Pergamon

Biochemical Pharmacology, Vol. 48, No. 1, pp. 101–109, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)E0111-W

VANINOLOL: A NEW SELECTIVE β_1-ADRENOCEPTOR ANTAGONIST DERIVED FROM VANILLIN

BIN-NAN WU, TSONG-LONG HWANG, CHING-FONG LIAO* and ING-JUN CHEN†

Department of Pharmacology, Kaohsiung Medical College, Kaohsiung; and
*Cell Signalling Laboratory, Institute of Zoology, Academia Sinica, Taipei, Taiwan, Republic of China

(*Received* 12 *October* 1993; *accepted* 25 *February* 1994)

Abstract—The β-adrenoceptor blocking properties of vaninolol ((±)4-[4′-(2-hydroxy-3-tert-butylaminopropoxy)-3′-methoxyphenyl]-3-buten-2-one), derived from vanillin, were first investigated under *in vivo* and *in vitro* conditions. Vaninolol (0.1, 0.5, 1.0 mg/kg, i.v.), as well as propranolol, produced a dose-dependent bradycardia response and a sustained pressor action in urethane-anesthetized normotensive rats. Vaninolol inhibited the tachycardia effects induced by (−)isoproterenol, but had no blocking effect on the arterial pressor responses induced by phenylephrine. These findings suggested that vaninolol possessed β-adrenergic blocking activity, but was without α-adrenergic blocking activity. In isolated guinea-pig tissues, vaninolol antagonized (−)isoproterenol-induced positive inotropic and chronotropic effects of the atria and tracheal relaxation responses in a concentration-dependent manner. The parallel shift to the right of the concentration–response curve of (−)isoproterenol suggested that vaninolol was a β-adrenoceptor competitive antagonist. The effect of vaninolol was more potent on the atria than on tracheal tissues, indicating it had some β_1-adrenoceptor selectivity. On the other hand, the order of the hydrophilicity was atenolol ≫ vaninolol > propranolol. In addition, vaninolol had a mild direct cardiac depression at high concentrations and was without intrinsic sympathomimetic activity (ISA). Furthermore, binding characteristics of vaninolol and other β-adrenoceptor antagonists were evaluated in [^{3}H]dihydroalprenolol binding to guinea-pig ventricular membranes. The order of potency of β-adrenoceptor antagonists in competing for the binding sites was (−)propranolol ≫ vaninolol ≥ atenolol. In conclusion, vaninolol was found to be a selective β_1-adrenoceptor antagonist with relatively low lipophilicity in comparison with propranolol, devoid of ISA, and had a mild myocardial depressant effect.

Key words: inotropic and chronotropic effects; intrinsic sympathomimetic activity; membrane stabilizing activity; myocardial depressant effect; octanol/water partition coefficient; radioligand binding study

β-adrenoceptor blocking drugs are widely used in the treatment of cardiovascular disorders, including hypertension, angina pectoris, supraventricular arrhythmias, hypertrophic cardiomyopathy and myocardial infarction. These agents are also helpful in treating non-cardiovascular conditions including migraine, glaucoma, thyrotoxicosis and gastrointestinal bleeding [1–3]. Although they share a common structural feature they differ in many aspects of their pharmacology, e.g. potency, selectivity for β_1- and β_2-adrenoceptors, ISA,‡ membrane stabilizing activity and lipid versus water solubility [4, 5]. Many β-adrenoceptor blocking agents are known to exhibit various degrees of non-specific membrane stabilizing action at concentrations usually higher than those which exert specific antagonistic and agonistic actions on the β-adrenoceptor [6]. Non-specific myocardial depression of the β-adrenoceptor blocking agents is closely related to membrane stabilizing action such as local anesthetic action, negative dromotropic action or antiarrhythmic action [6–8]. Non-specific myocardial depression or membrane stabilizing action has been demonstrated to correlate well with the octanol/water partition coefficients [7, 8]. A number of compounds showing selective β_1-adrenoceptors in animal experiments have been described: atenolol, acebutolol, metoprolol, tolamolol, H 87/07 and Cl 775 [9]. β-adrenoceptor blockers derived from natural products such as xanthone and flavone have been investigated by Chen *et al.* [10] and Kinsolving *et al.* [11], respectively. Vaninolol (Fig. 1) is a newly

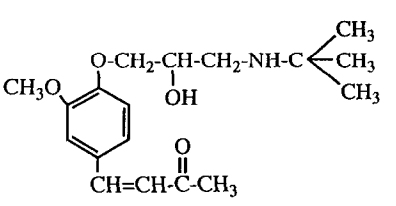

Fig. 1. Chemical structure of vaninolol.

† Corresponding author: Ing-Jun Chen, Department of Pharmacology, Kaohsiung Medical College, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan, Republic of China. FAX 886-7-3218309.

‡ Abbreviations: ISA, intrinsic sympathomimetic activity; vaninolol, (±)4-[4′-(2-hydroxy-3-tert-butylaminopropoxy)-3′-methoxyphenyl]-3-buten-2-one; [^{3}H]DHA, [^{3}H]dihydroalprenolol; CR, concentration ratios; K_d, equilibrium dissociation constant; B_{max} maximum binding capacity.

developed compound from vanillin designed to possess β-adrenoceptor antagonistic activities. In the present study the aim was to investigate the pharmacological properties of vaninolol, such as its ability to bind to β-adrenoceptors and its relative selectivity for β-adrenoceptors, ISA and lipid/water solubility.

MATERIALS AND METHODS

Drugs

(−)[propyl-1,2,3-^{3}H]dihydroalprenolol HCl (60 Ci/mmol) was purchased from New England Nuclear Corp. (MA, U.S.A.). (−)Alprenolol, (−)isoproterenol bitartrate, (−)phenylephrine HCl, (−)propranolol HCl, (±)atenolol (±)metoprolol tartrate, (±)propranolol HCl, mecamylamine HCl, phenoxybenzamine HCl and reserpine were all purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Protein assay dye was obtained from Bio-Rad Laboratories (Richmond, CA, U.S.A.). All other reagents used were from E. Merck (Darmstadt, Germany). (±)Vaninolol (synthesized in this laboratory), (−)isoproterenol bitarate, (−)phenylephrine HCl and mecamylamine HCl were dissolved in normal saline for *in vivo* experiments. Phenoxybenzamine and reserpine were dissolved in 95% absolute alcohol and 90% benzyl alcohol, respectively, and then diluted with distilled water.

In vivo *experiments*

Male Wistar rats, weighing 250–300 g (provided by the Experimental Animal Center, Cheng-Kung National University Medical College, Tainan, Taiwan) were anesthetized with urethane (1.5 g/kg, i.p.). Following tracheal cannulation, systemic arterial blood pressure and heart rates were recorded from the femoral artery with a pressure transducer (Gould, Model P50, U.S.A.). Body temperatures were maintained at 37°. A femoral vein was cannulated for i.v. injections. All drug solutions were administered in a volume of 0.4 mL/kg. Equivolumetric injections of the vehicle were administrated as a control. The magnitudes of the effects elicited after injections were evaluated by measuring the changes in arterial blood pressure and heart rate between the responses and basal blood pressure or heart rate.

β-adrenergic response. Rats were pre-treated with mecamylamine (5 mg/kg, i.v.), a ganglion blocking agent, to ensure a uniform initial heart rate. Isoproterenol (0.5 μg/kg) was administered via a femoral vein and the resultant tachycardia recorded as the control. A single dose of vaninolol was then administered intravenously. Ten minutes later, a further injection of isoproterenol was recorded and then expressed as a percentage of the control responses.

α-Adrenergic response. Rats were pre-treated with reserpine (5 mg/kg, i.p.) 24 hr prior to the injection of (−)phenylephrine (10 μg/kg, i.v.), followed 15 min later by the intravenous injection of a single dose of vaninolol, propranolol, or labetalol (1 mg/kg). Ten minutes later, a further injection of (−)phenylephrine was given.

In vitro *experiments*

Guinea-pigs (Hartley) of either sex, weighing between 350 and 500 g, were killed by a blow on the head. Their hearts and trachea were quickly excised and excess tissue was removed.

Isolated right atria. Spontaneously-beating right atria were dissected from the hearts and mounted in a 10 mL organ bath with one end fixed and the other end connected to a force displacement transducer (Grass, Model FT03). The frequency of contraction was measured on a separate channel by a tachometer (Coulbourn, Model S77-26) connected to a high-speed videograph (Coulbourn, AT L19-69). The experiments were carried out at 32.5° in a Krebs solution of the following composition (mM): NaCl 113, KCl 4.8, $CaCl_2$ 2.2, KH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 25, dextrose 11.0; bubbled with a 95% O_2–5% CO_2 mixture. The atrial strip was pre-stretched to a baseline tension of 0.2 g. The atria were equilibrated for 90 min in an aerated (95% O_2–5% CO_2) Krebs solution before the experimental protocols were initiated. For the assessment of β-adrenergic blocking activity a control cumulative concentration–response curve to the chronotropic effect of isoproterenol was established. The atria were then allowed a 30–60 min washout period to restabilize, after which time various concentrations of the test compound were incubated with the atrium 30 min before the cumulative concentration of the isoproterenol (3×10^{-10}–10^{-5} M) was added. All responses to isoproterenol were calculated as a percentage of the maximum control response to isoproterenol.

Isolated left atria. Quiescent left atria were dissected free of connective tissue and mounted in organ chambers under a resting tension of 0.5 g. Atria were bathed in an aerated Krebs solution (32.5°) and were driven at 2 sec intervals via two platinum electrodes placed at either side of the atrium. β-adrenoceptor antagonist activity was determined as follows. Cumulative concentration–response curves to the positive inotropic effects of isoproterenol were obtained in the absence and presence of various concentrations of a test compound. An incubation time of 30 min was allowed for the test compound. Data were calculated as a percentage of the increase in force induced by isoproterenol.

Isolated tracheal strips. The trachea was cleaned of extraneous connective tissue and cut into spiral strips as described by Constantine [12]. This spiral strip was cut into two equal segments and both were suspended in organ baths filled with 20 mL of Krebs solution. Temperature was maintained at 32.5° and the solution was gassed with 95% O_2–5% CO_2. An initial basal tension of 2 g was applied to each tracheal strip and the tissue was allowed to gain tone spontaneously until a steady level was reached (60 min). The tracheal preparations were pre-treated with phenoxybenzamine (50 μM for 30 min followed by thorough washout as described by O'Donnell and Wanstall [13]) to prevent extraneuronal uptake and to block α-adrenoceptors. For the determination of β-adrenoceptor antagonist activity, cumulative concentration–response curves to the relaxant effects

of isoproternol were obtained in the absence and presence of a test compound (60 min incubation time). Data were calculated as a percentage of the maximum relaxation induced by isoproterenol.

Intrinsic sympathomimetic activity. Animals were pre-treated with reserpine (10 mg/kg, i.p.) 24 hr prior to the experiment [14]. All preparations, including isolated right atria and left atria strips, were studied. The concentration–response curve was obtained by cumulative addition of (−)isoproterenol, propranolol, atenolol or vaninolol.

Calculations and analysis of results

CR, EC_{50} in the presence of antagonist/EC_{50} in the absence of antagonist, were corrected for any change in sensitivity to the agonist that was not due to the presence of the antagonist, using the appropriate correction factor. The correction was accomplished by multiplying the experimentally determined concentration ratios by correction factors derived from a separate series of experiments for each tissue and for each agonist as described by O'Donnell and Wanstall [15]. The CR values calculated from the experiments on left atrial strips and tracheal strips needed to be adjusted, but the CR for spontaneously-beating right atria did not. The β-adrenoceptor antagonist log(CR-1) was plotted against the log molar concentration of the antagonist (log[B]). pA_2 values were calculated from the equation $pA_2 = \log(CR-1) - \log[B]$ as proposed by Arunlakshana and Schild [16].

Receptor binding experiments

Hartley guinea-pigs (400–600 g) of both sexes were killed by decapitation. Various heart tissues were removed and prepared as detailed below.

Ventricular membrane preparation. The ventricles were placed in 10 vol. of ice-cold buffer (250 mM sucrose/50 mM Tris [hydroxymethyl] aminomethane–HCl/1 mM magnesium chloride, pH 7.4) and all subsequent procedures were carried out at 4°. The tissue was homogenized with three 12 sec pulses using a Polytron homogenizer (Kinematica, Model PT 3000, Switzerland). The homogenate was filtered with pressure through muslin and the filtrate centrifuged for 10 min at 1000 *g* to remove connective tissue, unbroken cells and cell debris. The supernatant was centrifuged again at 10,000 *g* for 12 min. This second supernatant was then centrifuged for 15 min at 30,000 *g* and the final pellet was resuspended in an assay buffer (75 mM Tris–HCl/25 mM $MgCl_2$, pH 7.4). Protein was determined by the method of Bradford [17]. This membrane preparation has been described by Ciaraldi and Marinetti [18].

Binding assays. The binding assay of [^{3}H]DHA was carried out as described by Lefkowitz *et al.* [19] with slight modifications. [^{3}H]DHA and ventricular membranes (200–300 μg) were incubated for 60 min at 25°, with and without the addition of 10 μM alprenolol, in a 75 mM Tris–HCl buffer comprising 25 mM $MgCl_2$, to give a final volume of 250 μL. In competitive binding experiments, the competing agent was added directly to the incubation mixture. The incubation was terminated by addition of 1 mL of ice-cold assay buffer followed by immediate filtration through Whatman GF/C glass fibre filters supported on a 12-port filter manifold (Millipore). The filters were immediately washed three times with 5 mL of ice-cold assay buffer and dried in an oven at 60° for 2 hr prior to adding 5 mL of Triton–toluene based scintillation fluid. Membrane-bound [^{3}H]DHA trapped in the filters was counted in a Wallac LKB 1211 rackbeta liquid scintillation counter with an efficiency of 41%. In each experiment, non-specifically bound [^{3}H]DHA was determined by incubating membrane protein and [^{3}H]DHA with 10 μM alprenolol. Specific binding was thus obtained by deducting this value from the total binding of [^{3}H]DHA for each sample.

Lipid/water solubility experiments

The octanol/water partition coefficients were determined according to the method of Hellenbrecht *et al.* [20]. Five milligrams of propranolol HCl, atenolol and vaninolol were dissolved in 100 mL of 0.15 M phosphate buffer (pH 7.0). The *n*-octanol was saturated with buffer and was allowed to stand overnight. The octanol–buffer volume ratio was 1:1 for propranolol HCl, atenolol and vaninolol. The mixture was shaken moderately on a mechanical shaker for 40 min at room temperature and then centrifuged at 3000 rpm for 10 min. Discarding the octanol layer, the drug concentration in the aqueous phase was assayed by spectrophotometer and a partition coefficient was obtained.

Statistical evaluation of data

Statistical analysis of the results was performed with an independent Student's *t*-test for unpaired observations and with a paired *t*-test in paired observations. Whenever a control group was compared with more than one treated group, the one-way analysis of variance (ANOVA) was used. The probability value $(P) < 0.05$ was considered to be significant in all experiments. Analysis of the data and plotting of the figures were done with the aid of software (SigmaStat and SigmaPlot, Version 5.0, Jandel, U.S.A.; PHARM/PCS, Version 4.2, MCS, U.S.A.) run on an IBM PC-AT computer.

RESULTS

Effects of vaninolol on blood pressure and heart rate

Intravenous injection of vaninolol (0.1, 0.5, 1.0 mg/kg) produced a sustained rise in blood pressure and a decrease in heart rate, sustained over 1 hr, in urethane-anesthetized normotensive Wistar rats. Administration of propranolol also showed the same effects as vaninolol (Fig. 2). In ganglion-blocked anesthetized rats, vaninolol (0.1 mg/kg, i.v.) exhibited a decrease in isoproterenol-induced cardioaccelerator responses. Whereas, injection with 0.5 mg/kg of vaninolol almost blocked the isoproterenol-induced tachycardia completely (Fig. 3). As shown in Fig. 4, phenylephrine (10 μg/kg, i.v.) was injected intravenously before and after i.v. injection of vaninolol, propranolol or labetalol (1.0 mg/kg). The pressor response to phenylephrine was not inhibited by vaninolol or propranolol, but administration of labetalol clearly showed the existence of a blockade.

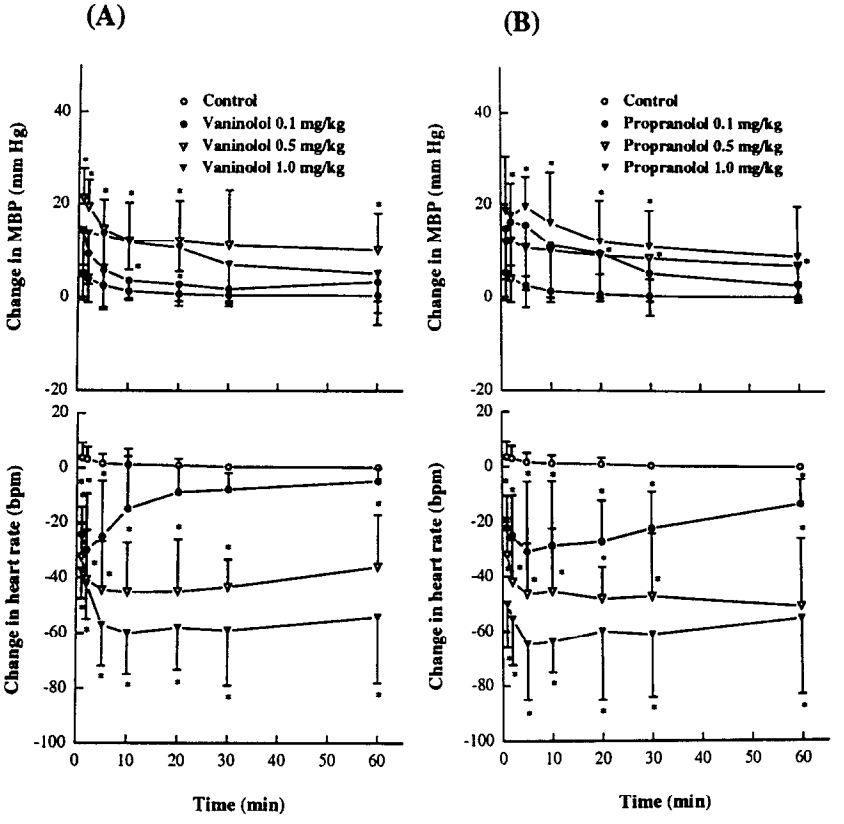

Fig. 2. Effects of vaninolol (A) and propranolol (B) on mean arterial blood pressure (MBP) and heart rate in normotensive Wistar rats, anesthetized with urethane. Normal saline was used as control. Each value represents mean ± SD, N = 10. *Significantly different from control, $P < 0.05$ (ANOVA followed by Student's *t*-test).

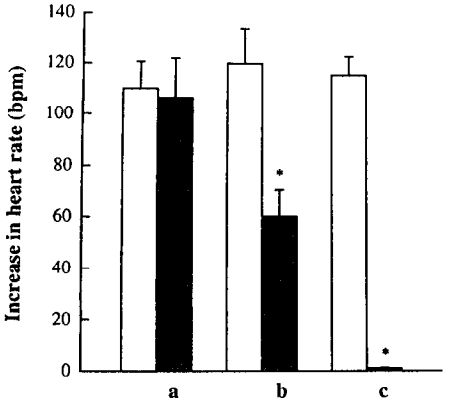

Fig. 3. Effects of intravenous injection of isoproterenol (0.5 μg/kg) in causing a tachycardia before (□) and after (■) i.v. injection of normal saline (a), vaninolol 0.1 mg/kg (b) and vaninolol 0.5 mg/kg (c) in the ganglion-blocked anesthetized rats. Each datum is the mean ± SD of six experiments. *$P < 0.05$ (paired Student's *t*-test).

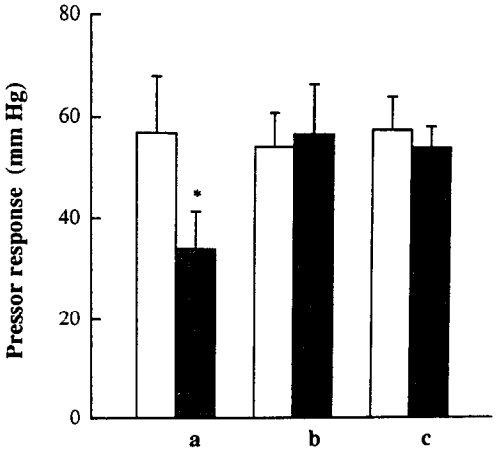

Fig. 4. Effects of intravenous injection of phenylephrine (10 μg/kg) in causing a pressor response before (□) and after (■) i.v. injection of labetalol (a), propranolol (b) and vaninolol (c) in reserpinized rats. Each datum is the mean ± SD of six experiments. *$P < 0.05$ (paired Student's *t*-test).

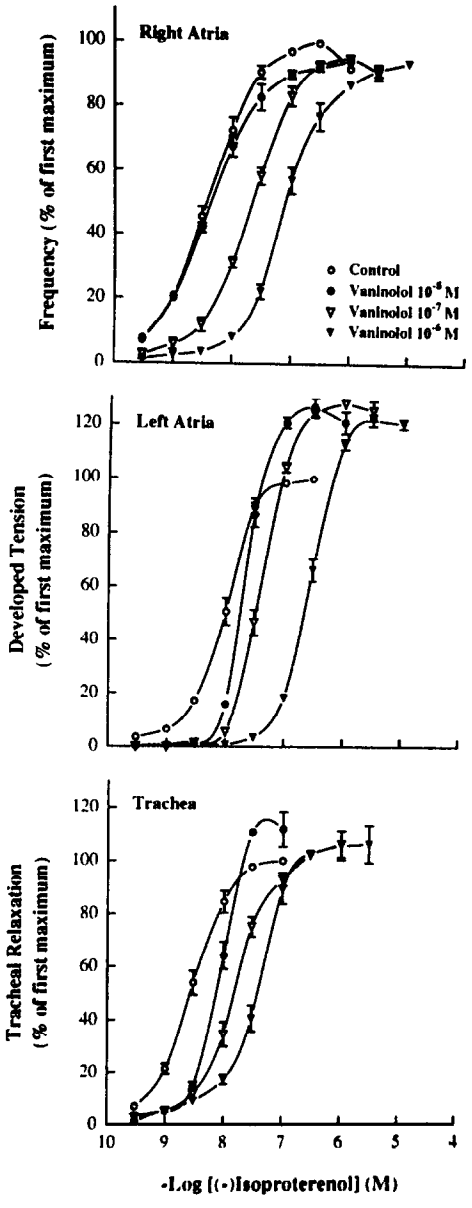

Fig. 5. The effects of vaninolol on responses in guinea-pig atria and trachea. Shown are the mean cumulative concentration–response curves for the positive chronotropic responses to (−)isoproterenol in spontaneously-beating guinea-pig right atria, positive inotropic responses to (−)isoproterenol in electrically driven guinea-pig left atria and relaxant effects to (−)isoproterenol in guinea-pig spontaneous tone tracheal strips in the absence or presence of vaninolol. Each value represents mean ± SEM from six to eight individual experiments.

Effects of vaninolol on β_1-adrenoceptor activity

Vaninolol antagonized isoproterenol-induced positive chronotropic actions in isolated guinea-pig right atrial strips. Vaninolol (10^{-8}–10^{-6} M) caused a dose-dependent parallel shift to the right of the isoproterenol concentration–response curves. The results of a typical experiment with right atria is illustrated in Fig. 5. In electrically driven guinea-pig left atrial strips vaninolol also antagonized isoproterenol-induced positive inotropic responses and produced dose-dependent rightward shifts of the cumulative concentration–response curves to isoproterenol (Fig. 5). Potential time-dependent changes in agonist potency were monitored by control experiments in which both the first and second isoproterenol concentration–response curves were carried out without antagonist. There was a decrease in the potency of isoproterenol in the second concentration–response curve that was statistically significant (data not shown). The CR for antagonists was corrected for this change in sensitivity. Vaninolol was more potent than atenolol, slightly more potent than metoprolol, and was less potent than propranolol in β_1-adrenoceptor blocking activity. The pA_2 values and slopes of regression lines are indicated in Table 1.

Effects of vaninolol on β_2-adrenoceptor activity

Vaninolol (10^{-8}–10^{-6} M) competitively antagonized isoproterenol-induced relaxation from the spontaneous tone of reserpinized guinea-pig tracheal strips. Vaninolol produced parallel shifts to the right of the agonist concentration–response curves (Fig. 5). The CR for antagonists was adjusted for the same reason that it was for left atrial strips. Vaninolol was more potent than atenolol or metoprolol, and was markedly less potent than propranolol in β_2-adrenoceptor blocking action. The pA_2 values and slopes of regression lines are indicated in Table 1.

β_1:β_2 selectivity of vaninolol

The β_1/β_2-selectivity ratio was obtained from the antilogarithm of the difference between the mean pA_2 values obtained from the right atria and trachea [21]. Vaninolol was 8.3 times more potent on right atria than on trachea, i.e. was selective for β_1-adrenoceptors. Atenolol and metoprolol were 33.9 and 20.4 times more potent on right atria than on trachea, respectively, i.e. were highly selective for β_1-adrenoceptors. Propranolol was only 1.9 times more potent on right atria than on trachea and was, therefore, considered to be non-selective (Table 1).

Lack of intrinsic sympathomimetic activity of vaninolol

The frequency of contraction of right atria and tension developed by left atrial strips from reserpinized guinea-pigs were measured against cumulatively increasing concentrations of vaninolol, atenolol, propranolol or isoproterenol. As shown in Fig. 6, isoproterenol produced concentration-dependent increases in heart rate and contractility with a maximum increase at 10^{-6} M. Vaninolol did not induce an increase in the heart rate or contractility, but caused negative inotropic and chronotropic effects in concentrations at 10^{-6} M or above. Propranolol also produced negative inotropic and chronotropic effects, and such depressant effects usually increased steeply with concentration, leading in most cases to arrest or inexcitability of the preparation at concentrations between 10^{-4} and 10^{-3} M, whereas, atenolol had nearly no depressant

Table 1. β-adrenoceptor blocking potency and β_1/β_2-selectivity of vaninolol and other β blockers on guinea-pig *in vitro* preparations

β blocker	β_1		β_2	
	pA_2 value*		pA_2 value*	
	Right atrium (slope)	Left atrium† (slope)	Trachea† (slope)	β_1/β_2-selectivity ratio
Vaninolol	7.68 ± 0.06 (0.92 ± 0.05)	7.53 ± 0.02 (0.82 ± 0.02)	6.76 ± 0.05 (0.97 ± 0.06)	8.3
Atenolol	7.23 ± 0.03 (0.97 ± 0.10)	7.31 ± 0.05 (0.85 ± 0.03)	5.70 ± 0.06 (0.96 ± 0.07)	33.9
Metoprolol	7.55 ± 0.09 (0.95 ± 0.07)	7.45 ± 0.04 (0.87 ± 0.09)	6.24 ± 0.07 (0.94 ± 0.04)	20.4
Propranolol	8.46 ± 0.06 (0.95 ± 0.04)	8.39 ± 0.09 (0.81 ± 0.05)	8.19 ± 0.12 (0.95 ± 0.08)	1.9

The pA_2 values and slope values were calculated from individual Schild plots by regression analysis. The β_1/β_2-selectivity ratio was obtained from the antilogarithm of the difference between the mean pA_2 values obtained from right atrium and trachea

* Each pA_2 value was the mean ± SEM of six to eight experimental results.

† pA_2 values were obtained from the formula $pA_2 = [\log(CR-1) - \log \text{molar concentration antagonist}]$ using CR values which were adjusted for changes in tissue sensitivity.

effects on the preparation at any of the concentrations tested (up to 3×10^{-4} M).

Effects of vaninolol on radioligand binding studies

[^{3}H]DHA bound to guinea-pig ventricular membranes in a saturable manner as illustrated in Fig. 7. The concentration dependence of [^{3}H]DHA binding was studied with labelled compound concentrations ranging from 0.1 to 30 nM. Scatchard analysis [22] to determine the affinity and number of binding sites is shown in the inset. K_d was 2.2 ± 0.5 nM (mean ± SEM), and B_{max} was 92.7 ± 11.2 fmol/mg protein (mean ± SEM) at 25°. The binding of [^{3}H]-DHA reached equilibrium in approximately 20 min and maintained it for up to 90 min (data not shown). Figure 8 demonstrates the competition curves of β-adrenoceptor antagonists for [^{3}H]DHA binding sites in the ventricular membranes. The IC_{50} value (mean ± SEM) of (−)propranolol, a non-selective β-antagonist, was 9.3 ± 1.8 nM. The IC_{50} value of atenolol, a selective β_1-antagonist, was 7.2 ± 1.2 μM, while that for vaninolol was 3.7 ± 1.0 μM. The order of potency of β-adrenoceptor antagonists in inhibiting [^{3}H]DHA binding was (−)propranolol ⩾ vaninolol ⩾ atenolol.

Effects of vaninolol on the octanol/water partition coefficient

The octanol/buffer partition coefficients of vaninolol, atenolol and propranolol were determined at pH 7.0 and their values were calculated to be 1.4, 0.005 and 5.5, respectively. Vaninolol had a low lipophilicity in comparison with propranolol, whereas atenolol had an extreme hydrophilicity.

DISCUSSION

Intravenous administration of vaninolol or propranolol produced a sustained pressor action and a dose-dependent bradycardia effect in urethane-anesthetized rats. The pressor response of propranolol in urethane-anesthetized rats was first reported by Dasgupta [23] and was confirmed later by Yamamoto and Sekiya [24] and Regoli [25]. Himori *et al.* [26] pointed out that the pressor response observed with β-blockers can be explained by both its inhibitory action on vasodilatory β_2-adrenoceptors and negating action on the peripheral vasodilatory component exerted by circulating epinephrine, leading to an increase in vascular tone. Vaninolol-induced pressor action might possibly be the same as, but weaker than, that of propranolol. Nevertheless, the precise mechanism for the paradoxically observed pressor action of vaninolol remains to be elucidated. Vaninolol has been shown to block (−)isoproterenol-induced tachycardia effects which indicates that the bradycardia effect of vaninolol is associated with β-adrenoceptor blocking activity. On the other hand, labetalol (a dual α- and β-adrenoceptor antagonist) revealed a significant inhibition of pressor responses to phenylephrine. However, vaninolol or propranolol had almost no effect on the pressor responses to phenylephrine, indicating that neither agent had a specific α-adrenoceptor blocking action.

To quantify the receptor selectivity of β-adrenoceptor antagonists, a comparison of their affinities for the β-receptor subtypes is required and pA_X values, usually pA_2[16], can be used for this purpose [15]. Harms [9] demonstrated that the guinea-pig atrium and trachea seem to be fairly good models for the study of β_1- or β_2-adrenoceptors selectivity. O'Donnell and Wanstall [15] pointed out that tissue selectivity (in terms of cardioselective and vascular selective) is not necessarily an accurate reflection of receptor selectivity. In recent years it has become evident that β_2-adrenoceptors, in addition to β_1-adrenoceptors, also exist and function in the heart, especially in the human heart, and even in the guinea-pig sinoatrial node; furthermore, the trachea

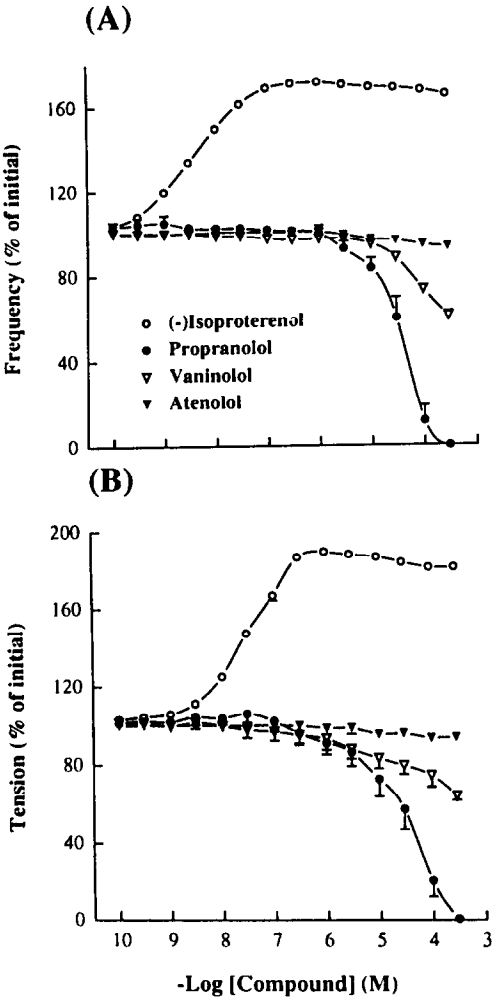

Fig. 6. Stimulant and depressant effects of (−)isoproterenol, propranolol, atenolol and vaninolol on frequency of spontaneously-beating right atria and tension developed by left atrial strips driven at 2 sec intervals from reserpinized guinea-pigs. Cumulative concentration–response curves were determined at 32.5° for four agents identified in panels (A) and (B). All measurements are expressed as mean percentages of the control frequency or force in the same preparation. Each point shows mean ± SEM from five to six individual experiments.

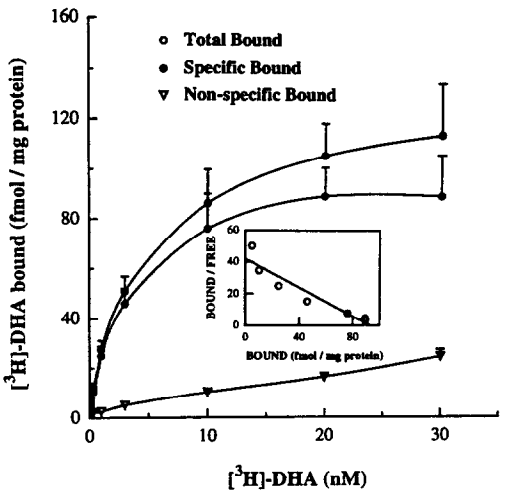

Fig. 7. [^{3}H]DHA binding curve with guinea pig ventricular membranes. The membrane fractions were incubated with increasing concentrations of [^{3}H]DHA (0.1–30 nM) in the presence (non-specific) and absence (total) of 10 μM (−)alprenolol. The inset shows a Scatchard analysis of specific [^{3}H]DHA binding to the membrane fraction. Each point represents the mean ± SEM of three experiments, each conducted in duplicate.

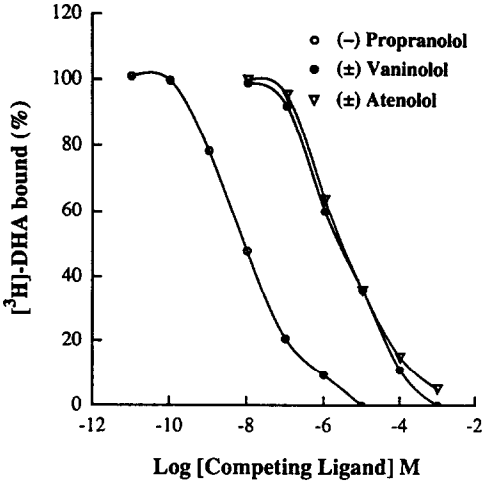

Fig. 8. Inhibition of [^{3}H]DHA specific binding to guinea pig ventricular membrane β-adrenoceptors by various β-adrenoceptor blocking agents. All incubations were performed as described in Materials and Methods. Data shown are the means of two or three independent duplicate determinations.

also relaxes partially through β_1-adrenoceptors [27]. In this study vaninolol and three β-adrenoceptor antagonists have been investigated on guinea-pig isolated atria and trachea in experiments designed to obtain pA_2 values which might best predict the selectivity of the agents between β_1- and β_2-adrenoceptors rather than their tissue selectivity. On guinea-pig isolated atria, vaninolol inhibited (−)isoproterenol-induced positive inotropic and chronotropic effects concentration-dependently. The parallel shift to the right of the concentration–response curve of (−)isoproterenol suggested that vaninolol was a β_1-adrenoceptor competitive antagonist. Vaninolol also inhibited (−)isoproterenol-induced relaxation from the spontaneous tone of guinea-pig tracheal preparations and caused a rightward shift of the concentration–response curve of (−)isoproterenol, suggesting a β_2-adrenoceptor competitive antagonism. Quantitative estimation of the degree of β_1-or β_2-adrenoceptor selectivity was

made using the method of Baird and Linnell [21], that is, the antilogarithm of the difference between the mean pA_2 values obtained from the right atria and trachea. The values of $\beta_1:\beta_2$ selectivity for vaninolol, atenolol, metoprolol and propranolol were 8.3, 33.9, 20.4 and 1.9, respectively (Table 1). The results obtained from the guinea-pig *in vitro* preparations indicated that in terms of β-adrenoceptors blocking potency and selectivity, propranolol was more potent, but non-selective; vaninolol was less potent, but β_1-selective; atenolol and metoprolol were also less potent, but highly β_1-selective.

In reserpinized guinea-pig experiments, vaninolol, atenolol and propranolol barely increased the frequency of spontaneously-beating right atria and myocardial contractile force by left atria. Therefore, vaninolol, atenolol and propranolol appeared to be devoid of ISA, in contrast to the reference drug isoproterenol (a β-adrenoceptor stimulating agent). At high concentrations, vaninolol and propranolol both produced negative inotropic and chronotropic effects, but vaninolol had less cardiodepressant action than propranolol. Atenolol had negligible cardiodepressant effects when the concentration reached 3×10^{-4} M. There is a general agreement that the cardiodepressant actions of β-blockers do not result from interaction with the β-adrenoceptors [28]. These actions have been correlated with the agents' ability to produce local anesthesia, to inhibit conduction velocity in nerve and muscle and also with their lipid solubility [6–8]. According to the lipid/water solubility experiments, vaninolol was observed to have less hydrophobilicity than propranolol. This result was positively demonstrated by the decreased cardiodepressant effects of vaninolol compared with those of propranolol.

The [^{3}H]DHA binding to guinea-pig ventricular membranes was saturable and of high affinity. The mean equilibrium dissociation constant ($K_{d25°}$) of [^{3}H]DHA, measured by Scatchard analysis of the saturation curve for the binding of increasing concentrations of [^{3}H]DHA, was 2.2 ± 0.5 nM. This value was very close to that reported by Rankin and Broadley [29] for [^{3}H]DHA binding to guinea-pig ventricular muscle membranes at 38°, where the K_d value was 4.9 ± 1.1 nM. The ranking order of inhibition for [^{3}H]DHA binding of β-blockers was (−)propranolol ⩾ vaninolol ⩾ atenolol. Chenieux-Guicheney *et al.* [30] described that selective β_1-antagonists were shown to have lower binding site affinities when compared to other blockers. This may be related to steric hindrance by the side chain at the aromatic end of these molecules. It should be noted that the structure of vaninolol (Fig. 1) also possessed a branched-side chain at the aromatic end of the molecule. This may have interfered with proper spatial relationships between other affinity determining groups of the blocker (such as the β-hydroxyl group, the cationic head and the aromatic moiety, according to Harms [9]) and their counterparts on the receptor [29]. It is no wonder that vaninolol revealed a lower binding affinity in a competing [^{3}H]DHA binding study.

In vivo or *in vitro* pharmacological examinations and radioligand binding studies have shown that vaninolol is a potent, chemically novel, relatively selective β_1-adrenoceptor blocking agent with low hydrophobicity in comparison to propranolol, no ISA, and little direct myocardial depression at high concentrations. To the best of the authors' knowledge, there is no β-adrenoceptor antagonist that is derived by combining the vanillin nucleus with an oxypropranolamine side chain to obtain vaninolol.

Acknowledgements—This work was supported by research grants from the National Science Council of the Republic of China, Taiwan (NSC 82-0115-C-001-0006 and NSC 82-0412-B-037-088).

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