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Capsinolol: the first β-adrenoceptor blocker with an associated calcitonin gene-related peptide releasing activity in the heart

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1 The β-adrenoceptor blocking and calcitonin gene-related peptide (CGRP)-releasing properties of capsinolol (N-[4-(2-hydroxy-3 (isopropylamino) propoxy)-3-methoxybenzyl] -nonanamide), derived from nonivamide, were investigated under *in vivo* and *in vitro* conditions.

2 Capsinolol (0.1, 0.5, 1.0 mg kg^{-1}, i.v.), as well as (±)-propranolol, produced a dose-dependent bradycardia response and a temporary pressor action in urethane-anaesthetized normotensive Wistar rats. These cardiovascular effects were different from the vagus reflex and parasympathetic efferent effects shown by capsaicin (0.1 mg kg^{-1}, i.v.) in the rat.

3 Capsinolol (1.0 mg kg^{-1}) inhibited the tachycardia effects induced by (−)-isoprenaline, but had no blocking effect on the arterial pressor responses induced by (−)-phenylephrine. The findings suggest that capsinolol possesses β-adrenoceptor blocking activity, but it has no α-adrenoceptor blocking activity.

4 In guinea-pig isolated tissues, capsinolol (10^{-8} to 10^{-6} M) antagonized (−)-isoprenaline-induced positive chronotropic and inotropic 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 (−)-isoprenaline suggests capsinolol is a β-adrenoceptor competitive antagonist.

5 Capsinolol (10^{-5} to 10^{-4} M) exhibited a positive cardiotonic effect that was not inhibited by (±)-propranolol and reserpine, but was inhibited by capsazepine (10^{-6} M) and $CGRP_{8-37}$ (10^{-6} M). This effect was independent of intrinsic sympathomimetic effects.

6 An immunoassay of released CGRP from guinea-pig isolated perfused heart indicated that capsinolol increases the release of CGRP and thus produces positive cardiotonic effects.

7 In conclusion, capsinolol is a non-selective β-adrenoceptor antagonist with capsaicin-like cardiotonic properties unrelated to traditional intrinsic sympathomimetic effects. It is suggested that capsinolol causes CGRP release from cardiac sensory neurones via a non-adrenergic mechanism and then activates CGRP receptors on cardiac muscle.

Keywords: Capsaicin analogues; β-adrenoceptor blocker; capsazepine; cardiotonic activity; calcitonin gene-related peptide

Introduction

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide, Figure 1), is a pungent compound produced by chilli peppers and related plants of the Capsicum family. Early investigations of its pharmacological effects showed a wide spectrum of activities (Buck & Burks, 1986; Holzer, 1991), including a decrease in heart rate caused by a sensory C-fibre-evoked parasympathetic efferent effect (Chahl & Lynch, 1987) and an increase in cardiac contractility force caused by the release of CGRP (calcitonin gene-related peptide) from sensory nerves (Franco-Cereceda *et al.*, 1988; 1989).

The stimulated release of endogenous CGRP by capsaicin is not generally associated with the positive chronotropic and inotropic activities necessary for the treatment of cardiac disease. However, CGRP has been shown to improve cardiac performance in patients with congestive heart failure (Gennari *et al.*, 1990). Recently, capsaicin has also been found to have antiarrhythmic and antiischaemic activity (D'Alonzo *et al.*, 1995).

β-Adrenoceptor blockers have received enormous attention because of their clinical benefits in the treatment of hypertension, ischaemic heart disease, and certain arrhythmias (Hoffman & Lefkowitz, 1991). However, it is known that depression of the myocardial function or heart failure may limit the clinical benefit of conventional β-blockers. Since the most serious adverse responses to β-adrenoceptor blockade are bradycardia and heart failure, β-adrenoceptor antagonists with partial intrinsic sympathomimetic activity (ISA) are desired to reduce these untoward cardioinhibitory effects (Hoffman & Lefkowitz, 1991). The cardiotonic CGRP, released by capsaicin, has not previously been used to reduce this β-adrenoceptor-mediated cardioinhibitory activity. Earlier studies of capsaicin and its derivatives in the cardiovascular system encouraged us to search for a new family of β-adrenoceptor blockers derived from capsaicin/nonivamide and which could be used in the treatment of cardiovascular diseases without the risk of bradyarrhythmia and heart failure (Chen *et al.*, 1992; 1994).

The oxypropanolamine side chain is a key-group of β-adrenoceptor blockers (Hoffman & Lefkowitz, 1991). The phenolic hydroxyl group of capsaicin and nonivamide also provides an opportunity to introduce 4-0-ether-linked oxypropanolamine side chains. Taking into consideration the parasympathetic efferent and sensory cardiotonic effects of capsaicin and this possible oxypropanolamine-introducing β-adrenoceptor blocking activity, capsinolol (N-[4-(2-hydroxy-3-(isopropylamino) propoxy)-3-methoxybenzyl]-nonanamide; Figure 1) was developed. This compound, a member of the guaiacoxypropanolamine family and a derivative of capsaicin or nonivamide (Chen *et al.*, 1994), was designed to display capsaicin-like cardiotonic properties unrelated to a sympathomimetic effect and to reduce the undesired cardioinhi-

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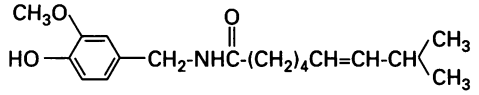
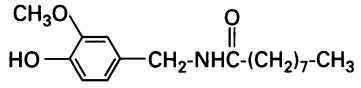
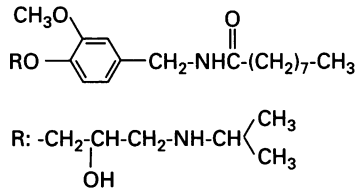


Capsaicin (8-methyl-N-vanillylamide)



Nonivamide (N-nonanoyl vanillylamide)



Capsinolol
(N-[4-(2-hydroxy-3-(isopropylamino)propoxy)-
3-methoxybenzyl]nonanamide)

Figure 1 Chemical structures of capsaicin, nonivamide, and capsinolol.

bitory effect of β-adrenoceptor blockers. Originally, guaiacoxypropanolamine derivatives of capsaicin were developed to combine the advantages of β-adrenoceptor blockade and an intrinsic positive cardiotonic effect, derived from capsaicin, in one molecule, and thus compensate the untoward circulatory effects of β-adrenoceptor blockers (Chen *et al.*, 1994). This study has been carried out to investigate the mechanism of action of capsinolol, including a study of its inhibitory effect on (−)-isoprenaline-induced cardiovascular effects, capsaicin-like positive chronotropic activity, CGRP releasing activity and CGRP activated cardiotonic activity.

Methods

Measurement of heart rate and blood pressure

Measurement of blood pressure and heart rate have been described previously (Yeh *et al.*, 1993; Chen *et al.*, 1993). Briefly, male Wistar rats, weighing 250–350 g were anaesthetized with urethane (1.5 g kg^{-1}, i.p.). Following tracheal cannulation, systemic arterial blood pressure and heart rate were recorded from the femoral artery with a pressure transducer (GOULD Inc, Model P50, U.S.A.) connected to a pressure processor amplifier (GOULD Inc, 13-4615-52, U.S.A.) and displayed on a recorder (GOULD Inc, 8188-4402, U.S.A.). Body temperature was maintained at 37°C. The femoral vein was cannulated for i.v. injections. All drug solutions were administered in a volume of 0.4 ml kg^{-1}. Equivolumetric injection of the vehicle was administered as a control. The magnitudes of the effects elicited after injections were evaluated by measuring the changes in arterial blood pressure and heart rate as the difference between the peak responses and basal blood pressure or heart rate.

Adrenoceptor blocking response in vivo

In the test for β-adrenoceptor blocking effects and possible capsaicin derived vagus reflex or parasympathetic efferent effects of capsinolol *in vivo*, some pretreatments were performed on the rats before the experiments. Bilateral cervical vagotomies were performed 1 h before the experiments. (−)-Isoprenaline (0.2 μg kg^{-1}) was administered via a femoral vein and the resultant tachycardia recorded as the control. A single dose of capsinolol and the vehicle was then administered intravenously. Ten minutes later, a further injection of (−)-isoprenaline was recorded and then expressed as % of the control responses.

In the test for α-adrenoceptor blocking responses *in vivo*, rats were pretreated with reserpine (5 mg kg^{-1}, i.p.) 24 h before the injection of (−)-phenylephrine (10 μg kg^{-1}, i.v.), followed 15 min later by the intravenous injection of a single dose of capsinolol, (±)-propranolol, or labetalol (1 mg kg^{-1}). Ten minutes later, a further injection of (−)-phenylephrine was given.

β-Adrenoceptor blocking response in the guinea-pig isolated atria

This experiment was carried out as previously described (Wu *et al.*, 1994a). Guinea-pigs (Hartley) of either sex weighing between 350 and 500 g, were killed by a blow on the head. Their hearts were quickly excised and excess tissue was removed. 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, U.S.A.). The frequency of contraction was measured on a separate channel by a tachometer (Coulbourn, Model S77-26, U.S.A.) connected to a high-speed videograph (Coulbourn, AT L19-69, U.S.A.). The experiments were carried out at 32.5°C and contained 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 95% O_2 and 5% CO_2. The atrial strip was prestretched to a baseline tension of 0.2 g. As a β-agonist, (−)-isoprenaline was administered to the preparation in a cumulative fashion after an equilibration period of 90 min in Krebs solution and a concentration-response curve 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 atria 15 min before (−)-isoprenaline was added. The activity of the test compound was expressed as a pA_2-value, which was calculated from the parallel shifts of the cumulative concentration-response curve of (−)-isoprenaline (Arunlakshana & Schild, 1959).

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°C) and were driven at a frequency of 2 Hz and at 2 ms intervals via two platinum electrodes placed at either side of the atrium under a resting tension 1.0 g. β-Adrenoceptor antagonist activity was determined as follows. Cumulative concentration-response curves to the positive effects of (−)-isoprenaline were obtained in the absence and presence of various concentrations of a test compound. Data were calculated as a percentage of the increase in force induced by (−)-isoprenaline.

β-Adrenoceptor blocking response in the guinea-pig isolated trachea

The isolated guinea-pig trachea was cleaned of extraneous connective tissue and cut into spiral strips as described previously (Wu *et al.*, 1994a,b). Each 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 37°C and the solution was gassed with 95% O_2 and 5% CO_2. An initial basal tension of 2.0 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 previously (O'Donnell & Wanstall, 1979) to prevent extraneuronal uptake and to block α-adrenoceptors. To determine β-adrenoceptor antagonist activity, cumulative concentration-response curves to the relaxant effects of (−)-isoprenaline were obtained in the absence and presence of a test compound

(30 min incubation time). Data were calculated as a percentage of the maximum relaxation induced by (−)-isoprenaline.

Intrinsic sympathomimetic response

In the test for possible intrinsic sympathomimetic and noradrenaline-releasing activities of capsinolol, reserpine (10 mg kg^{-1}, i.p.) was administered 24 h before the experiment. In addition, (±)-propranolol (10^{-7} M) was added to the bath 10 min before the experiment to block the possible stimulating activity of capsinolol on β-adrenoceptors. Finally, capsinolol was cumulatively added to the bath to induce atrial contractions.

Sensory neurone stimulation and CGRP release

In the test for possible intrinsic sensory neurone activating and CGRP releasing activities of capsinolol in the guinea-pig isolated atria, after the application for 30 min of capsazepine (10^{-6} M), a competitive capsaicin receptor antagonist on the sensory neurone, and $hCGRP_{8-37}$ (10^{-6} M), a CGRP antagonist, capsinolol was cumulatively added to the bath to induce atrial contractions.

Immunoreactivity of released CGRP from guinea-pig isolated perfused hearts

Adult guinea-pigs, weighing 250–350 g, were killed by a blow to the head. Their hearts and aortae were quickly excised and excess tissue was removed. After rapid cannulation of the aorta, heparin (100 u) in 5 ml of Tyrode solution was infused into the coronary circulation. The heart was then excised and infused according to Langendorff's method with a pressure of 70 cmH_2O (Broadley, 1979). The Tyrodes' solution contained (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.0, $MgCl_2$ 1.05, $NaHCO_3$, 11.9, NaH_2O_4 0.42, ascorbic acid 0.057 and either glucose 5.6 or lactic acid 5.0, as substrate. The solution was warmed to 37°C and gassed with 95% O_2 and 5% CO_2 immediately before infusion. Capsinolol (5, 10 and 100 μM) and capsaicin (1.0 μM) were infused into the coronary circulation. Perfusate fractions (5 min) were collected on ice with a final concentration of acetic acid of about 0.2 M. The perfusate samples were desalted, by use of SEPPAK C_{18} cartridges (Waters Corporation, Massachusetts, U.S.A.) lyophilized and redissolved in 0.5 ml of appropriate buffer to determine CGRP-like immunoreactivity by radioimmunoassay with an antiserum raised against hCGRP-α (Peninsula, CA, U.S.A.).

Sensory denervation

In order to stop the sensory neurone from functioning and releasing CGRP, capsaicin (100.0 mg kg^{-1}) was co-administered with ketamine (2.0 mg kg^{-1} day^{-1}) subcutaneously for 1 week to induce denervation of the sensory neurones in guinea-pigs. The isolated atria obtained from the denervated animals were then used to test the atrial contractility of capsinolol.

Drugs

(−)-Isoprenaline bitartrate, (−)-phenylephrine HCl, (±)-propranolol HCl, (±)-metoprolol tartrate, (±)-pindolol, (±)-alprenolol, labetalol, phenoxybenzamine HCl, reserpine, capsaicin and $hCGRP_{8-37}$ were all purchased from Sigma Chemical Co. Capsinolol was synthesized by our laboratory (Department of Pharmacology, Kaohsiung Medical College).

Pharmacological calculations and analysis of mechanical results

Concentration-ratios (CR), EC_{50} in the presence of antagonist, and EC_{50} in the absence of antagonist were corrected for any change in sensitivity to the agonist that was not due to the

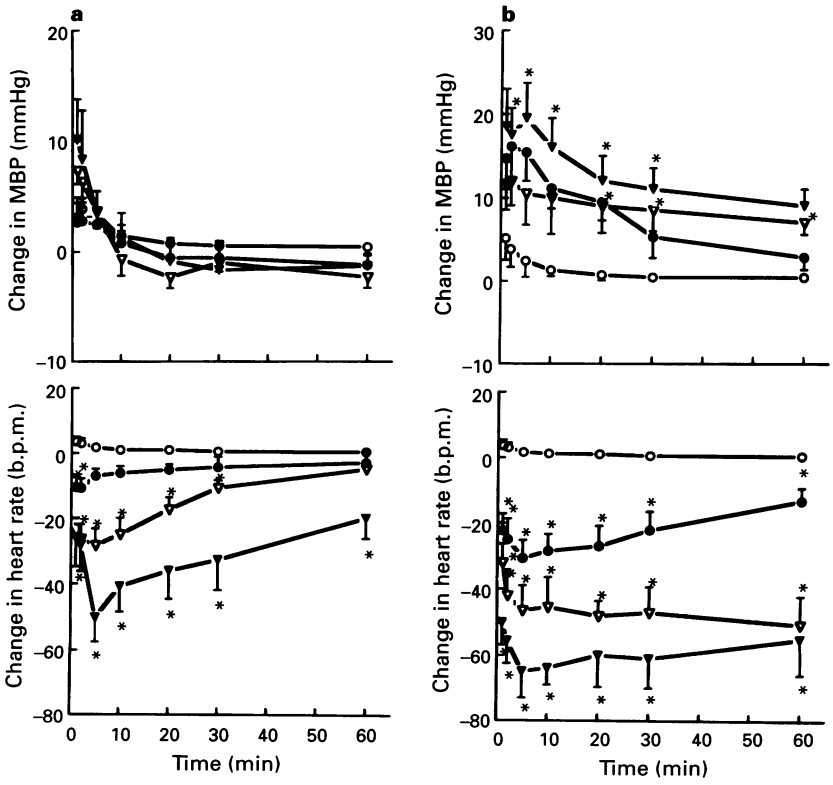


Figure 2 Effects of intravenous injection of capsinolol (a) and (±)-propranolol (b) on blood pressure and heart rate in normal rats. (○) Vehicle control; (●) 0.1 mg kg^{-1}, (▽) 0.2 mg kg^{-1}, (▼) 0.5 mg kg^{-1} capsinolol (a) or propranolol (b). Each point is the mean and vertical lines show s.e. Statistically significant differences compared with capsaicin control by Student's *t* test are shown by $*P<0.05$.

presence of the antagonist, by 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 (1979). The concentration-ratio (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 & Schild (1959). The β_1/β_1-selectivity ratio was obtained from the antilogarithm of the difference between the mean pA_2 values obtained from right atria and trachea.

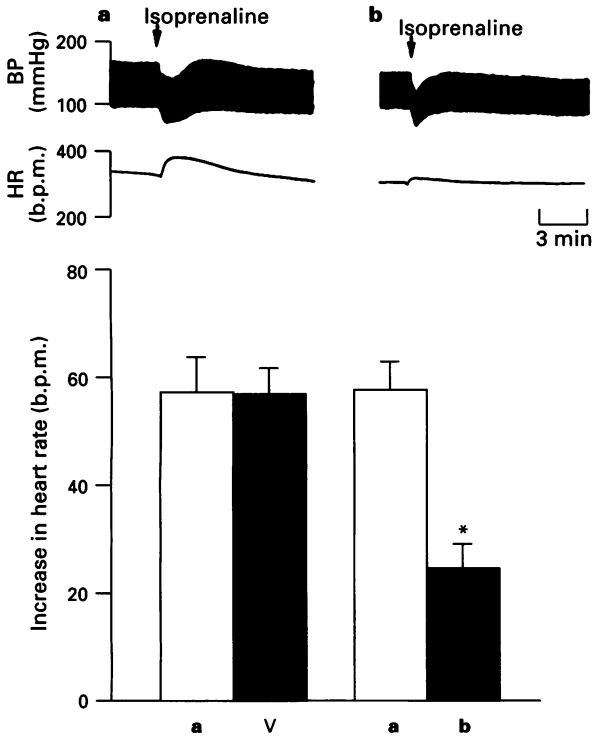


Figure 3 Effects of intravenous injection of (−)-isoprenaline ($0.2\,\mu g\,kg^{-1}$) on blood pressure and heart rate before (a) and after (b) the intravenous injection of capsinolol ($1.0\,mg\,kg^{-1}$) and its vehicle (V) in the vagotomized urethane-anaesthetized rat. Each column shows the mean and vertical lines indicate s.e. (*$P<0.05$, $n=6$).

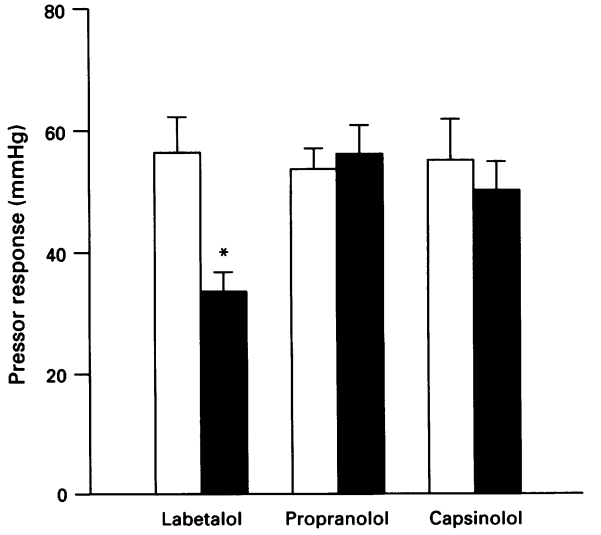


Figure 4 Effects of intravenous injection of (−)-phenylephrine ($10\,\mu g\,kg^{-1}$) on blood pressure before (open columns) and after (solid columns) the intravenous injection of capsinolol, (±)-propranolol and labetalol at $1.0\,mg\,kg^{-1}$ in the urethane-anaesthesized reserpine-treated rats. Each column shows the mean and vertical lines indicate s.e. (*$P<0.05$, $n=6$).

Statistics

All values in the text and figures are expressed as mean ± s.e. Statistical differences were evaluated by Student's t test in unpaired samples and by paired Student's t test in paired

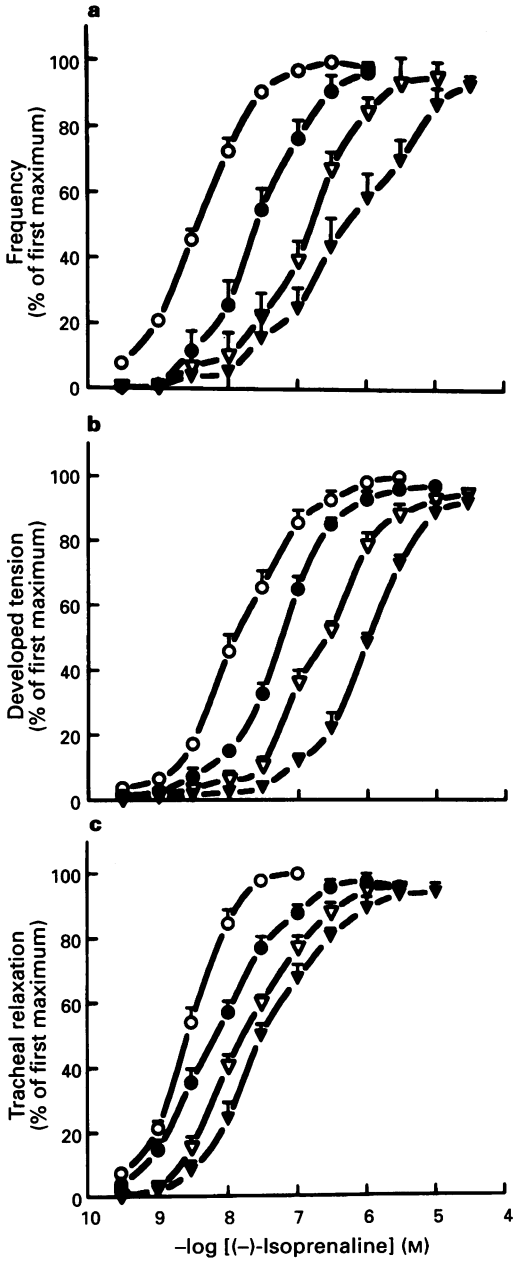


Figure 5 Antagonism of (−)-isoprenaline by capsinolol in guinea-pig right atria (a), left atria (b) and trachea (c). Shown are the mean cumulative concentration-response curves for the positive chronotropic responses of (−)-isoprenaline in (a) isolated spontaneously-beating right atria ($n=6$) and (b) electrically-driven left atria ($n=6$), and (c) relaxant effects of (−)-isoprenaline in guinea-pig isolated trachea ($n=10$) in the absence or presence of capsinolol ((○) vehicle control; (●) 10^{-8} M, (▽) 10^{-7} M, (▼) 10^{-6} M capsinolol). Each point is the mean and vertical lines show s.e. of individual experiments.

samples. Whenever a control group was compared with more than one treated, the one way analysis of variance (ANOVA) was used. Probability values (P) smaller than 0.05 were considered to be significant. 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 capsinolol on heart rate and blood pressure

Intravenous injections of capsinolol (0.1, 0.5, 1.0 mg kg^{-1}) and (±)-propranolol (0.1, 0.5, 1.0 mg kg^{-1}) stimulated a temporary rise in blood pressure and a decrease in heart rate which was sustained over 1 h in urethane-anaesthetized normotensive Wistar rats (Figure 2). In contrast, both capsaicin and nonivamide induce hypotensive and vagus reflex activities (Yeh *et al.*, 1993; Lo *et al.*, 1994).

In vagotomized anaesthetized rats, capsinolol (1.0 mg kg^{-1}, i.v.) exhibited a decrease in (−)-isoprenaline (0.2 μg kg^{-1})-induced cardioaccelerator responses (Figure 3). In the reserpine-treated rats, (−)-phenylephrine (10 μg kg^{-1}) was injected intravenously before and after intravenous injection of capsinolol, (±)-propranolol or labetalol, all at 1.0 mg kg^{-1}. As shown in Figure 4, the pressor response to (−)-phenylephrine was not inhibited by capsinolol or (±)-propranolol, but administration of labetalol clearly showed the existence of a blocking effect.

Effects of capsinolol on β_1-adrenoceptor activity

Capsinolol antagonized (−)-isoprenaline-induced positive chronotropic actions in guinea-pig isolated right atrial strips. Capsinolol (10^{-8}–10^{-6} M) caused a dose-dependent parallel shift to the right of the (−)-isoprenaline concentration-response curves. In electrically driven left atrial strips of the guinea-pig, capsinolol also antagonized (−)-isoprenaline-induced positive inotropic responses and produced dose-dependent rightward shifts of the cumulative concentration-response curves to isoprenaline (Figure 5). Potential time-dependent changes in agonist potency were monitored by control experiments in which both the first and second (−)-isoprenaline concentration-response curves were carried out without antagonist. There was a decrease in the potency of (−)-isoprenaline in the second concentration-response curve that was statistically significant (data not shown). The CR for antagonists was corrected for this change in sensitivity. Capsinolol was more potent than (±)-metoprolol, and was less potent than (±)-propranolol as a β_1-adrenoceptor blocker. The pA_2 values and slopes of regression lines are presented in Table 1. Capsinolol is obviously a non-selective β-adrenoceptor blocker with a lower β_1/β_2-selectivity ratio-value, in comparison with (±)-propranolol. The relative order of β_1/β_2-selectivity of these 3 drugs is (±)-metoprolol > > (±)-propranolol > capsinolol.

Effects of capsinolol on β_2-adrenoceptor activity

Capsinolol (10^{-8}–10^{-6} M) competitively antagonized (−)-isoprenaline-induced relaxation from the spontaneous tone of reserpine-treated guinea-pig tracheal strips. Capsinolol produced parallel shifts to the right of the agonist concentration-response curves (Figure 5). The CR for antagonists was adjusted for the same reason that it was for left atrial strips. Capsinolol was more potent than (±)-metoprolol, and was markedly less potent than (±)-propranolol as a β_2-adrenoceptor blocker. The pA_2 values and slopes of regression line are presented in Table 1. The estimated β_1/β_2-selectivity ratio value (1.3) indicates that capsinolol is a non-selective β-adrenoceptor blocker.

Cardiotonic effects of capsinolol

The frequency of contraction of right atria and tension developed by left atrial strips from reserpine-treated guinea-pigs were measured against cumulatively increasing concentrations of capsinolol, (±)-propranolol, (±)-alprenolol, (±)-pindolol or (−)-isoprenaline. As shown in Figure 6, (−)-isoprenaline produced concentration-dependent increases in heart rate and contractility with a maximum increase at 10^{-6} M. (±)-Propranolol produced negative inotropic and chronotropic effects, and such depressant effects usually increased steeply with concentration, leading in most cases to arrest or unexcitability of the preparation at concentrations between 10^{-4} and 10^{-3} M. The relative ability of these β-adrenoceptor blockers to produce this effect at 10^{-4} M was (±)-propranolol > (±)-pindolol > (±)-alprenolol. Capsinolol (10^{-6} to 3×10^{-5} M) caused an increase in heart rate and contractile force directly, but larger concentrations of capsinolol had no additional effects. These effects of capsinolol were not affected by (±)-propranolol (10^{-7} M) and reserpine (10 mg kg^{-1}, i.p.) (data not shown), but were inhibited by sensory denervation and pretreatment with a CGRP antagonist (10^{-6} M) and capsazepine (10^{-6} M), a capsaicin antagonist. Capsazepine shifted the capsinolol concentration-response curves both rightward and downward, and the chronotropic response started at 3×10^{-5} M. Results for $CGRP_{8-37}$ were also significantly different from control, but those for (±)-propranolol were not (Figures 7 and 8).

Immunoreactivity of CGRP from guinea-pig isolated perfused heart

The stimulant effects of capsinolol on the release of CGRP-like immunoreactivity from the guinea-pig isolated perfused heart are shown in Figure 9. In comparison with the control group,

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

	β_1 pA_2 values[a]		β_2 pA_2 values[a]	
β-blocker	Right atrium (slope)	Left atrium[b] (slope)	Trachea[b] (slope)	β_1/β_2-selectivity ratio
Capsinolol	8.16±0.21 (0.78±0.08)	8.05±0.14 (0.77±0.04)	8.04±0.84 (0.71±0.14)	1.3
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
Metoprolol	7.55±0.09 (0.95±0.07)	7.45±0.04 (0.81±0.05)	6.24±0.07 (0.94±0.04)	20.4

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. [a]Each pA_2 value was the mean±s.e. of six to eight experimental results. [b]pA_2 values were obtained from the formula $pA_2 = [\log(CR-1) - \log$ molar concentration antagonist] using concentration-ratio (CR) values which were adjusted for changes in tissue sensitivity values.

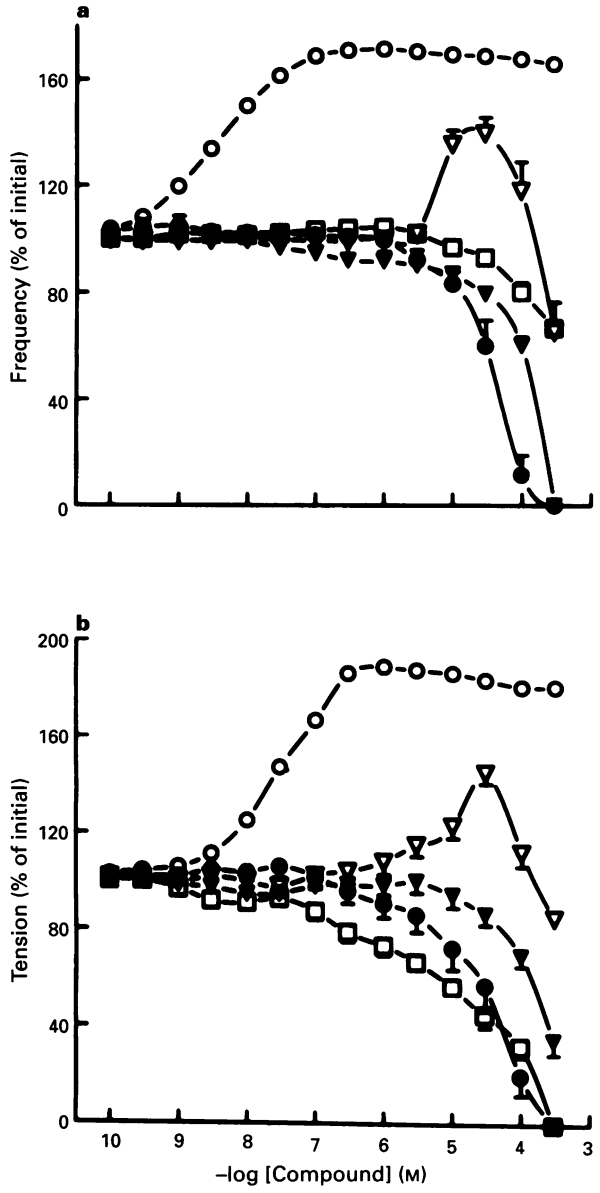


Figure 6 Positive chronotropic (a) and inotropic (b) effects of capsinolol (▽) in comparison with (−)-isoprenaline (○), (±)-propranolol (●), (±)-alprenolol (▼) and (±)-pindolol (□). Each point is the mean and vertical lines show s.e. (*$P<0.05$, $n=7$).

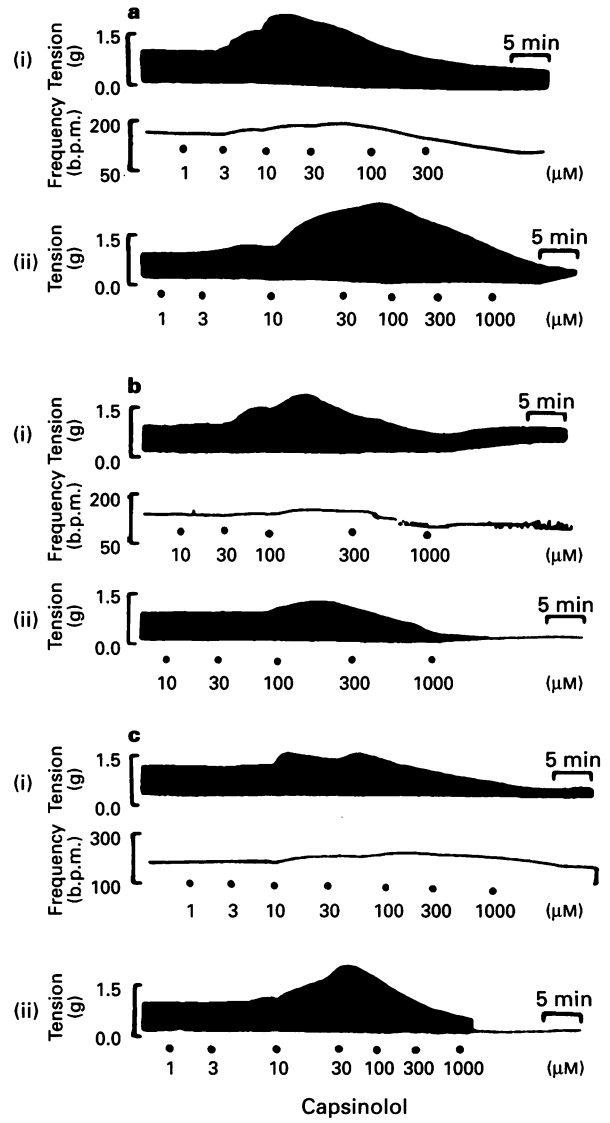


Figure 7 Typical recording of inhibitory effects of (b) capsazepine (10^{-6} M), (c) a calcitonin gene-related peptide (CGRP) antagonist $CGRP_{8-37}$ (10^{-6} M), and (a) vehicle (control) on capsinolol-induced cardiotonic activities in isolated spontaneously-beating right atria (upper trace, i) and electrically-driven left atria (lower trace, ii). Dots indicate the time of application of capsinolol (1.0–1000 μM).

capsinolol (5, 10, and 100 μM) perfused for 5 min increased the release of CGRP-like immunoreactivity to 48.9±5.2, 119.6±10.6, and 258.1±30.4 fmol per fraction, respectively, but was less effective than capsaicin (1.0 μM) in the release of immunoreactivity (356.7±39.3 fmol per fraction, not shown in Figure 9).

Discussion

Bradycardia and heart failure are normal responses to β-adrenoceptor blockade; however, in patients with partial or complete atrioventricular conduction defects, β-adrenoceptor antagonists may cause life-threatening bradyarrhythmias (Hoffman & Lefkowitz, 1991). In order to prevent the possibility of heart failure, particular caution is indicated in patients who are taking calcium channel blockers. Several β-blockers (e.g., pindolol and acebutolol) partially activate β-adrenoceptors in the absence of catecholamines. However, the intrinsic activities of these drugs are far weaker than for a full agonist such as isoprenaline. These β-blockers are said to have intrinsic sympathomimetic activity (ISA). Traditionally, ISA means a β-adrenoceptor blocker with partial β-adrenoceptor agonist activity. Substantial ISA in full is counterproductive to the response desired from a β-adrenoceptor antagonist. Obviously, a slight residual cardiotonic activity due to ISA may prevent profound bradycardia or negative inotropy in the resting heart (Hoffman & Lefkowitz, 1991). The results in our present study indicate that capsinolol reveals the desired cardiotonic activity (Figures 7 and 9) and sustained bradycardia effect (Chen *et al.*, 1994) of (±)-pindolol and (±)-acebutolol (Clark *et al.*, 1982), but this cardiotonic effect of capsinolol is effected by a CGRP releasing activity, a mechanism of action different from ISA induced by β-blockers.

Intravenous injection of capsaicin, significantly, produced a triphasic effect on blood pressure: an initial sharp reduction (effect A), a transient increase in pressure (effect B), and a delayed fall (effect C) in blood pressure (Donnerer & Lembeck, 1982). Nonivamide, a synthetic capsaicin, has also been shown to reveal similar but less potent activities in previous studies (Yeh *et al.*, 1993; Lo *et al.*, 1994). In contrast to capsaicin/nonivamide, intravenous capsinolol revealed a sustained bra-

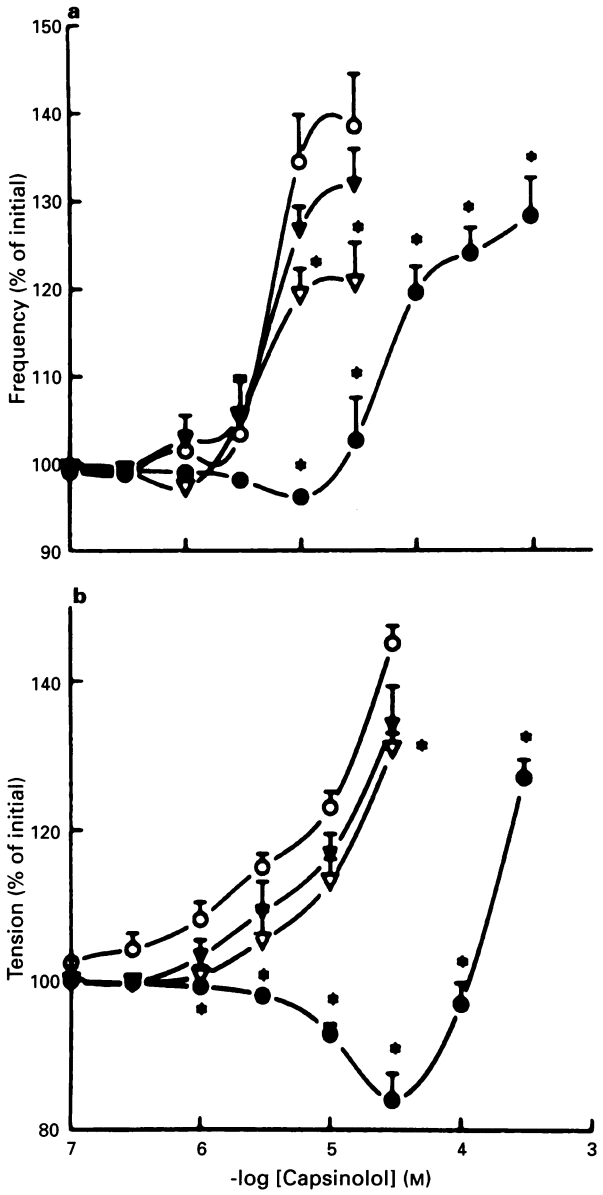


Figure 8 Inhibitory effects of capsazepine (10^{-5} M) and calcitonin gene-related peptide$_{8-37}$ ($CGRP_{8-37}$) (10^{-7} M) on capsinolol-induced cardiotonic activities in isolated spontaneously-beating right atria (a) and electrically-driven left atria (b). (○) Capsinolol; (▼) propranolol pretreatment; (▽) $CGRP_{8-37}$ pretreatment; (●) capsazepine pretreatment. Each point is the mean of 8 experiments; vertical lines show s.e. Statistically significant differences from the control: $^*P<0.05$, $n=8$ (paired Student's t test).

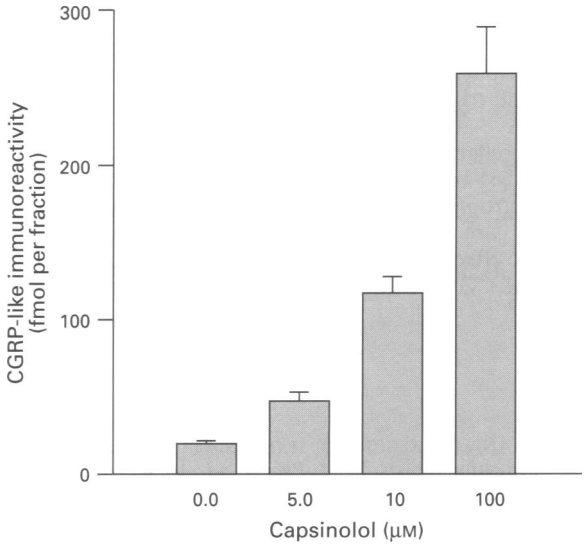


Figure 9 Stimulating effects of capsinolol (5.0, 10, and 100 μM) on the release of calcitonin gene-related peptide (CGRP)-like immunoreactivity from perfused isolated guinea-pig heart. Each column shows the mean and vertical lines s.e. of 3 experiments.

dycardia effect but did not exhibit a temporary triphasic blood pressure change and biphasic bradycardia effects (Yeh *et al.*, 1993; Chen *et al.*, 1994). This indicates that a capsaicin-derived parasympathetic efferent effect is not evident and the possible β-adrenoceptor blocking effect derived from oxypropanolamine is predominant in capsinolol. In addition capsinolol did not inhibit the hypertensive effect of (−)-phenylephrine, which indicates that capsinolol is not an α-adrenoceptor blocker. However, capsinolol could inhibit (−)-isoprenaline-induced tachycardia which indicates that the bradycardia effect of capsinolol may be related to its β-adrenoceptor blocking activity.

Acute intravenous administration of capsinolol or (±)-propranolol produced a temporary pressor action and a dose-dependent bradycardia effect in urethane-anaesthetized rats. A pressor response of (±)-propranolol in urethane-anaesthetized rats has been shown previously by Sekiya *et al.* (1974). Himori *et al.* (1984) proposed that the pressor response observed with this β-blocker is due to its inhibitory action on the vasodilator component exerted by circulating adrenaline which leads to an increase in vascular tone. Capsinolol-induced temporary pressor action might possibly be by the same mechanism as that of (±)-propranolol.

In the test for cardiotonic ISA and noradrenaline releasing activity of capsinolol, application of (±)-propranolol and pretreatment with reserpine were found to be unable to prevent the cardiotonic effect of capsinolol in the guinea-pig isolated atria. Pretreatment with capsaicin made the atrium insensitive to capsinolol-induced cardiotonic activity which indicates that this effect was dependent on the sensory neurone function. Furthermore, application of capsazepine, a competitive capsaicin receptor antagonist, significantly inhibited this cardiotonic activity of capsinolol.

Franco-Cereceda *et al.* (1988) showed that capsaicin, similar to CGRP, caused positive inotropic and chronotropic effects on the guinea-pig right atria. This cardiotonic effect of capsaicin may be secondary to the release of endogenous CGRP from local sensory nerve endings within the heart (Franco-Cereceda *et al.*, 1988). CGRP has been shown to enhance adenylate cyclase activity resulting in an elevation of cyclic AMP content and then increase calcium influx and potassium permeability (Ohmura *et al.*, 1990). Moreover, Castle (1992) suggested that capsaicin may have a second direct action on cardiac myocytes to prolong the action potential associated with inhibition of the K^+ current. The electrophysiological results from our examination of capsaicin in cultured vascular smooth muscle cells of rat aorta also indicated that capsaicin may cause inhibition of voltage-dependent K^+ channels (Lo *et al.*, 1995).

In the present study, capsinolol revealed positive inotropic and chronotropic effects, which were inhibited by pretreatment with a CGRP antagonist. Capsinolol, like capsaicin, also increased the release of CGRP-like immunoreactivity from the guinea-pig isolated perfused heart. These findings suggest that capsinolol shows both β-blocking and capsaicin-like activity resulting in the activation of capsaicin receptors. The data shown in Figures 7 and 8 suggest that capsazepine competes with capsinolol on the capsaicin receptor and $CGRP_{8-37}$ competes with released CGRP on the myocyte receptor. The fact that $CGRP_{8-37}$ is less potent than capsazepine may be due to hindered tissue penetration to its receptor sites. Its cardiotonic effect is thus indirectly effected by the release of CGRP

via capsaicin-sensitive sensory neurotransmission. The results in the present study also indicate that capsinolol, as a β-adrenoceptor blocker with capsaicin-like activity, may depress automatic and triggered rhythms not only through depressant effects on the β-adrenoceptor but also by a capsaicin-like antiarrhythmic effect, as described by D'Alonzo *et al.* (1995). Further studies on the antiarrhythmic effect and direct depressant electrophysiological effects of our non-pungent capsinolol that are unrelated to its positive inotropic and chronotropic effects on the guinea-pig atrial tissue, caused by the release of CGRP from sensory C-fibres, need to be done.

The effects of other guaiaoxypropanolamines, which also act as β-adrenoceptor blockers but show different responses, have been studied recently in our laboratory (Wu *et al.*, 1994a,b; 1996; Lin *et al.*, 1996).

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