shows some resemblance both to papaverine and to the aminopyrimidine moiety of cyclic AMP and cyclic GMP. Yohimbine is an indolealkylamine alkaloid with a structure similar to that of reserpine.

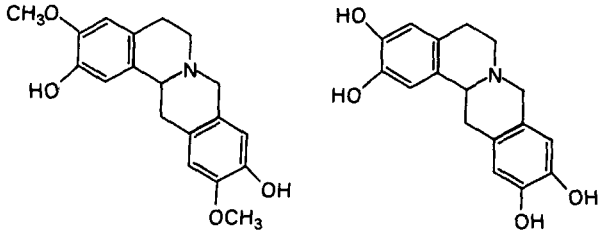
(−)-Govadine and (±)-THP ((±)-2,3,10,11-tetrahydroxytetrahydroprotoberberine HBr), two tetrahydroprotoberberine alkaloids, have, respectively, been isolated from *Corydalis govaniane* Wall (Mehra et al 1976) and prepared from natural xylopinine by *O*-demethylation (Schmutz 1959). Their biological activities have not, however, been studied. We recently found that (±)-govadine and (±)-THP inhibited noradrenaline-induced contraction of rat thoracic aortae in a large scale screening test. In this study we have investigated the selectivity of these two agents for several types of receptor in various tissues and tried to elucidate their mechanisms of action.

Correspondence: F. N. Ko, Pharmacological Institute, College of Medicine, National Taiwan University, No. 1 Jen-Ai Road, 1st Section, Taipei, Taiwan.

Materials and Methods

Materials

(±)-Govadine and (±)-THP [(±)-2,3,10,11-tetrahydroxytetrahydroprotoberberine HBr] (Fig. 1) were synthesized as described previously (Chen et al 1994). Noradrenaline HCl, isoprenaline HCl, yohimbine HCl, prazosin HCl, phentolamine HCl, U46619 (9,11-dideoxy-9α,11α-methanoepoxyprostaglandin $F_{2\alpha}$), angiotensin II acetate, histamine dihydrochloride, 5-hydroxytryptamine creatinine sulphate (5-HT), trichloroacetic acid and Dowex-1 resin (100–200 mesh; 8% cross-linkage formate) were obtained from Sigma Chemical Co. Phenylephrine HCl was purchased from Denmarks Apotekerforening. Leukotriene C_4 (LTC_4) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were obtained from Biomol Research Laboratories. Cyclic (c) AMP and cGMP enzyme immunoassay kits were from Cayman Chemical Co. *myo*-[2-^{3}H]Inositol was purchased from



(±)-Govadine (±)-THP

FIG. 1. Chemical structures of (±)-govadine and (±)-THP.

Amersham. (±)-Govadine and (±)-THP were dissolved in dimethylsulphoxide (DMSO) and the final concentration of DMSO in the bathing solution did not exceed 0·1% and had no effect on muscle contraction.

Rat aortic contraction

Wistar rats of either sex, 250–300 g, were killed by a blow to the head. The thoracic aorta was isolated and excess fat and connective tissue were removed. The vessels were cut into rings of about 5 mm in length and mounted in organ baths containing 5 mL Krebs solution of composition (mM): NaCl 118·4, KCl 4·7, $MgSO_4$ 1·2, KH_2PO_4 1·2, glucose 11·7, $CaCl_2$ 1·9 and $NaHCO_3$ 25·0. The tissue bath solution was maintained at 37°C and oxygenated with 95% O_2–5% CO_2. Two stainless steel hooks were inserted into the aortic lumen, one was fixed whereas the other was connected to a transducer. Aortae were equilibrated in the medium for 90 min with three changes of Krebs solution and maintained under an optimum tension of 1 g before specific experimental protocols were initiated. Contractions were recorded isometrically via a force-displacement transducer connected to a Grass polygraph. In some experiments the endothelium was removed by rubbing with a cotton ball and the absence of acetylcholine-induced relaxation was taken as an indicator that vessels were denuded successfully. Aortae were allowed to equilibrate for 15 min with (±)-govadine or (±)-THP before the generation of a cumulative concentration–response curve with each agonist for 15–30 min at 3 min intervals. Results are expressed as percentage of the maximum control response for each agonist before the addition of (±)-govadine or (±)-THP.

The contractile effects of calcium were studied in rings stabilized in K^+ (80 mM) solution without Ca^{2+}. Calcium was then added from stock dilutions to furnish the desired concentrations, and the effect of each Ca^{2+} concentration was recorded. The maximum tension attained at a Ca^{2+} concentration of 3 mM was considered as 100%. The high-K^+ solution was prepared by substituting NaCl with an equimolar amount of KCl.

cAMP and cGMP assay of rat aortae

The cAMP or cGMP content of aortic rings was assayed as previously described (Itoh et al 1982; Kauffman et al 1987). After incubation of aortic rings with DMSO (0·1%), forskolin, sodium nitroprusside, (±)-govadine or (±)-THP for 2 min, the aortic rings were rapidly frozen in liquid nitrogen and stored at −80°C until homogenization in trichloroacetic acid (10%; 0·5 mL) and EDTA (4 mM) using a Potter glass/glass homogenizer. The homogenate was centrifuged at 10 000 *g* for 5 min and the supernatant was removed and extracted with diethyl ether (4 × 3 vols), and the cAMP or cGMP content was then assayed using enzyme immunoassay kits. The precipitate was used for protein assay (Lowry et al 1951). cAMP and cGMP levels were expressed as pmol (mg protein)$^{-1}$.

Measurement of [^{3}H]inositol monophosphate

[^{3}H]Inositol monophosphate was determined by the procedure described by Hirata et al (1990). Briefly, rat thoracic aortae were exposed to Krebs solution containing 10 μCi mL^{-1} [^{3}H]*myo*-inositol for 3 h and oxygenated with a 95% O_2–5% CO_2 mixture. The tissues were then transferred to tubes containing fresh Krebs solution and DMSO (0·1%), (±)-govadine, (±)-THP or prazosin for 15 min, and saline or noradrenaline (3 μM) was added and the tubes incubated for another 15 min. LiCl (10 mM) was added 5 min before noradrenaline to inhibit metabolism of inositol monophosphate (Berridge et al 1982). Aortae were then frozen in liquid nitrogen and homogenized in trichloroacetic acid (10%; 1·3 mL). After centrifugation, supernatant (1 mL) was collected and trichloroacetic acid was removed by washing with diethyl ether (4 × 3 vols). The inositol monophosphate in the aqueous phase was analysed by application of the sample to a column of Dowex-1 ion-exchange resin (1 mL) according to the method of Neylon & Summers (1987). The pellets of the tissues were resuspended in NaOH (1·0 M) and assayed for protein according to the method of Lowry et al (1951).

Guinea-pig tracheal contraction

Tracheae from guinea-pigs were dissected out, transferred to a dish containing Krebs solution and cut transversely between the segments of cartilage. Several of these, usually approximately five, were tied together to form a chain, which was then mounted in Krebs solution at 37°C, oxygenated with 95% O_2–5% CO_2. One end of the chain was attached to a fixed pin in the bath, the other to a force-displacement transducer connected to a Grass polygraph. Resting tension on each tissue was set at 1 g. Tracheae were left to equilibrate for at least 1 h and washed periodically. Cumulative concentration-response curves of spasmogens were obtained by application of various concentrations of each spasmogen for 15–18 min at 3-min intervals. Responses were found to be reproducible with this procedure. Tracheal rings were pre-incubated with DMSO (0·1%), (±)-govadine or (±)-THP for 15 min, then various concentrations of spasmogens were added at 3-min intervals. Results are expressed as percentage of the maximum control response for each agonist.

Rat right and left atria

Strips (4 × 6 mm) of right and left atria were quickly dissected from the hearts of male WKY rats (250–300 g) and placed in an organ bath containing Tyrode solution (10 mL) oxygenated with 95% O_2–5% CO_2 and kept at 36·0±0·2°C. The composition of Tyrode solution was (mM): NaCl 137, KCl 5·4, $MgCl_2$ 1·1, $NaHCO_3$ 11·9, NaH_2PO_4 0·33, dextrose 11 and $CaCl_2$ 2. Contractions of spontaneously beating right atria and electrically driven left atria strips were measured by connecting one end of the preparation using a fine silk thread to a force displacement transducer (Type BG 25, Gould) and tension was recorded on a Gould 2200S recorder. A pre-load of 500 mg was used. The left atria strips were stimulated at a frequency of 2 Hz

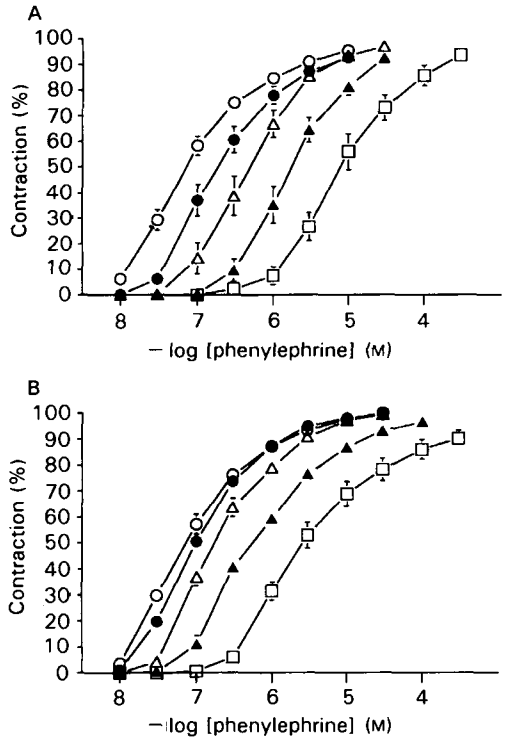


FIG. 2. Cumulative concentration-response curves to phenylephrine in intact rat thoracic aortae. DMSO (0·1%, control) (○), (±)-govadine (A) or (±)-THP (B) was pre-incubated with aortae for 15 min, then cumulative concentrations of phenylephrine were added. Each point represents the means ± s.e. (n = 8). (±)-Govadine: ● 0·3, △ 1, ▲ 3, and □ 10 μM. (±)-THP: ● 0·3, △ 1, ▲ 3, and □ 10 μM.

by rectangular pulses of 1 ms duration at supramaximal intensity via an isolated Grass SD9 stimulator.

Data analysis

In each experiment, agonist dose–response curves in the presence of (±)-govadine or (±)-THP were related to the control dose–response curve, the maximum response of which was taken as 100%. In most experiments, three to four concentrations of (±)-govadine or (±)-THP were tested and the slopes of the resulting Schild plots were used to assess competitive antagonism. The concentration of agonist necessary to give a half-maximum response in the presence of each concentration of antagonist was divided by the concentration giving a half-maximum response in the absence of antagonist, to determine the dose ratio (DR). Data were plotted by the method of Arunlakshana & Schild (1959) as the − log (antagonist concentration) (M) vs log (DR − 1). When DR was 2, the −log (antagonist concentration) was taken as the pA_2 value from the Schild plot (MacKay 1978).

The experimental results are expressed as the means ± s.e. and accompanied by the number of observations. Statistical significance was assessed by Student's *t*-test and *P* values less than 0·05 were considered significant.

Results

α-Adrenoceptor antagonism in rat aortae

α-Adrenoceptor antagonistic activities of (±)-govadine and (±)-THP were evaluated against concentration–response curves to phenylephrine and noradrenaline in rat thoracic aortae. (±)-Govadine (0·3–10 μM) and (±)-THP (0·3–10 μM) produced a parallel, rightward shift of the curve consistent with competitive blockade (Fig. 2). The pA_2 values of (±)-govadine and (±)-THP against phenylephrine were 6·74±0·08 (slope 1·21±0·10) and 6·06±0·10 (slope 1·18±0·09), respectively (Table 1). These concentration-related shifts by (±)-govadine and (±)-THP were not affected in rat thoracic aorta in which the endothelium had been removed (Table 1). The pA_2 values of (±)-govadine and (±)-THP were 6·83±0·09 (slope 1·08±0·07) and 6·25±0·06 (slope 1·10±0·10), respectively, against phenylephrine, and 6·57±0·07 (slope 1·03±0·09) and 5·93±0·06 (slope 1·12± 0·08), respectively, against noradrenaline in rat aortae denuded of endothelium (Table 1).

Concentration–response curve analysis of phenylephrine-induced contraction of endothelium denuded aortae was also performed for α_1-adrenoceptor antagonism by (±)-govadine and (±)-THP in comparison with prazosin, phentolamine and yohimbine. (±)-Govadine and (±)-THP were more potent than yohimbine ($pA_2 = 6·05 \pm 0·05$) but were less potent than phentolamine ($pA_2 = 7·54 \pm 0·11$) and prazosin (9·27 ± 0·12). In all cases the Schild slopes were not significantly different from 1·0.

(±)-Govadine (3–30 μM) and (±)-THP (3–30 μM) also produced a concentration-related shift in the concentration–response curves for clonidine in rat aortae denuded of endothelium. An unconstrained Schild plot was derived from shifts in concentration–response curves in individual tissues. The pA_2 values against α_2-adrenoceptor were 5·50 ± 0·13 (n = 5) and 5·41 ± 0·11 (n = 5) and the slopes of Schild plots were 1·21 ± 0·13 and 1·01 ± 0·10, respectively.

Table 1. Potencies of α_1-adrenoceptor antagonists against contractions to phenylephrine and noradrenaline in rat thoracic aorta.

	(±)-Govadine	(±)-THP	Phentolamine	Yohimbine	Prazosin
Intact aorta					
Phenylephrine	6·74 ± 0·08	6·06 ± 0·10			
Denuded Aorta					
Phenylephrine	6·83 ± 0·09	6·25 ± 0·06	7·54 ± 0·11	6·05 ± 0·05	9·27 ± 0·12
Noradrenaline	6·57 ± 0·07	5·93 ± 0·06			

DMSO (0·1%, control) or various concentrations of (±)-govadine (0·3, 1, 3 and 10 μM), (±)-THP (0·3, 1, 3 and 10 μM), phentolamine (0·3, 1, 3 and 10 μM) or yohimbine (1, 3 and 10 μM) was pre-incubated with aortae for 15 min, then cumulative concentrations of phenylephrine or noradrenaline were added to induce the muscle contractions. Potencies are expressed as pA_2 values (means ± s.e., n = 6–8) calculated from the respective Schild plot.

Table 2. Effects of (±)-govadine, (±)-THP and prazosin on the accumulation of [^{3}H]inositol monophosphate in rat thoracic aortae by noradrenaline.

Treatment	[3H]Inositol monophosphate (counts min^{-1} (mg protein)$^{-1}$)
Resting	1440 ± 99
Noradrenaline (3 μM)	3116 ± 158
(±)-Govadine (30 μM)	1548 ± 117*
(±)-THP (30 μM)	1759 ± 115*
Prazosin (3 μM)	1484 ± 109*

Segments of rat aortae were pre-incubated with DMSO (0·1%, for resting and control), (±)-govadine, (±)-THP or prazosin for 15 min, then saline (for resting) or noradrenaline was added for another 15 min. Values presented are means ± s.e. (n = 4). *$P < 0.001$ compared with the control value.

To see if signal transduction after α_1-adrenoceptor activation was blocked by (±)-govadine and (±)-THP, rat thoracic aortae were labelled with [^{3}H]*myo*-inositol. The accumulation of [^{3}H]inositol monophosphate in rat aortae was increased in the presence of noradrenaline (3 μM). This increase was markedly suppressed by (±)-govadine (30 μM), (±)-THP (30 μM) or prazosin (3 μM) (Table 2). A high concentration of (±)-govadine (30 μM) or (±)-THP (30 μM) did not, however, block the increase in tension produced by U46619, $PGF_{2\alpha}$, 5-HT, angiotensin II, endothelin and calcium (80 mM K^+ depolarization) in rat aortae denuded of endothelium (Table 3).

Effects of (±)-govadine and (±)-THP on the cAMP and cGMP formation in rat aorta

The cyclic nucleotide content of the aortae was measured by enzyme immunoassay. As shown in Table 4, forskolin and sodium nitroprusside markedly elevated cAMP and cGMP levels, respectively, in rat aorta. (±)-Govadine (30 μM) and (±)-THP (30 μM) did not exert any effect on the levels of these cyclic nucleotides.

Antagonism against carbachol, histamine, LTC_4, neurokinin A and isoprenaline in guinea-pig trachea

In guinea-pig trachea, carbachol, histamine, LTC_4 and neurokinin A caused contractions of tracheal smooth muscle. (±)-Govadine (30 μM) and (±)-THP (30 μM) did not depress these concentration-response curves. (±)-Govadine (30 μM) and (±)-THP (30 μM) also had no effect on the isoprenaline-induced relaxation of guinea-pig trachea pre-contracted by carbachol (3 μM) (Table 3).

β_1-Adrenoceptor blockade in rat right and left atria

(±)-Govadine (30 μM) and (±)-THP (30 μM) did not affect the sinus nodal rate and contractility in isolated right atrium nor the contractility in isolated left atrium from the rat. They also did not affect the inotropic (for right and left atria) and chronotropic (for right atrium) concentration-response curves for isoprenaline in rat right and left atria (Table 3 for right atria).

Discussion

These studies have demonstrated that (±)-govadine and (±)-THP, two tetrahydroprotoberberine alkaloids, inhibited the contractile responses of rat aortae to the adrenoceptor agonists phenylephrine and noradrenaline. The signal transduction after α_1-adrenoceptor activation was also investigated and (±)-govadine and (±)-THP were found to inhibit the [^{3}H]inositol monophosphate formation caused by noradrenaline. They act as selective α-adrenoceptor antagonists without affecting the contraction of rat aorta caused by the thromboxane receptor agonist (U46619), $PGF_{2\alpha}$, 5-HT, angiotensin II, endothelin or high-potassium depolarization. They also had no apparent effects on the LTC_4 receptor, β_2-adrenoceptor and histamine, neurokinin A and muscarinic receptors in guinea-pig trachea. β_1-Adrenoceptors of rat right and left atria were not affected by (±)-govadine and (±)-THP. (±)-Govadine and (±)-THP, however, also blocked the α_2-adrenoceptor in vascular smooth muscle. On the basis of the pA_2 values of functional studies, and assuming the pA_2 values against clonidine-induced aortic contractions is 1·0, they are about 20 and 6 times more selective

Table 3. Activities of (±)-Govadine (30 μM) and (±)-THP (30 μM) at receptors other than α-adrenoceptor.

Tissue (response)	Competing drug	Concentration ratio (±)-Govadine	(±)-THP	n
Rat aortae (contraction)	U46619	1·47 ± 0·29	1·19 ± 0·18	8
	$PGF_{2\alpha}$	1·21 ± 0·14	1·15 ± 0·12	8
	5-HT	1·52 ± 0·17	1·86 ± 0·18	8
	Angiotensin II	1·26 ± 0·21	1·31 ± 0·24	8
	Endothelin	1·17 ± 0·10	1·13 ± 0·09	8
	Calcium	1·14 ± 0·09	0·97 ± 0·16	8
Guinea-pig trachea (contraction)	Carbachol	1·02 ± 0·06	1·30 ± 0·18	6
	Histamine	1·43 ± 0·06	1·46 ± 0·29	6
	LTC_4	1·00 ± 0·04	1·06 ± 0·06	6
	Neurokinin A	0·97 ± 0·10	1·11 ± 0·14	6
Guinea-pig trachea (relaxation)	Isoprenaline	1·17 ± 0·40	1·01 ± 0·26	8
Rat right atria (rate)	Isoprenaline	0·98 ± 0·12	1·06 ± 0·11	6
Rat right atria (force)	Isoprenaline	1·13 ± 0·11	1·17 ± 0·15	6

The concentration of agonist necessary to give a half-maximum response was defined as the EC50 value. The concentration ratio was calculated from EC50 values of each agonist in the presence and absence of (±)-govadine or (±)-THP and represented as means ± s.e., n = number of estimates.

Table 4. Effects of (±)-govadine and (±)-THP on cAMP and cGMP formation in rat thoracic aortae.

Treatment	cAMP (pmol (mg protein)$^{-1}$)	cGMP (pmol (mg protein)$^{-1}$)
Control	2·92 ± 0·37	3·64 ± 0·36
Forskolin (1 μM)	6·62 ± 0·73*	–
Sodium nitroprusside (1 μM)	–	7·96 ± 0·31*
(±)-Govadine (30 μM)	3·00 ± 0·37	3·72 ± 0·47
(±)-THP (30 μM)	2·98 ± 0·35	3·80 ± 0·38

After pre-incubation of aortic rings in Krebs solution for 5 min, DMSO (0·1%, control), forskolin, sodium nitroprusside, (±)-govadine or (±)-THP was added for another 2 min and the reaction was stopped by immersing the tissue into liquid nitrogen. The cAMP and cGMP contents of the rat aortae were then measured. Results are expressed as the means ± s.e. (n = 5). *$P < 0.001$ compared with the respective control.

towards the α_1- than the α_2-adrenoceptor. All these data indicate that (±)-govadine and (±)-THP are selective α_1-adrenoceptor blocking agents.

The vascular endothelium plays an important role in controlling vascular tone via the secretion of both relaxant and contractile factors (Vanhoutte et al 1986; Schulz & Triggle 1994). Endothelial cells respond to a variety of neurochemical and physical stimuli to release endothelium-derived relaxing factor (EDRF) and prostacyclin (PGI_2). Endothelium modulates the vasoconstrictor responses to many agonists, and EDRF is mainly responsible for these effects (Egleme et al 1984; Palmer et al 1987). The α-adrenoceptor antagonistic action of (±)-govadine and (±)-THP persisted in aortae denuded of endothelium. Thus, the α-adrenoceptor antagonism of (±)-govadine and (±)-THP were independent of endothelium and was not mediated by either EDRF or PGI_2. It has been reported that vascular endothelium modified the mode of antagonism of prazosin and doxazosin, as reflected in the noradrenaline and phenylephrine concentration–response curves, but not the antagonism of phentolamine and yohimbine (Alosachie & Godfraind 1986). Prazosin and doxazosin act as non-competitive antagonists against noradrenaline and phenylephrine in the presence of endothelium and as competitive antagonists after removal of endothelium. The change in the mode of antagonism of prazosin and doxazosin after removal of endothelium was related to a change in agonist efficacy and receptor reserve between intact and rubbed preparations. Phentolamine and yohimbine were not sensitive to the decrease in receptor reserve in intact preparations and their mode of antagonism was, therefore, not affected by endothelium. Analysis of the concentration–response curves of (±)-govadine and (±)-THP revealed that competitive antagonism against noradrenaline and phenylephrine occurred in aortae in the presence or absence of endothelium. Thus, vascular endothelium modifies the mode of antagonism of prazosin and doxazosin but not those of (±)-govadine, (±)-THP, phentolamine and yohimbine.

Cyclic nucleotides are very important in the relaxing of vascular smooth muscles (Murad 1986). cAMP can dilate vascular smooth muscle either by causing phosphorylation of myosin light-chain kinase (Adelstein & Eisenberg 1980), or by increasing calcium uptake by the sarcoplasmic reticulum (Scheid et al 1979), or by acting in other ways to reduce free cytosolic calcium (Kamm & Stull 1985). cGMP inhibits Ca^{2+} influx and Ca^{2+} release, augments Ca^{2+} sequestration and reduces the sensitivity of contractile elements to Ca^{2+} (Karaki et al 1988). Forskolin and sodium nitroprusside have been shown to be potent relaxing agents in vascular smooth muscles. Forskolin increases the level of cAMP via activation of adenylate cyclase (Ousterhout & Sperelakis 1987); sodium nitroprusside produces a prompt, dose-dependent increase in the cGMP level by directly activating guanylate cyclase (Gruetter et al 1979). Neither cAMP nor cGMP content was changed by (±)-govadine or (±)-THP. This indicates that the α-adrenoceptor antagonistic effects of (±)-govadine and (±)-THP are not mediated by increase of cellular cyclic nucleotide concentrations.

It is concluded that (±)-govadine and (±)-THP are selective vascular α-adrenoceptor antagonists. Their structural novelty may provide an original chemical basis for the development of new α-adrenoceptor blockers.

Acknowledgement

This work was supported by a research grant from the National Science Council of the Republic of China (NSC84-2331-B002-310 M03).

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