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# Comparison of the parasympatholytic activity of ACC-9358 and disopyramide

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**1** ACC-9358 (N-[(3,5-di(pyrrolidinylmethyl)-4-hydroxy)benzoyl]aniline) is a newly developed analogue of changrolin, an antiarrhythmic agent used in the Peoples Republic of China. Since changrolin and other antiarrhythmic agents exert parasympatholytic activity which may limit their clinical usefulness, it was of interest to examine the parasympatholytic effects of ACC-9358. For comparative purposes we also studied the parasympatholytic activity of disopyramide.

**2** In guinea-pig isolated ileal strips, disopyramide, 3–30 $\mu$M, and ACC-9358, 100–300 $\mu$M, competitively antagonized carbachol-induced contractions with $pA_2$ values of 5.78 and 4.17, respectively.

**3** In guinea-pig isolated right atria, disopyramide 3–30 $\mu$M, competitively antagonized methacholine-induced slowing of spontaneous beating with a $pA_2$ value of 5.99 whereas ACC-9358, 3–300 $\mu$M, produced no significant muscarinic blockade in this preparation.

**4** Disopyramide (1.9–15 mg kg$^{-1}$, i.v.), but not ACC-9358 (7.5–1.5 mg kg$^{-1}$, i.v.), significantly increased rat pupil diameter *in vivo*.

**5** Disopyramide and ACC-9358 blocked vagal-induced reductions in heart rate in dogs anaesthetized with pentobarbitone. $ED_{50}$ values were approximately 0.65 and 11.25 mg kg$^{-1}$, respectively.

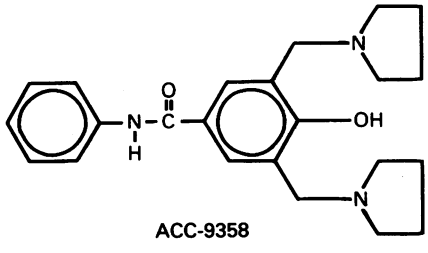
**6** We conclude that ACC-9358 possesses significantly less parasympatholytic activity than disopyramide.

## Introduction

Many antiarrhythmic agents alter the activity of the autonomic nervous system. For some agents this activity is a component of the therapeutic profile of the drug, for example, β-adrenoceptor blockade by propranolol is an important aspect of its mechanism of antiarrhythmic action. However, the autonomic effects of several other antiarrhythmic drugs are undesirable. Noradrenaline release and adrenergic neuronal blockade by bretylium, α-adrenoceptor blockade by quinidine and the anticholinergic activity of quinidine and disopyramide limit the clinical usefulness of these agents (Bigger & Hoffman, 1980).

Changrolin, a novel class I antiarrhythmic agent (Xu & Carmeliet, 1981) used in the Peoples Republic of China (Xu & Qu, 1978: Li *et al.*, 1979), has also been reported to possess parasympatholytic activity *in vivo* (Chen *et al.*, 1979). Stout *et al.* (1983, 1984, 1985) have studied the structure-activity relationships of a series of synthetic analogues of changrolin in an attempt to identify a compound which has antiarrhythmic activity similar to changrolin, but with less parasympatholytic activity than changrolin. From these studies one compound, N-[(3,5-di (pyrrolidinylmethyl)-4-hydroxy)benzoyl]aniline (ACC-9358, Figure 1), has been selected for clinical development (Stout *et al.*, 1985).

The objectives of this study were to examine the parasympatholytic activity of ACC-9358 both *in vitro*




ACC-9358

**Figure 1** Structure of ACC-9358.

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and *in vivo* and compare this activity with that of disopyramide, the antiarrhythmic agent for which anticholinergic activity is most clinically restrictive.

## Methods

### In vitro

*Guinea-pig ileum* Male Hartley guinea-pigs (300–400 g) were killed by a sharp blow to the head. A midline abdominal incision was made to expose the ileocaecal junction. An approximately 7 cm section of ileum was isolated immediately proximal to the ileocaecal junction. The 2–3 cm of ileum nearest the caecum were discarded. The remaining tissue was placed in a physiological saline solution (PSS) and refrigerated overnight. The composition of PSS was (in mmol $l^{-1}$): NaCl 120, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 0.57, $KH_2PO_4$ 1.2, $CaCl_2$ 1.96, dextrose 11.1 and hexamethonium bromide 0.1. The next day each ileal strip was transferred into fresh oxygenated (95% $O_2$, 5% $CO_2$) PSS at room temperature. A 1 cm segment of ileum was mounted on a muscle holder and placed in a 30 ml vertical muscle bath containing oxygenated PSS maintained at 37°C. Developed tension was recorded on a Gould 2800S physiological recorder using a Gould-Statham UC-2 or UC-3 force transducer. Under a resting tension of 1 g, each tissue was allowed to stabilize for 30 min.

Following stabilization, cumulative concentrations of carbachol ($2 \times 10^{-8}$M–$5 \times 10^{-6}$M final concentration) were added to each muscle bath. Each successively higher concentration of carbachol was added when the steady state response to the previous concentration was reached. Each muscle bath was then flushed with fresh PSS and carbachol administration was repeated. The ileal responses to the second series of carbachol administrations were used as pretreatment control values since preliminary experiments indicated that responses to a second and third carbachol dosing series were nearly identical, whereas ileal responses to the first carbachol sequence were often exaggerated. Following the control (second) carbachol dosing series, each muscle bath was flushed with fresh PSS. Vehicle or a single concentration of ACC-9358 or disopyramide was then added and 45–60 min later a third cumulative dosing of carbachol was carried out.

*Guinea-pig right atrium* Male Hartley guinea-pigs (300–500 g) were killed by a sharp blow to the head. The heart was rapidly removed and placed in oxygenated (95% $O_2$, 5% $CO_2$) Krebs-Henseleit (K-H) solution. The composition of the K-H solution was (mmol $l^{-1}$): NaCl 118.4, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, $CaCl_2$ 2.5, dextrose 11.7 and $Na_2EDTA$ 0.03. The right atrium was isolated, mounted on a muscle holder and placed in a 30 ml vertical muscle bath containing K-H solution maintained at 37°C. Under a diastolic tension of 0.5 g, developed tension was recorded on a Gould 2800S physiological recorder with a Gould-Stratham UC-2 or UC-3 force displacement transducer to monitor spontaneous rate.

Following a 60 min equilibration period, during which the atria were washed several times with fresh K-H solution, cumulative concentrations of methacholine ($10^{-7}$M–$10^{-4}$M final concentration) were added to each muscle bath. Each successively higher concentration of methacholine was added when the atrium had reached a steady state response to the previous concentration. After each atrium reached a maximum response (i.e., no spontaneous rate) methacholine was washed out and the atrium was allowed to attain its initial beating frequency. At this time, either vehicle or a single concentration of ACC-9358 or disopyramide was added and 60 min later a second cumulative dosing of methacholine was performed.

### In vivo

*Rat pupil diameter* The pupil diameter of female Sprague Dawley rats (150–220 g) was measured with an American Optical stereo star zoom microscope (at 10 × magnification) equipped with a micrometer disc in one eyepiece which was calibrated for this study. Each rat was hand-held in the supine position with the experimenter's hand resting on the stage of the microscope. The right eye of each animal was illuminated with a constant intensity light from a Model 650 American Optical illuminator fixed to the base of the microscope. Pupil diameter was determined before drug or saline administration and at predetermined times following intravenous injection of a fixed volume (0.01 ml $g^{-1}$ body weight) of isotonic saline, ACC-9358 or disopyramide. Each animal received either saline or a single dose of ACC-9358 or disopyramide.

*Vagally-induced bradycardia* Mongrel dogs (8 to 16 kg, either sex) were anaesthetized with pentobarbitone, 30 mg $kg^{-1}$ i.v., followed by a constant infusion, 4 mg $kg^{-1}$ $h^{-1}$, beginning 60 min after induction of anaesthesia. Dogs were ventilated via a cuffed endotracheal tube with a Harvard Apparatus respirator at 20 cycles $min^{-1}$ and 15 cc $kg^{-1}$ stroke volume. A femoral vein was catheterized for administration of test compound. Electrodes were attached to the limbs for recording lead II of the ECG. Each vagus nerve was isolated in the cervical region and cut between two ties. Platinum hook electrodes were inserted into the distal portion of the right vagus nerve for electrical stimulation to produce a slowing of heart rate. The voltage which caused maximum slowing of heart rate (frequency 1 Hz, pulse duration 2.5 ms) was

determined for each dog and was increased by 20V to provide supramaximal stimulation voltage. The frequency of supramaximal stimulation required to decrease heart rate by approximately 60 beats $min^{-1}$ after 10 s of stimulation was determined for each animal.

The pretreatment control response to vagal stimulation was taken as the average of three responses obtained at 1 min intervals. ACC-9358 or disopyramide were administered as bolus injections of 0.5 mg $kg^{-1}$, i.v., each min; isotonic saline was administered at an equal volume and rate (1 ml $min^{-1}$). Fifty seconds after each bolus injection, vagal stimulation was performed and the heart rate response was recorded on a Beckman R-611 strip chart recorder.

*Drugs*

Carbamylcholine chloride (carbachol) and methacholine chloride were purchased from Sigma Chemical Co. (St. Louis, MO). Disopyramide phosphate (racemic mixture) was a gift from the G.D. Searle Co. (Skokie, IL). Atropine sulphate (injectable) was manufactured by Burroughs Wellcome Co. (Research Triangle Park, NC). ACC-9358 was synthesized as the free base in the Medicinal Chemistry Department of American Critical Care (McGaw Park, IL).

*Data analysis*

Unless otherwise indicated, all data are expressed as the mean ± s.e.mean.

In guinea-pig ileum and right atrium studies, agonist (carbachol or methacholine) $EC_{50}$ values were determined by least squares analysis for each tissue in the presence and absence of test compound (ACC-9358 or disopyramide) or vehicle. Schild analysis (Arunlakshana & Schild, 1959) was performed on the resulting concentration-ratios to determine whether ACC-9358 or disopyramide were competitive antagonists in these models and, if so, their potencies ($pA_2$ values). A two-tailed Student's *t* test for unpaired data was used to compare drug-induced with vehicle-induced mean concentration ratios. A *P* value less than 0.05 indicated a significant difference.

In rat pupil diameter studies, changes from pretreatment control values were determined at 5, 10, 15, 20, 25 and 30 min after drug or saline administration. A one-way repeated measures analysis of variance (ANOVA) model (Winer, 1971) was used to test the hypothesis that mean values were unchanged over time. Calculations were performed using the SAS GLM procedure (SAS Institute, 1982). If there was evidence of a statistically significant time effect, Dunnett's two-sided test (Winer, 1971) was used to deter-

**Table 1** Concentration-ratios, slope ratios and Schild analysis for vehicles, disopyramide and ACC-9358 in the carbachol-stimulated guinea-pig ileum

| *Treatment* | *n* | *Concentration ratio* (X ± s.e.) | *Slope ratio* (X ± s.e.) | *Schild analysis* *Slope (± 95% CI)* | *$pA_2$ (± 95% CI)* |
|---|---|---|---|---|---|
| Vehicles | | | | | |
| $H_2O$[a] | 5 | 1.36 ± 0.13 | 0.99 ± 0.18 | | |
| Low HCl[b] | 5 | 1.29 ± 0.16 | 0.93 ± 0.10 | | |
| High HCl[c] | 4 | 1.35 ± 0.20 | 0.98 ± 0.05 | | |
| *Disopyramide* | | | | | |
| 3 μM | 5 | 2.83 ± 0.44[d] | 0.80 ± 0.17 | | |
| 10 μM | 6 | 8.54 ± 1.65[d] | 0.90 ± 0.13 | 0.99 ± 0.29 | 5.78 ± 0.25 |
| 30 μM | 5 | 18.59 ± 3.84[d] | 0.81 ± 0.07 | | |
| *ACC-9358* | | | | | |
| 3 μM | 6 | 1.22 ± 0.13 | 0.89 ± 0.06 | | |
| 10 μM | 6 | 1.15 ± 0.07 | 0.94 ± 0.09 | 0.40 ± 0.25[e] | |
| 30 μM | 5 | 2.02 ± 0.27[d] | 0.96 ± 0.18 | 0.59 ± 0.19[f] | |
| 100 μM | 5 | 2.41 ± 0.22[d] | 0.98 ± 0.14 | 0.74 ± 0.51[g] | 4.17 ± 0.34[g] |
| 300 μM | 6 | 4.22 ± 0.42[d] | 1.40 ± 0.16 | | |

[a]Vehicle control for all disopyramide concentrations.
[b]Vehicle control for 3, 10, 30 and 100 μM ACC-9358.
[c]Vehicle control for 300 μM ACC-9358.
[d]Significantly different from appropriate vehicle control value or unity ($P < 0.05$).
[e]Calculated using all 5 concentrations.
[f]Calculated using the highest 3 concentrations.
[g]Calculated using the highest 2 concentrations.

mine which mean values obtained at various time-points after drug administration were significantly different from the pretreatment control mean. The mean of the maximum pupil diameter following saline or drug treatment was compared to the pretreatment mean using Student's $t$ test for paired data. The significance level for ANOVA, Dunnett's and Student's tests was set at $P < 0.05$.

In vagally-induced bradycardia studies, heart rate responses to vagal stimulation were determined each minute following commencement of drug or saline administration. Mean responses over time (or cumulative dose of ACC-9358 or disopyramide) were compared to pretreatment control responses using the ANOVA and Dunnett's procedures as described above.

## Results

### *Guinea-pig ileum*

In the concentration range of 0.02 to 2.0 μM, carbachol caused a concentration-dependent increase in tension development in the guinea-pig ileum. In all experiments ($n = 58$) the $ED_{50}$ of carbachol alone was $3.5 \pm 0.2 \times 10^{-7}$M.

The relative antimuscarinic activity of disopyramide and ACC-9358 in the guinea-pig ileum was compared by examining the effects of these agents (and the appropriate vehicles) on the carbachol concentration-response curve. None of the three vehicles used in this study caused a significant shift in the carbachol concentration-response curve (Table 1). Disopyramide, however, caused a concentration-dependent rightward parallel shift in the carbachol concentration-response curve. Schild analysis of this effect indicated that disopyramide was a competitive antagonist of carbachol in this tissue with a $pA_2$ value of 5.78 ± 0.25 (Table 1). At 3 and 10 μM, ACC-9358 had no effect on the carbachol concentration-response curve whereas at 30,100 and 300 μM, ACC-9358 caused a rightward parallel shift in the curve. Schild analysis of these effects indicated that ACC-9358 may be a competitive antagonist of carbachol in the guinea-pig ileum, but only at the two highest concentrations tested (Table 1). There was a large degree of variability in the data at 100 and 300 μM ACC-9358 and the slope of the Schild plot using concentration-ratios determined at these concentrations of ACC-9358 was not

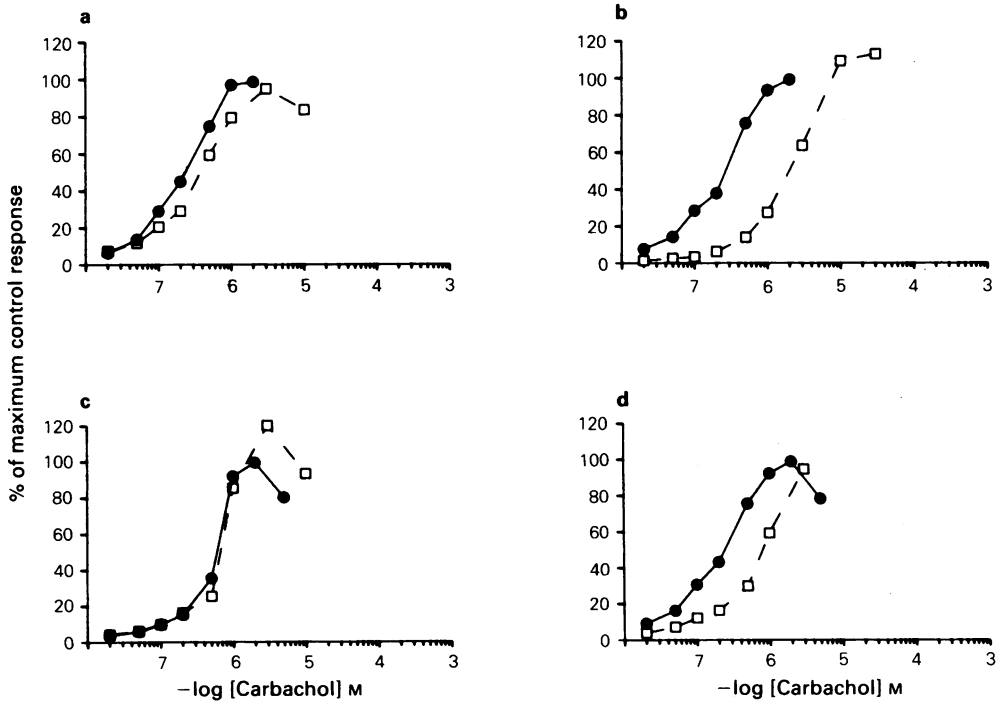


**Figure 2** Concentration-response curves for carbachol in guinea-pig isolated ileum before (●) and after exposure to vehicle (□, a), disopyramide 10 μM (□, b) or ACC-9358 10 μM (□, c) or 300 μM (□, d). Each point represents the mean response to each concentration of carbachol ($n = 5$–6 for each group).

**Table 2** Concentration ratios, slope ratios and Schild analysis for vehicles, disopyramide and ACC-9358 in the methacholine-treated guinea-pig right atrium

| *Treatment* | *n* | *Concentration ratio* (X ± s.e.) | *Slope ratio* (X ± s.e.) | *Schild analysis* Slope (± 95% CI) | $pA_2$ (± 95% CI) |
|---|---|---|---|---|---|
| *Vehicle* | | | | | |
| [a]HCl 0.05 N | 13 | 1.16 ± 0.26 | 1.02 ± 0.05 | | |
| *Disopyramide* | | | | | |
| 3 μM | 5 | 5.48 ± 1.13[b] | 0.96 ± 0.10 | | |
| 10 μM | 3 | 9.23 ± 2.55[b] | 0.88 ± 0.03 | | |
| 30 μM | 7 | 32.06 ± 6.52[b] | 1.02 ± 0.16 | 1.02 ± 0.27 | 5.99 ± 0.27 |
| *ACC-9358* | | | | | |
| 3 μM | 4 | 0.95 ± 0.03 | 0.82 ± 0.11 | | |
| 10 μM | 4 | 1.65 ± 0.65 | 0.97 ± 0.06 | | |
| 30 μM | 6 | 2.37 ± 0.61 | 1.11 ± 0.24 | | |
| 100 μM | 4 | 2.24 ± 0.44 | 0.95 ± 0.02 | | |
| 300 μM | 7 | 2.50 ± 0.82 | 1.10 ± 0.26 | ND | ND |

[a]Vehicle used for all disopyramide and ACC-9358 concentrations.
[b]Significantly different from vehicle control value or unity ($P < 0.05$).
ND = Not determined.

different from unity. Comparison of calculated $pA_2$ values indicated that ACC-9358 was 41 fold less potent than disopyramide.

Carbachol concentration-response curves in the presence or absence of vehicle (water), 10 μM disopyramide or 10 or 300 μM ACC-9358 are shown in Figure 2. This figure clearly illustrates the marked difference in antimuscarinic activity between equimolar (10 μM) concentrations of disopyramide and ACC-9358 and that, even at a 30 times higher concentration, ACC-9358 shifted the carbachol concentration-response curve to a smaller extent than 10 μM disopyramide.

None of the vehicles or any concentration of disopyramide or ACC-9358 significantly altered baseline tension or the maximum response to carbachol.

*Guinea-pig right atrium*

In the concentration range of 0.1–100 μM, methacholine produced a concentration-dependent slowing of spontaneously beating guinea-pig right atria. In all experiments ($n = 53$) the $ED_{50}$ of methacholine alone was $7.6 \pm 1.6 \times 10^{-6}$M.

Analysis of the effect of several concentrations of disopyramide or ACC-9358 indicated that 3, 5 and 10 μM disopyramide caused rightward parallel shifts in the methacholine concentration-response curves with a $pA_2$ value of 5.99 ± 0.27, whereas at 3–100 μM, ACC-9358 had no significant effect on the atrial response to methacholine (Table 2). These results indicate that disopyramide, but not ACC-9358, is a competitive muscarinic antagonist in the methacholine-treated guinea-pig right atrium.

Figure 3 illustrates the effects of the vehicle (0.05 N HCl), 3 and 30 μM disopyramide and 100 μM ACC-9358 on the methacholine concentration-response curve in guinea-pig right atria. The vehicle had no effect on either the control spontaneous atrial rate or on the bradycardia induced by methacholine. Although both disopyramide and ACC-9358 slightly reduced control spontaneous atrial rate, only disopyramide caused a rightward shift in the methacholine concentration-response curve (Figure 3).

*Rat pupil diameter*

Saline, ACC-9358 (7.5, 15.0 mg $kg^{-1}$) or disopyramide (1.9, 3.8, 7.5, 15.0 mg $kg^{-1}$) were administered as an intravenous bolus and their effects on rat pupil diameter were determined for 30 min after administration. Mean pretreatment diameters ranged from 0.46 ± 0.02 to 0.52 ± 0.01 mm in the seven experimental groups with no significant differences between groups.

The maximum response to each treatment, illustrated in Figure 4, was reached at 30, 25, 20, 20, 10, 5 and 5 min following administration of saline, 7.5 and 15.0 mg $kg^{-1}$ ACC-9358, 1.9, 3.8, 7.5 and 15.0 mg $kg^{-1}$ disopyramide, respectively. At all doses tested, disopyramide significantly increased pupil diameter compared to pretreatment control values. Conversely, neither saline nor ACC-9358 significantly affected pupil diameter at any time period within 30 min after administration.

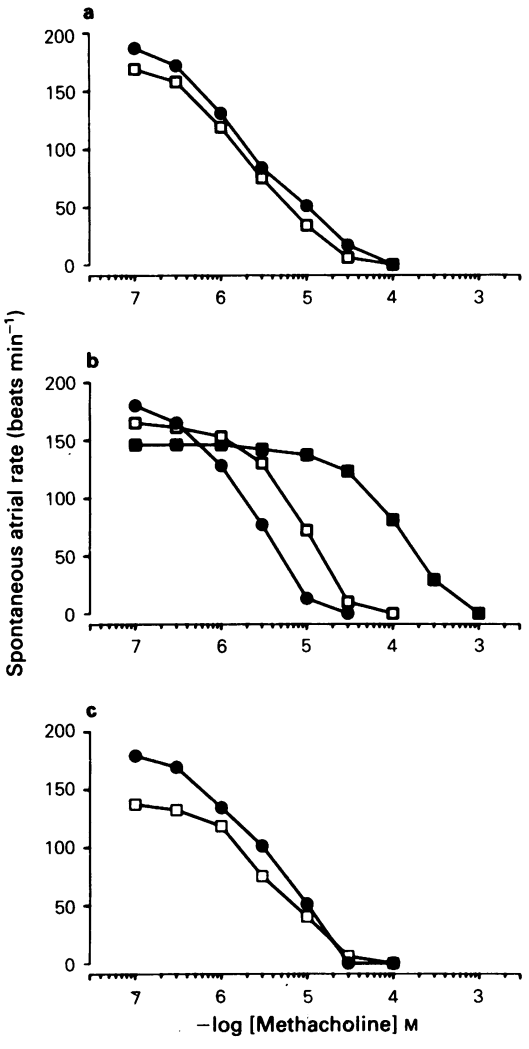


**Figure 3** Concentration-response curves for methacholine in guinea-pig isolated right atria before (●) and after exposure to vehicle (□, a), disopyramide 3 μM (□) and 30 μM (■, b), or ACC-9358 100 μM (□, d). Each point represents the mean response to each concentration of methacholine ($n = 13$ for vehicle, $n = 5-7$ for disopyramide, $n = 7$ for ACC-9358).

To establish further the validity of this procedure for determining anticholinergic activity we also evaluated the effects of atropine in this preparation. Intravenous bolus injections of 12.5, 25.0, 50, or 100 μg kg$^{-1}$ atropine significantly increased pupil diameter within 5–10 min of administration to 1.1, 2.2, 3.2, 4.2 mm, respectively, from pretreatment values of 0.48 ± 0.03 to 0.51 ± 0.02 mm.

*Vagally-induced bradycardia*

Under the conditions of this study, stimulation of the vagus nerve in a pentobarbitone-anaesthetized dog reduced spontaneous heart rate by approximately 60 beats min$^{-1}$. The effect of repeated bolus intravenous injections (each minute) of isotonic saline, ACC-9358 (0.5 mg kg$^{-1}$ min$^{-1}$) or disopyramide (0.5 mg kg$^{-1}$ min$^{-1}$) on the heart rate response to vagal stimulation was determined for a maximum of 30 min of saline or drug administration. Mean pretreatment heart rates were 170 ± 8, 168 ± 14 and 162 ± 9 beats min$^{-1}$ in saline-, disopyramide- and ACC-9358-treated dogs, respectively, with no significant differences between the three experimental groups. Over the time period of these experiments both ACC-9358 and disopyramide lowered spontaneous heart rate to no less than 140 beats min$^{-1}$. At this heart rate a 60 beat min$^{-1}$ decrease in rate could readily be achieved and therefore the direct bradycardiac effects of these agents were not considered likely to account for any of the observed diminution of heart rate responses to vagal stimulation.

The effect of vagal stimulation on spontaneous heart rate before and at various times following continuous administration of saline, ACC-9358 or disopyramide is illustrated in Figure 5. At no time did isotonic saline alter the bradycardiac response to vagal stimulation. Both ACC-9358 and disopyramide, however, significantly attenuated the effects of vagal stimulation. The parasympatholytic effect of disopyramide occurred at an earlier time (and consequently a lower cumulative dose) than did that of ACC-9358. In this regard, disopyramide and ACC-9358 inhibited the vagally-induced bradycardiac effect by 50% at a cumulative dose of approximately 0.65 and 11.25 mg kg$^{-1}$, respectively, thereby indicating that the parasympatholytic activity of disopyramide is approximately 17 fold greater than that of ACC-9358 in this *in vivo* preparation.

## Discussion

It is well known that pharmacological agents can exert parasympatholytic activity via several mechanisms. These include: (1) an antimuscarinic action by competitive or non-competitive blockade of muscarinic receptors located in the postsynaptic membrane of the effector organ, or (2) a vagolytic action through suppression of impulse conduction along efferent vagal nerves, inhibition of acetylcholine synthesis or storage, inhibition of release from pre- or postganglionic nerve terminals, or by blockade of nicotinic (and, to a small extent, muscarinic) receptors in parasympathetic ganglia. In the present study we examined the effects of ACC-9358 and disopyramide

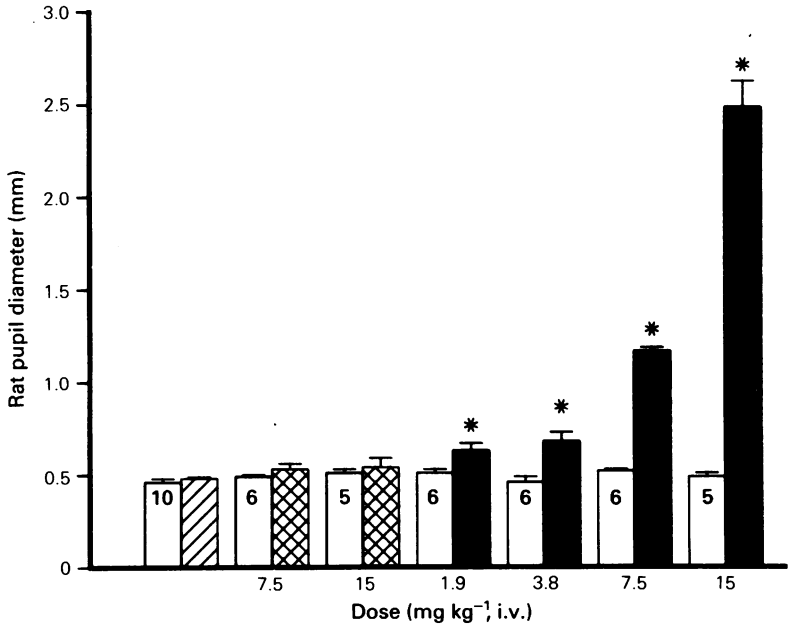


**Figure 4** Effect of saline (hatched column), disopyramide (solid column) or ACC-9358 (cross-hatched column) on rat pupil diameter. Each bar represents the mean of the maximum pupil diameter observed within 30 min of saline or drug administration; s.e.mean shown by vertical lines. ($n$ = number shown in pretreatment column for each group.) *Indicates $P < 0.05$ compared to pretreatment value (open column) using Student's $t$ test for paired data.

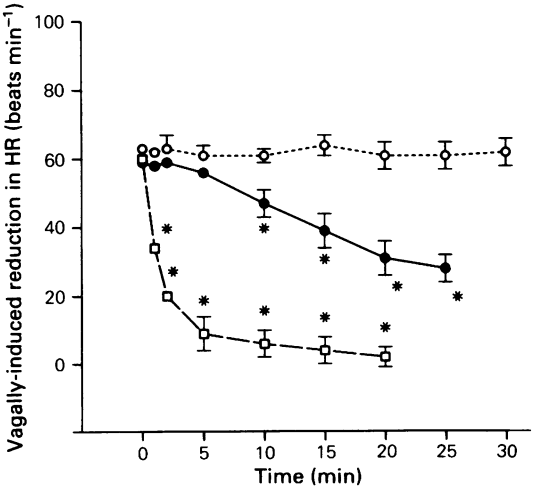


**Figure 5** Effect of saline (○), disopyramide (□) or ACC-9358 (●) on vagally-induced bradycardia in the pentobarbitone-anaesthetized dog. Each point represents the mean decrease in heart rate produced by vagal stimulation ($n = 4-5$ for each group), s.e.mean shown by vertical lines.
*$P < 0.05$ compared to pretreatment value using Dunnett's two-sided test.

under conditions which specifically allowed for the evaluation of antimuscarinic activity (*in vitro* experiments) and under conditions in which an antimuscarinic or vagolytic action could account for an observed parasympatholytic effect (*in vivo* experiments).

With the possible exception of very high concentrations in the guinea-pig ileum, the results of our *in vitro* studies indicated that ACC-9358 was not a competitive antagonist of exogenously-administered muscarinic agonists in either the ileum or right atrium. Conversely, disopyramide clearly was a competitive muscarinic antagonist in both the ileum and right atrium with a $pA_2$ value of 5.78 and 5.99, respectively, in these tissues. A $pA_2$ value of 5.78 for racemic disopyramide in intact ileal strips compares well with values previously reported for the R and S isomers of disopyramide in isolated longitudinal muscle strip preparations from guinea-pig ileum (Giacomini *et al.*, 1980). These investigators reported a $pA_2$ of 5.74 and 6.25 for the R and S isomers, respectively. Furthermore, a $pA_2$ of 5.99 for racemic disopyramide in guinea-pig isolated right atria also agrees well with the inhibition constant ($K_i$) of $8.5 \times 10^{-7}$M (analogous to a $pA_2$ of 6.07) for racemic disopyramide derived from tritiated quinuclidinyl benzilate ([$^3$H]-QNB) displacement studies with crude homogenates of guinea-

pig right atria (Mirro *et al.*, 1980). Thus, under conditions in which disopyramide exerted antimuscarinic activity virtually identical to that previously reported, ACC-9358 exerted little, if any, antimuscarinic activity.

In rat pupil diameter studies, doses of ACC-9358 up to 15 mg $kg^{-1}$ had no mydriatic effects. Conversely, disopyramide in doses as low as 1.9 mg $kg^{-1}$ produced a significant mydriatic effect. These results indicate that, in the rat, ACC-9358 had neither antimuscarinic nor vagolytic activity. However, since pupil diameter is modulated by both parasympathetic and sympathetic activity, it is theoretically possible that ACC-9358 exerted either (1) α-adrenergic *and* muscarinic-cholinergic antagonism, or (2) suppression of both parasympathetic and sympathetic nerve activity, thereby resulting in no net change in pupil diameter. The former possibility is unlikely since available evidence indicates that ACC-9358 is devoid of α-antagonist properties (unpublished observation) and the present studies indicate little, if any, muscarinic antagonist properties.

The possibility that ACC-9358 suppressed both parasympathetic and sympathetic neural activity cannot be ruled out at present. Accordingly, since ACC-9358 lacked appreciable antimuscarinic activity, its inhibition of vagally-induced bradycardia in the dog is probably due to a direct vagolytic action. Supporting this direct depression of vagus nerve activity are preliminary experiments showing that ACC-9358 reduced the hindlimb vasoconstrictor response to stimulation of the lumbar sympathetic chain in the anaesthetized dog (unpublished observation). Thus, in sufficiently high doses, ACC-9358 can be shown to suppress both parasympathetically- and sympathetically-mediated responses. It should be pointed out, however, that the doses required to exert vagolytic ($ED_{50}$ = 11.25 mg $kg^{-1}$) or sympatholytic ($ED_{50}$ = 25.0 mg $kg^{-1}$) activity are well above those which exert antiarrhythmic activity (2–3 mg $kg^{-1}$) in the pentobarbitone-anaesthetized dog (Reynolds *et al.*, 1984). Although the precise mechanism of the vagolytic and sympatholytic activity of ACC-9358 is unknown, it may be due to a local anaesthetic action since ACC-9358 has been shown to be a potent sodium channel blocker in canine isolated Purkinje fibres (Wine & Brown, 1985).

Depending on the tissue studied, ACC-9358 possessed approximately 1/40 the antimuscarinic activity of disopyramide and exhibited vagolytic activity *in vivo* only at very high doses. In light of the fact that ACC-9358 is approximately equipotent to disopyramide in the ouabain-induced ventricular tachycardia and crush-stimulation-induced atrial flutter experimental arrhythia models (Reynolds *et al.*, 1984), these results suggest that the incidence of anticholinergic side effects of ACC-9358 should be significantly lower than those encountered with disopyramide.

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