PHARMACOLOGY OF dl-5-METHYL-8-(2-HYDROXY-3-t-BUTYLAMINOPROPOXY) COUMARIN HYDROCHLORIDE (CS-359), A NEW β -ADRENERGIC BLOCKING AGENT

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Abstract— β -Adrenergic blocking activity and other pharmacological properties of CS-359, 5-methyl-8-(2-hydroxy-3-t-butylaminopropoxy) coumarin were compared with those of propranolol. CS-359 inhibited the positive chronotropic and the smooth muscle relaxing responses to isoproterenol. The cardiac effect of stellate ganglion stimulation in anesthetized dogs was also abolished. CS-359 given orally to conscious dogs inhibited the positive chronotropic response to isoproterenol given i.v. The β -adrenergic blocking activity of this compound was 2 to 3 times more potent than that of propranolol, both of which were devoid of any intrinsic stimulant activity. Aconitine- and ouabain-induced arrhythmias in anesthetized dogs were protected by CS-359. As to local anesthetic action on frog sciatic nerve and guinea-pig cornea, CS-359 had one-tenth the activity of propranolol. Concerning percent reduction of the maximum rate of rise of action potential recorded intracellularly from the ventricular muscle fiber, CS-359 was 3 times less potent than propranolol. It reduced heart rate but had only minor effects on the blood pressure and respiration in anesthetized dogs. These data suggest that CS-359 is a more specific β -adrenergic blocking agent with a greater potency and a less cardiac depression than propranolol.

Since classification of adrenoceptors into α - and β -types by Ahlquist (1), first proof in support of this classification was the observation of the ability of dichloroisoproterenol (DCI) to block selectively β -adrenoceptors (2). Thereafter, numerous other β -adrenoceptor antagonists have been reported, including methoxamine (3), pronethalol (4), MJ-1999 (5), propranolol (6), butoxamine (7), H56/28 (8), LB-46 (9) and ICI-50172 (10). Propranolol is the first compound to be tested successfully regarding effectiveness particularly for the treatment of angina pectoris, various cardiac arrhythmias, phaeochromocytoma and hypertension. Its strong myocardial depression is however, considered to be an unfavorable effect and presumably does not relate to the β -adrenergic blocking activity (11-13).

The present paper describes the pharmacology of CS-359: dl-5-methyl-8-(2-hydroxy-3-t-butylaminopropoxy) coumarin hydrochloride ($C_{17}H_{23}NO_4 \cdot HCl$, M.W. 341.83) as compared to propranolol. CS-359 was selected as the most promising of a series of coumarin derivatives (14). This agent differs structurally from propranolol in that the naphthalene

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ring and the N-isopropyl group of propranolol are replaced by a coumarin ring and a N-tertiary-butyl group respectively. Results obtained in this study show this compound to be a β -adrenoceptor antagonist of a considerably higher potency but relatively weak in cardiac depression in comparison with propranolol. The chemical structure of CS-359 is given in Fig. 1.

FIG. 1. Chemical structure of CS-359.

MATERIALS AND METHODS

1. β -Adrenergic blocking effects

1-1. Anesthetized dogs

1-1-1. Chronotropic response of anesthetized dogs

Adult mongrel dogs of both sexes, weighing 7 to 14 kg were anesthetized with sodium pentobarbital 30 mg/kg i.v. The trachea was cannulated for artificial respiration, 10 ml/kg of tidal volume at a rate of 20 per min, and both vagi were severed. Blood pressure at the femoral artery was measured by a pressure transducer (Nihon Kohden, LPU-0.5) and heart rate by a cardi tachometer (Nihon Kohden, RT-2) triggered by the pulse wave or the R wave of ECG (ABL-1). Blood pressure and heart rate were simultaneously recorded on an ink-writing oscillograph (Nihon Kohden, WI-380). Drug solution dissolved in saline was given i.v. into the femoral vein through polyethylene tubing. Isoproterenol was used as an agonist and percent inhibition by the antagonists was measured regarding the positive chronotropic response to isoproterenol.

1-1-2. Vascular response of anesthetized dogs

Femoral blood flow in both sides was measured by an electromagnetic flowmeter, noncannulating type (Nihon Kohden, MF-25). For drug administration, a fine vinyl tubing was indwelt in the artery proximal to the flow meter and fixed there by use of bio-adhesive (Alon Alpha A). Drug solution was injected in a volume of 0.1 ml in 10 sec. The mean systemic blood pressure was monitored at the carotid artery by a pressure transducer (Nihon Kohden, LPU-0.5) for the purpose of maintaining the blood pressure at the same level throughout the experiment by transfusion of fresh blood from time to time. Isoproterenol was administered as an agonist and 0.01 μ g/kg was found sufficient for detecting antagonism of β -adrenergic blocking agents without having any effect on systemic blood pressure. Vascular responses to isoproterenol which had been injected at 2 min and thereafter at 10 min intervals after administration of β -adrenergic blocking agent were obtained, and expressed in terms of a percent decrease to the control.

1-1-3. Resistance to inflation of the lung of anesthetized dogs

The method devised by Konzett and Rössler (15) was modified in experiments as fol-

FIG. 2. Schematic diagram showing experimental setup for measuring resistance to inflation of the lung of anesthetized dogs.

lows. The animals were anesthetized with sodium pentobarbital 30 mg/kg i.v. and both vagi were severed. The spontaneous respiratory movement was completely prevented with gallamine, after which the animals were artificially ventilated with air of a constant volume by respiration pump (Natsume, KN-50). The tidal volume was 200 ml at a rate of 20 per min, which overflowed through the side tube of the tracheal cannula against 10 cm hydrostatic pressure. The volume of overflowed air was measured by a pneumotachograph (Nihon Kohden, MFP-1T) in the ventilatory circuit as shown in Fig. 2. Blood pressure and heart rate as well as the volume of overflowed air were simultaneously recorded on an ink-writing oscillograph (Nihon Kohden, WI-260). Drugs were injected into the femoral vein. Increase in the overflowed air indicates the resistance increase in the respiratory circuit and any bronchospasm induced by 0.1 to 1.0 $\mu\text{g/kg}$ of acetylcholine is reflected in the change in the air way resistance, which is antagonized by the prophylactic isoproterenol.

1-2. Unanesthetized dogs

Adult beagle dogs of both sexes, weighing 8 to 10 kg were trained for the unstrained experiment. A vinyl tubing was chronically indwelt into the ear vein for administration of the agonist, l-isoproterenol. The tubing was flushed with saline containing heparin of 20 units/ml every day. Fasted animals were used. Fresh solution of l-isoproterenol containing 0.2 $\mu\text{g/kg}$ in 0.1 ml was prepared from a stock solution just prior to the experiment. Gelatin capsules containing 0.03, 0.1, 0.3 and 1.0 mg/kg of CS-359 or propranolol respectively mixed with starch as a vehicle were given orally. For control experiment, capsules of 2 mg/kg of starch were given. Heart rate was calculated from ECG

(ABL-I) recorded by biopotential electrodes. Each animal was challenged with three control injections of isoproterenol followed by 0.5 ml flush of heparin-treated saline. After CS-359 or propranolol the animal was subsequently challenged by isoproterenol at 30 min intervals for the first 3 hr and 1 hr intervals for the next 3 hr. Percent inhibition of the positive chronotropic response was calculated.

2. *Intrinsic stimulant activity*

Adult mongrel dogs of both sexes, weighing 6 to 12 kg were treated with a daily dose of reserpine 0.5 mg/kg for 2 days, and another 1.5 mg/kg i.v. of guanethidine at 1.5 hr before the experiment. The animals were anesthetized with sodium pentobarbital 20 mg/kg i.v. The trachea was cannulated for artificial respiration and both vagi were severed. CS-359 or propranolol was given in cumulative doses in a sequence of 0.1, 0.3, 1.0, 3.0, 5.0 and 10.0 mg/kg i.v. Blood pressure and heart rate were recorded as previously described. Effects of the β -adrenergic blocking agents on heart rate were studied in the above reserpinized dogs.

3. *Effects on the positive chronotropic response to sympathetic nerve stimulation*

The right stellate ganglion of vagotomized dogs was exposed at the first intercostal space. The preganglionic fibers of the stellate ganglion were ligated. The postganglionic nerve was stimulated intermittently by bipolar platinum electrodes for periods of 10 sec with square-wave impulses of supramaximal voltage, 1 msec duration and 10 cps. Reproducible tachycardias were recorded. The stimulation was applied before, 3 min after i.v. administration of CS-359 or propranolol and thereafter at 20 min intervals for 2 hr. Blood pressure and heart rate were recorded as previously described. Three experiments were performed with each antagonist.

4. *Antiarrhythmic activity*

Antiarrhythmic activities of CS-359 and propranolol were compared with those of ajmaline, quinidine and procainamide in dogs anesthetized with sodium pentobarbital 30 mg/kg i.v. Arrhythmia was induced as follows: (1) Aconitine-induced atrial arrhythmia was produced by the cup method (16). Test drugs were administered by i.v. infusion at a constant rate to block the arrhythmia within a period of 10 to 30 min. (2) Ouabain-induced ventricular arrhythmia was produced by the modified method of Lucchesi and Hardman (17). A total amount of 10 mg/kg of the test drugs was infused at a constant speed during a period of 20 min. The minimum effective dose was determined when the sinus rhythm was recovered and sinus bradycardia was affirmed during right vagal stimulation (18).

5. *Quinidine-like activity*

So-called quinidine-like activity, reduction in the maximum rate of rise of the intracellular action potential was compared among CS-359, propranolol and quinidine using the isolated canine right ventricular muscle (19). The preparation was fixed on a paraffin block in the bath containing 35 ml of Tyrode's solution, maintained at 37°C and circulated by bubbled gases (95% oxygen and 5% carbon dioxide). The driving electrodes used were a pair of Ag-AgCl wires inserted into the preparation at its edge. The resistances

of the recording glass microelectrodes filled with 3 M KCl ranged from 5 to 15 megohms.

6. Local anesthetic activity

Local anesthetic activity was compared among CS-359, propranolol and local anesthetics, procaine and lidocaine as follows: (1) Frog sciatic nerves were stripped of their sheaths under magnification and were placed in a chamber with three compartments at room temp. They were stimulated at the one end, and monophasical action potentials were recorded from the other. The segment of nerve in the central compartment was bathed in frog Ringer's solution which contained various concentrations of CS-359 or other drugs (The composition of solution was as follows: NaCl 6.7, KCl 0.2, CaCl_2 0.2, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 0.13, Na_2HPO_4 0.3 g/l at pH 7.12–7.16). The height of the fastest wave of action potential was measured before and after exposure to each concentration of the drug for 30 min. (2) Local anesthesia of the guinea-pig cornea was determined according to the method of Chance and Lobstein (20). The concentration of any drug required to cause 50% reduction in spike amplitude of action potential of the sciatic nerve or control response to the cornea stimulation was calculated according to the method of Litchfield and Wilcoxon (21).

7. Effects on the cardiovascular system in anesthetized dogs

Adult mongrel dogs of both sexes, weighing 8 to 13 kg were anesthetized with sodium pentobarbital 30 mg/kg i.v. Blood pressure at the femoral artery was measured by a pressure transducer (Nihon Kohden, LPU-0.5). Heart rate was measured by a cardi tachometer (Nihon Kohden, RT-2) triggered by the pulse wave. Respiratory movement was measured by a pneumotachograph (Nihon Kohden, MFP-1T) connected to an endotracheal tube. All parameters mentioned above were recorded on an ink-writing oscillograph (Nihon Kohden, WI-380). ECG was recorded by an electrocardiograph (Nihon Kohden, Cardiofax MC-3). CS-359 or propranolol was infused at a rate of 0.2 mg/kg/min for 60 min (total 12 mg/kg) through a cannula in the femoral vein.

8. Acute toxicity

The LD50 was determined in 6 groups of 10 aggregated mice, weighing 20 ± 2 g, 7 days after i.v. or p.o. administration. The LD50 and confidence limits were calculated according to the method of Litchfield and Wilcoxon (21).

9. Drugs

The following drugs were used: CS-359 (*dl*-5-methyl-8-(2-hydroxy-3-*t*-butylamino-propoxy) coumarin hydrochloride), propranolol (propranolol hydrochloride, prepared in our laboratories), isoproterenol (*dl*-isoproterenol sulfate, C.H. Boehringer Sohn), *l*-isoproterenol (*l*-isoproterenol hydrochloride, Nikken Chemicals), reserpine (Ciba), gallamine (Teikoku Chemicals), acetylcholine (Daiichi Seiyaku), aconitine (Nakarai Chemicals), ouabain (Tokyo Kasei), ajmaline (Hitachi Chemicals), quinidine sulfate (Tokyo Kasei), procainamide hydrochloride (Daiichi Seiyaku), procaine hydrochloride and lidocaine hydrochloride. All drugs were dissolved in physiological saline in the desired concentrations. Doses of drugs refer to the salt listed above.

RESULTS

1. β -Adrenergic blocking effects

1-1. Anesthetized dogs

1-1-1. Effects of CS-359 and propranolol on the positive chronotropic response to isoproterenol in anesthetized dogs (Figs. 3-5)

Dose-response curve of isoproterenol was first determined, where the responses were as a percentage of the maximum response to isoproterenol. Dose-response curves of isoproterenol were shifted to the right paralleling the various doses of CS-359 and propranolol as shown in Fig. 3. Each circle with a vertical bar represents the mean of five animals and the standard error. Similar relations among dose-response curves were observed for prevention of depressor responses to isoproterenol. CS-359 was approx. 3 times more potent than propranolol.

FIG. 3. Displacement of dose-response curves of isoproterenol by treatment with i.v. cumulative doses (mg/kg) of CS-359 (A) and propranolol (B), which is indicated by the number on the curve. Each circle with a vertical bar represents the mean $\pm$ S.E. of 5 cases.

Percentage inhibition of tachycardia induced by 0.3 $\mu\text{g}/\text{kg}$ of isoproterenol was determined with a single dose of each β -adrenergic blocking agent from 3 to 100 $\mu\text{g}/\text{kg}$. Mean value and standard error for five animals at each dose level of each antagonist are shown in Fig. 4. The ED_{50} of CS-359 and propranolol for inhibition of tachycardia induced by this dose of isoproterenol were 12.5 and 26.5 $\mu\text{g}/\text{kg}$ respectively. CS-359 reduced the heart rate by approx. 15% with 100 $\mu\text{g}/\text{kg}$. Responses to isoproterenol recovered 2 hr after 100 $\mu\text{g}/\text{kg}$ i.v. of both CS-359 and propranolol as shown in Fig. 5.

FIG. 4. Dose-response curves for β -adrenergic blocking activity of CS-359 and propranolol on the positive chronotropic response to 0.3 $\mu\text{g}/\text{kg}$ of isoproterenol. Ordinate is the degree of blockade (%). Abscissa is the dose of β -adrenergic blocking agent in logarithmic scale. Each circle with vertical bar represents the mean $\pm$ S.E. of 5 cases.

FIG. 5. Blocking effect of CS-359 on the positive chronotropic response induced by isoproterenol (A) and electrical stimulation of the right stellate ganglion (B).

1-1-2. *Effects of CS-359 and propranolol in the femoral vascular bed* (Figs. 6-8)

An intra-arterial injection of propranolol in a dose range from 0.3 to 10 $\mu\text{g}/\text{kg}$ caused a transient but dose-dependent increase in the blood flow, while the vasodilator response to CS-359 was very slight. The maximum inhibitory effect on isoproterenol-induced vasodilator response was observed at 2 min as shown in Fig. 7. Dose-response curves of inhibitory effects at 2 min after administration of β -adrenergic blocking agents were compared in Fig. 8, which showed a 2.2 times higher potency of CS-359 than propranolol. The inhibitory effect of 3 $\mu\text{g}/\text{kg}$ of CS-359 persisted for about 40 min, this being generally proportionate to the degree of maximum inhibitory effect.

FIG. 6. Direct action of CS-359 and propranolol and the β -adrenergic blocking effect on vasodilator response induced by isoproterenol (0.01 $\mu\text{g}/\text{kg}$) in the canine femoral vascular bed.

FIG. 7. Time-effect curves for β -adrenergic blocking activity of CS-359 and propranolol (3 $\mu\text{g}/\text{kg}$) on the vasodilator response induced by isoproterenol (0.01 $\mu\text{g}/\text{kg}$) in the canine femoral vascular bed. Ordinate is degree of blockade (%). Abscissa is time after i.a. administration of β -adrenergic blocking agent. Each circle with a vertical bar represents the mean $\pm$ S.E. of 5 cases.

FIG. 8. Dose-response curves for β -adrenergic blocking activity of CS-359 and propranolol on the vasodilator response induced by isoproterenol ($0.01 \mu\text{g/kg}$) in the canine femoral vascular bed. Ordinate is the degree of blockade (%). Abscissa is the dose of β -adrenergic blocking agent in logarithmic scale. Each circle with a vertical bar represents the mean $\pm$ S.E. of 5 cases.

I-1-3. Effects of CS-359 and propranolol on the resistance to lung inflation (Fig. 9)

The bronchospasm induced by acetylcholine was slightly potentiated after treatment with either CS-359 or propranolol in a dose range from 3 to $100 \mu\text{g/kg}$, which can pro-

FIG. 9. Antagonistic effect of isoproterenol on acetylcholine-induced bronchoconstriction before and after CS-359 and propranolol in anesthetized dogs. Overflow represents the amount of air expired against 10 cm hydrostatic pressure.

bably be ascribed to the reduction of sympathetic reflex by systemic administration of acetylcholine. The bronchospasm induced by $3 \mu\text{g}/\text{kg}$ of acetylcholine was inhibited with $3 \mu\text{g}/\text{kg}$ of isoproterenol as shown in Fig. 9. Doses of CS-359 and propranolol required to suppress the response to isoproterenol by 50% were 28.1 ($n=5$) and 40.1 $\mu\text{g}/\text{kg}$ ($n=5$) respectively. According to the parallel line assay (22), however, there was not any significant difference between these two drugs.

1-2. Unanesthetized dogs (Fig. 10)

The resting heart rate of the five dogs ranged from 80 to 100 beats/min with a phasic sinus arrhythmia associated with respiration. The effect of *l*-isoproterenol on the heart

FIG. 10. Time-effect curves for β -adrenergic blocking activity of CS-359 and propranolol given orally on the positive chronotropic response to 0.2 $\mu\text{g}/\text{kg}$ of *l*-isoproterenol in unanesthetized dogs. Ordinate is degree of blockade (%). Abscissa is time after oral administration of β -adrenergic blocking agent. Each circle with a vertical bar represents the mean $\pm$ S.E. of 5 cases (A). Dose-response curves for β -adrenergic blocking activity of CS-359 and propranolol 1 hr after administration. Ordinate is the degree of blockade (%). Abscissa is the dose of β -adrenergic blocking agent. Each circle with a vertical bar represents the mean $\pm$ S.E. of 5 cases (B).

rate presented a linear relationship with the log dose scale in abscissa. Increase in the heart rate by approx. 100 beats/min was repeatedly observed with the particular dose of 0.2 $\mu\text{g/kg}$ among doses from 0.006 to 0.6 $\mu\text{g/kg}$ given at 30 min intervals. Time-effect curves for CS-359 and propranolol are shown in Fig. 10 (A). The maximum inhibition appeared 30 min to 1.5 hr after p.o. administration of CS-359 and propranolol. Onset time of the effect of CS-359 was slightly earlier than that of propranolol. Dose-response curves for β -adrenergic blocking activity 1 hr after administration of the β -adrenergic blocking agents are shown in Fig. 10 (B), where CS-359 was 2.3 times more potent than propranolol.

2. Assessment of intrinsic stimulant activity

The average heart rate of reserpinized dogs was 94 ± 5.0 ($n=10$). Cumulative doses of CS-359 and propranolol were given i.v. in reserpine-treated, vagotomized dogs. In a dose range from 0.01 to 10.0 mg/kg, the heart rate was not significantly modified with CS-359 and propranolol.

3. Effects of CS-359 and propranolol on the positive chronotropic response produced by electrical stimulation of the right stellate ganglion (Fig. 5)

A typical record of tachycardia produced by electrical stimulation of the right stellate ganglion is shown in Fig. 5. Systemic blood pressure was slightly elevated. CS-359 antagonized tachycardia caused by endogenous catecholamine in a way similar to the i.v. administration of isoproterenol.

4. Antiarrhythmic effects on aconitine-induced atrial and ouabain-induced ventricular arrhythmias (Tables 1 and 2)

For inducing atrial arrhythmia with aconitine, the cup method was used which provided a sustained atrial arrhythmia of a relatively uniform severity for several hours without any significant failure in cardiac function. The total amount of ouabain for inducing a sustained ventricular tachycardia was 60 to 80 $\mu\text{g/kg}$. When ventricular tachycardia ensued, supramaximal stimulation of the right vagal nerve did not in any way affect the ventricular rhythm. Concerning the antiarrhythmic effect on aconitine-induced atrial

TABLE 1. Antiarrhythmic effects on aconitine-induced atrial arrhythmias.

Drug	No. of effective cases ^a	Effective dose (mg/kg)	Duration ^b (min)
	No. of experiments		
CS-359	5/7	6.2 ± 1.4 ($n=5$)	7 ± 3
Propranolol	6/6	0.24 ± 0.05 ($n=6$)	40 ± 9
Ajmaline	8/8	3.2 ± 0.3 ($n=8$)	17 ± 3
Quinidine	5/6	15.3 ± 1.7 ($n=5$)	9 ± 3
Procainamide	3/10	48.0 ($n=3$)	8

^aDrugs were infused until the following three effects were attained; 1) complete recovery of A-V conduction, 2) reduction of heart rate under 200 beats per min, 3) disappearance of sinus arrhythmia. Each value represents the mean $\pm$ S.E. of dose for full protection or duration of full protection.

TABLE 2. Antiarrhythmic effects on ouabain-induced ventricular arrhythmias.

Drug	No. of effective cases	Effective dose ^a (mg/kg)	Duration ^b (min)
	No. of experiments		
CS-359	5/5	5.0±1.4 (n=5)	20 (n=1) >90 (n=4)
Propranolol	5/5	3.4±0.8 (n=5)	>90 (n=5)
Ajmaline	5/7	4.5±1.4 (n=5)	>90 (n=5)
Quinidine	5/5	13.4±2.0 (n=5)	9 (n=3) >90 (n=2)

^aMinimum amount of drugs infused until the following three effects were attained ; 1) reversion to normal sinus rhythm or A-V nodal rhythm, 2) regularity of R-R interval and pulse wave, 3) failure of right vagal stimulation to induce repetitive ventricular ectopic automaticity. ^bAverage duration of complete protection after i.v. injection of 10 mg/kg of test drugs.

arrhythmia, CS-359 was significantly less potent than propranolol but more potent than quinidine and procainamide. For suppressing ouabain-induced arrhythmia, CS-359 showed nearly the same potency as propranolol and ajmaline, but was twice as potent as quinidine. The antiarrhythmic dose of CS-359 was almost the same on both aconitine-induced atrial and ouabain-induced ventricular arrhythmias. Heart rate, systolic and diastolic blood pressure were not significantly affected by the antiarrhythmic dose of CS-359, while that of propranolol caused a decrease in the heart rate. Results are summarized in Tables 1 and 2.

FIG. 11. Quinidine-like activity assessed on the electrically-driven isolated canine ventricular muscle. Ordinate is the percent change in maximum rate of rise of action potential (%). Abscissa is the concentration of the drug in logarithmic scale. Each circle with a vertical bar represents the mean±S.E. of 8 cases.

5. Quinidine-like activity (Fig. 11)

Retardation of the maximum rate of rise of intracellular action potential was recorded with CS-359, propranolol and quinidine by use of the canine right ventricular myocardium. The myocardium was electrically driven at 1 cps. The control value of the maximum rate of rise was 183.5 ± 11.8 V/sec ($n=22$). Dose-response curves are shown in Fig. 11, CS-359 was approx. 3 times less potent than propranolol and equal to quinidine.

6. Local anesthetic activity (Table 3)

Local anesthetic activities of CS-359, propranolol and other local anesthetics were determined by the two methods. The results are summarized in Table 3. The local anesthetic activity was dose-dependently increased. The drug concentration required to produce 50% reduction in the amplitude of evoked action potential or response to mechanical corneal stimulation and relative potency is shown in Table 3. Propranolol was the most effective, i.e., 10 times more potent than CS-359, and 4 to 8 times more potent than procaine.

TABLE 3. Local anesthetic activity.

Drug	Isolated frog sciatic nerve			Guinea-pig cornea		
	n ^a	ED50 (g/ml)	Potency ^b	n	ED50 (g/ml)	Potency
CS-359	12	9.5×10^{-4} (6.3-15)	0.08	5	1.8×10^{-2} (1.03- 3.15)	0.1
Propranolol	10	7.5×10^{-5} (4.2-13)	1	5	1.8×10^{-3} (1.0 - 3.2)	1
Procaine	8	5.7×10^{-4} (3.3-9.8)	0.13	5	6.8×10^{-3} (3.9 -11.9)	0.26
Lidocaine				5	4.3×10^{-3} (2.4 - 7.7)	0.42

^aNumber of experiments which were performed at each drug concentration.

^bRelative potency (propranolol : 1). ^cValues in parentheses represent 95% confidence limits.

7. Effects of CS-359 and propranolol on the cardiovascular system in anesthetized dogs (Fig. 12)

CS-359 and propranolol were infused continuously into the femoral vein at a rate of 0.2 mg/kg/min for 60 min, to a total of 12 mg/kg, of each drug for five animals. The systemic blood pressure, heart rate, respiratory movement and ECG were recorded. Both CS-359 and propranolol reduced the systemic blood pressure and heart rate but increased the respiratory rate and PQ interval of ECG. Time-effect curves for the blood pressure and heart rate are shown in Fig. 12. In the case of propranolol, two animals died due to cardiovascular collapse at approx. 35 min (7 mg/kg). Lethal dose of CS-359 determined by continuous infusion at the same rate was 33 mg/kg ($n=5$). It is concluded that the cardiovascular depressive effect of CS-359 is less marked than that of propranolol.

8. Acute toxicity to mice (Table 4)

Both CS-359 and propranolol given i.v. produced convulsions with marked respiratory depression at lethal doses. As shown in Table 4, acute toxicity of CS-359 to mice was slightly lower than that of propranolol.

FIG. 12. Graphs showing arterial blood pressure (A) and heart rate (B) as a percentage of control value after i.v. infusion of CS-359 and propranolol in anesthetized dogs. Ordinate is percent change (control : 100%). Abscissa is increasing dose of β -adrenergic blocking agent (infusion rate : 0.2 mg/kg/min). Each circle with a vertical bar represents the mean $\pm$ S.E. of 5 cases. Note, 2 out of 5 cases died at 7 mg/kg of propranolol (†).

TABLE 4. Acute toxicity in mice.

Strain	Route of administration	Sex	LD50 (mg/kg)	
			CS-359	Propranolol
RFVL	i.v.	male	33.1 (30.1-36.4)	25.6 (24.2-29.1)
		female	31.6 (29.5-33.8)	23.9 (22.3-25.6)
	p.o.	male	676 (604-757)	550 (470-644)
		female	692 (563-851)	525 (465-593)
ddy	i.v.	male	45.5 (42.5-48.7)	28.0 (24.1-32.5)

^aValues in parentheses represent 95% confidence limits.

DISCUSSION

β -Adrenergic blocking activity and other pharmacological properties of CS-359, 5-methyl-8-(2-hydroxy-3-t-butylaminopropoxy) coumarin hydrochloride were examined in the present study. β -Adrenergic blocking activity, especially competitive antagonism, specificity and organ selectivity, and other pharmacodynamic activities such as intrinsic adrenergic stimulant activity, local anesthetic, quinidine-like and antiarrhythmic effects, and oral route efficacy were compared with propranolol (23-25).

When CS-359 was administered i.v., intra-arterially or orally, a dose-dependent antagonism was obtained not only on the cardiac stimulation induced by either isoproterenol or stimulation of stellate ganglion but also on the relaxation of vascular and bronchial smooth muscles induced by isoproterenol, which indicates that CS-359 blocks the typical responses to β -receptor stimulation. No special selectivity for a particular population of β -receptors (26) was observed by use of either CS-359 or propranolol as these compounds blocked equally all these β -responses. In contrast to these compounds, practolol (ICI-50172) is cardioselective (27) and butoxamine blocks β_2 -receptors (28).

Over a wide range of doses, CS-359 shifted the dose-response curves of positive chronotropic and hypotensive responses to isoproterenol, to the right. The data suggest that the antagonism is of a competitive nature. CS-359 is 2 to 3 times more potent than propranolol in various organs studied; heart, vascular and bronchial smooth muscles. Furthermore, there is no qualitative difference between CS-359 and propranolol in the mode of antagonism and organ selectivity. Previously (14), the authors confirmed that the β -adrenergic blocking activity of CS-359 was confined to the levorotatory form similar to other β -adrenergic blocking agents.

In these experiments, neither CS-359 nor propranolol within a wide dose range employed produced increase in heart rate in reserpinized dogs. CS-359 was devoid of intrinsic stimulant activity as was propranolol (29), while this property is observed using DCI, pronethalol, alprenolol (H56/28), pindolol (LB-46) and practolol (ICI-50172) (30). Opinions differ widely as to the clinical importance of the intrinsic stimulant property and this remains to be studied (8, 24, 31).

At a higher dose level which is far greater than the β -adrenergic blocking dose, CS-359 had also antiarrhythmic activity in dogs in aconitine-induced atrial and ouabain-induced ventricular arrhythmias. CS-359 was similarly effective to ouabain-induced arrhythmia with propranolol but much less potent than the latter against aconitine-induced arrhythmia. β -Adrenergic blocking activity did not parallel the antiarrhythmic potency. In a previous report, the authors stated that a relationship was not found between β -adrenergic blocking activities and antiarrhythmic activities against these arrhythmias among several β -adrenergic blocking agents (16, 32). It is expected that any β -adrenergic blocking agent will prevent or abolish arrhythmias caused by exogenously applied sympathomimetic amines or by increased sympathetic activity (33-35). It is less clear however why some of them, but not all, are also effective against aconitine- and ouabain-induced arrhythmias which are assumed to be largely independent of adrenergic mechanisms. Some

β -adrenergic blocking agents including propranolol are potent local anesthetics (36). This property is the result of membrane stabilization, which is thought to be associated with either the direct negative inotropic or quinidine-like action of these drugs (37). Local anesthetic and quinidine-like activities most likely contribute to the mechanisms involved in abolishing arrhythmias which are independent of adrenergic influence (13). As a local anesthetic, CS-359 had one-tenth of the activity of propranolol and half that of procaine, as tested on the frog sciatic nerve and guinea-pig cornea. As to quinidine-like action, by measuring reduction in the maximum rate of rise of action potential of ventricular muscle, CS-359 was 3 times less potent than propranolol and generally equal to quinidine.

CS-359 given i.v. to anesthetized dogs caused bradycardia and mild hypotension. The bradycardia was attributed to the blockade of resting sympathetic tone since CS-359 did not affect the heart rate in dogs pre-treated with reserpine. The fall in blood pressure with CS-359 may be due to decreased cardiac output, as this compound does not produce any change in peripheral resistance.

These data suggest that CS-359 is a more specific β -adrenergic blocking agent with a greater potency and relatively less cardiac depressant properties as compared to propranolol.

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