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EFFECT OF A NEW ADRENERGIC BETA RECEPTOR BLOCKER ACC-9089 ON TRANSMEMBRANE POTENTIAL OF CANINE PURKINJE FIBERS IN COMPARISON WITH PROPRANOLOL

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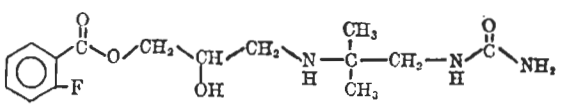
ABSTRACT ACC 0.1 mg/l and propranolol, which did not cause any changes in the transmembrane potential, diminished or eliminated the increases in diastolic depolarization and spontaneous firing rate induced by epinephrine. Resting membrane potential increased in ACC 0.3 mg/l but was unchanged in higher concentrations. Propranolol up to 10 mg/l had no effect on resting potential. The voltage time course of repolarization was unaffected by ACC in 0.3-10 mg/l, yet markedly shortened by propranolol. ERP markedly decreased in propranolol 3 mg/l, but was unchanged in ACC 3 mg/l and slightly decreased in ACC 10 mg/l. Only in 10 mg/l did ACC decrease $\dot{V}_{max}$, overshoot and APA. These direct effects of ACC 10 mg/l were much less than those of propranolol 3 mg/l. It is concluded that ACC is a potent β receptor blocker with only slight direct membrane depressant effects.

KEY WORDS anti-arrhythmie agents; ACC-9089; propranolol; Purkinje fibers; diastolic depolarization; membrane responsiveness; effective refractory period; duration of action potentials

ACC-9089 (ACC) is a newly synthesized fast acting adrenergic β blocker with a short duration of action which may be used for the treatment or diagnosis of certain cardiac arrhythmias. It has the following structure:



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β receptor blocking agents currently used as anti-arrhythmic drugs are of 2 varieties: with and without direct membrane effects[1]. Some believed that blockade of sympathetic influences may be the most important mechanism of anti-arrhythmic action of β receptor blockers[2]. Lack of direct membrane effects may increase the therapeutic/toxic concentration ratio. We studied the effects of ACC on the electrical activity of normal Purkinje fibers to determine whether it is devoid of direct membrane effects.

METHODS

Dogs were anesthetized with sodium pentobarbital. The hearts were excised and placed in cool, oxygenated Tyrode's solution. The false tendons together with the attached papillary muscles were dissected from both ventricles and mounted in a 4-ml chamber superfused with Tyrode's solution equilibrated with 95% O_2 + 5% CO_2, 15 ml/min at 37.0±0.5℃.

The preparations were stimulated with rectangular pulses 1-3 ms in duration and 1.5-2.0 times threshold voltage at a cycle length of 700 ms, unless otherwise mentioned, through a pair of Teflon-coated Ag wire electrodes positioned on the papillary muscle. When determining refractory periods or membrane response curves a 2nd test stimulus was applied to the tissue through the same electrodes. The stimuli were suitably isolated from ground.

The recording instrumentation was similar to that described previously[3]. Transmembrane action potentials were recorded through glass capillary microelectrodes filled with 3 *M* KCl with tip resistances of 10-20 MΩ. The microelectrodes were coupled with Ag-AgCl wire to

Tab 1. Effect of ACC on transmembrane action potentials of Purkinje fibers, $\bar{x}\pm SD$. Compared with before: *p>0.05, **p<0.05, ***p<0.01

Concn (mg/l)		0.3	1.0	3.0	10.0
No. of prepns		10	10	11	10
RP	before	-91.6±2.5	-91.5±2.1	-91.7±2.6	-93±4
(mV)	after	-93.6±2.5***	-91.9±2.3*	-90±4*	-93±3*
OS	before	32±4	30±5	32±3	33±4
(mV)	after	32±5*	31±4*	32±4*	31±4**
APA	before	126±6	124±6	126±5	128±6
(mV)	after	128±4*	125±6*	124±5**	125±6***
APD_{60}	before	240±36	248±25	262±24	242±36
(ms)	after	239±35*	249±24*	265±26*	239±35*
APD	before	345±47	359±35	374±33	341±35
(ms)	after	345±44*	361±33*	373±37*	341±37*
$\dot{V}_{max}$	before	712±149	672±131	694±119	718±113
(V/s)	after	723±146*	667±144*	653±111***	663±96**

a high input impedence amplifier with capacitance neutralization. The output of this amplifier was monitored on an oscilloscope and photographed from a second one (Tektronix D 12 dual-beam oscilloscope) with an oscillographic camera (Tektronix C-5C). The maximal rate of depolarization ($\dot{V}_{max}$) of all action potentials was determined by an electronic differentiator[4].

The tissue was first superfused with Tyrode's solution until control membrane potential recordings were obtained. It was then superfused with Tyrode's solution containing ACC or propranolol-HCl for 15-30 min. In some experiments, the tissues were returned to control solution again and stayed there for another 30 min or more and the course of recovery from the influence of the drug was observed. Several features of the transmembrane potentials were measured and compared[5,6].

For experiments on responses of Purkinje fibers to epinephrine, the K^+ concentration in the Tyrode's solution was 2.7 m*M*, and tissues were driven at a cycle length of 2 s. The drive stimulus was interrupted periodically to assess automaticity of the Purkinje fiber. The superfusate was then changed to Tyrode's solution containing epinephrine bitartrate 0.5 mg/l and maintained for 7-10 min. Automaticity of the Purkinje fibers was again assessed by stopping the drive. The tissue was then superfused with control Tyrode's solution until automaticity returned to control. Superfusion with Tyrode's solution containing epinephrine bitartrate 0.5 mg/l and a test drug (either ACC or propranolol) was then started and continued for 7-10 min. Purkinje fiber automaticity was evaluated again. EDTA 5 μ*M* was added to the reservoir to prevent oxidation of epinephrine.

RESULTS

Effects of β receptor blockers on resting and action potential of Purkinje fibers

1. ACC: The concentrations of ACC studied were 0.3, 1, 3 and 10 mg/l. The data shown in Tab 1 were from experiments in which the microelectrode remained in the same cell throughout the control period and during exposure to the drug. Results from 4 preliminary experiments with ACC 0.1 mg/l showed no change in any features of the transmembrane potential.

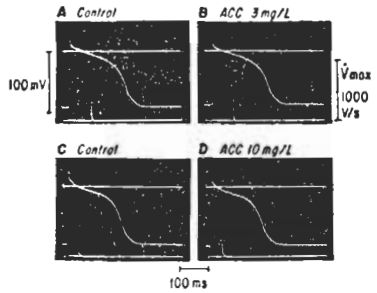

Fig 1. Effects of ACC on action potential of Purkinje fibers. A & B from one experiment, C & D from another. B & D 12 min after superfusion with ACC 3 & 10 mg/l, respectively. These concentrations had slight depressant effect on $\dot{V}_{max}$ and action potential amplitude (Tab 1).

ACC 0.3 mg/l caused a small but significant increase in the transmembrane resting potential (RP). This effect occurred within 10–20 min after exposure to the drug and returned to control within 10 min after washing the drug out with control Tyrode's solution. Higher concentrations of ACC up to 10 mg/l exerted no significant effect on RP (Fig 1 and Tab 1).

ACC 0.3 and 1.0 mg/l had no effect on the overshoot (OS) or amplitude of the action potential (APA); 3 and 10 mg/l yielded only very slight depressant effects (Tab 1). $\dot{V}_{max}$ was also slightly but significantly decreased by 3 and 10 mg/l. All effects of ACC were reversed by superfusing the fibers in drug-free Tyrode's solution.

ACC gave no significant effect on repolarization of the action potential; it did not alter

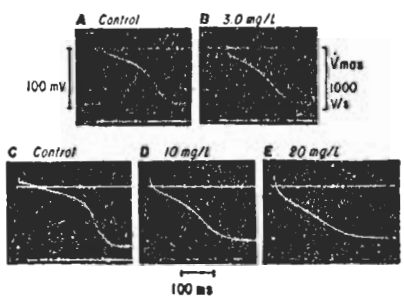

Fig 2. Effects of propranolol on the Purkinje fiber action potential. A & B from one experiment, C–E from another. A & C: controls. B, D & E: propranolol 3, 10 & 20 mg/l, respectively.

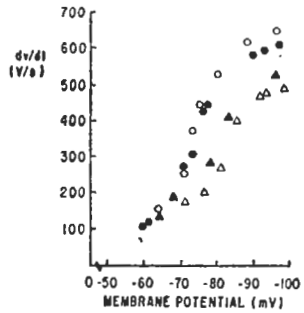

Fig 3. Relation between the maximum rate of depolarization during the upstroke (ordinate) and the level of membrane potential in control solution (●), ACC 0.3 mg/l (○), 3 mg/l (▲) and 10 mg/l (△).

duration to −60 mV (APD_{-60}) or total action potential duration (APD), even at 10 mg/l (Fig 1 and Tab 1).

2. Propranolol: We did 1 or 2 experiments for each of 4 concentrations (1, 3, 10 and 20 mg/l) of propranolol. The results were substantially the same as those previously described[7]. APD_{-60} and APD were shortened. and $\dot{V}_{max}$, OS and APA progressively decreased as the concentration of drug was raised. At 10 mg/l there was a small decrease in RP. In the presence of propranolol 20 mg/l, the plateau phase disappeared and a relative prolongation of the terminal portion of phase 3 occurred (Fig 2).

Effects of ACC on membrane responsiveness The effect of ACC on $\dot{V}_{max}$ varied with the concentration used (Tab 1). $\dot{V}_{max}$ decreased at concentrations above 1 mg/l and tended to increase in 0.3 mg/l (although $p > 0.05$). Since $\dot{V}_{max}$ is dependent on the level of membrane potential[8], we also determined the effects of various concentrations of ACC on the relation between $\dot{V}_{max}$ and the level of membrane potential. This was determined by applying test stimuli to the fiber at different times during phase 3 and hence eliciting action potentials at different levels of membrane potential. The results of one of the experiments are shown in Fig 3. The curve was slightly shifted to the left and up at 0.3 mg/l and shifted to the right and down at 3 and 10 mg/l.

Davis and Temte reported that concentrations above 1 mg/l depressed it (shifted the curve downward and to the right)[7].

Effects of β receptor blockers on duration of effective refractory period (ERP)

1. ACC: In order to determine the drug effects on the duration of the ERP, 2 microelectrodes were located in Purkinje fibers in the false tendon, one very close to the site of attachment of the false tendon with the papillary muscle (proximal fiber) and the other in the false tendon several mm away from this attachment (distal fiber). The stimulating electrodes were placed on the surface of the papillary muscle near the tendinous end. Purkinje fibers were excited by propagated action potential from the papillary muscle[9]. The tissue was driven at a cycle length of 700 ms. A test stimulus was applied at various preselected times throughout repolarization after a driving stimulus and was given every 8 th driving stimulus. Graded responses[10] were usually observed during control determinations of the ERP. They were premature responses with slow upstroke velocities, small amplitudes and short durations. By the end of the ERP, the premature response elicited at the distal fiber abruptly increased in magnitude and assumed a contour similar to that at the proximal site. Photographs were then taken and the time from the upstroke of the basic response to the upstroke of the test response at the proximal fiber was measured and was taken as the duration of the ERP.

In 6 experiments with concentrations of ACC 3 mg/l, there was no change in the duration of the ERP. In 5 experiments with ACC 10 mg/l, there was a slight decrease of 9 ±7ms ($p > 0.05$) in the duration of the ERP (Fig 4). Graded responses still occurred in the presence of ACC.

2. Propranolol: Quite different results were noted with propranolol. The ERP was greatly shortened and graded responses were abolished in the presence of propranolol 3 mg/l. Our data is similar to Davis and Temte's data[7].

Effects of β receptor blockers on response to epinephrine

1. ACC: The effect of low concentrations of ACC on the response of Purkinje fibers to epinephrine was studied in 8 experiments. Two indices were used. One was the slope of diastolic depolarization and the other was the spontaneous firing rate[11]. Purkinje fibers that were superfused with normal Tyrode's solution ($K^+ = 2.7$ mM) had spontaneous rates of 1–30/min and slopes of diastolic depolarization of 0.3–1.3 mV/s. After exposure to epinephrine (0.5 mg/l) for 7–10 min, the automatic rate increased more than twofold and the slope of

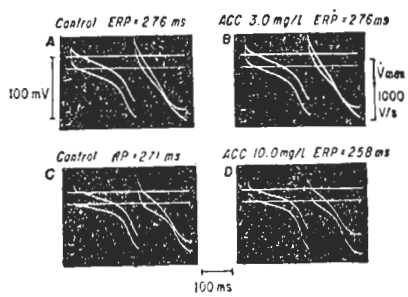

Fig 4. Determination of effective refractory period of Purkinje fibers. Top traces are recordings from proximal site; lower traces from distal site. Each panel shows the shortest premature coupling interval resulting in a response propagating from the proximal to the distal site. A &B from one experiment, C & D from another. B: 22 min in ACC 3 mg/l. D: 16 min in ACC 10 mg/l.

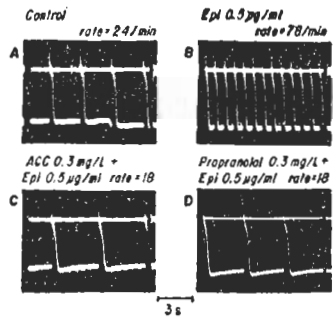

Fig 5. Effects of ACC and propranolol on positive chronotropic effect of epinephrine. A: control firing rate of unstimulated Purkinje fiber bundle in Tyrode's containing 2.7 mM KCl. B: epinephrine 0.5 μg/ml increased the firing rate from 24 to 78/min. Addition of either ACC 0.3 mg/l (C) or propranolol 0.3 mg/l (D) completely prevented this positive chronotropic effect.

phase 4 depolarization increased 2- to 4-fold. After washout of epinephrine, both indices returned to the control state. Subsequently, Tyrode's solution containing ACC 0.1–0.3 mg/l as well as epinephrine was superfused. The spontaneous firing rate was only slightly increased (+20%) in solutions containing ACC 0.1 mg/l and the effect of epinephrine was always completely abolished by concentrations of ACC 0.3 mg/l. The slope of phase 4 depolarization also was only slightly increased (+60% – 130%) in the presence of ACC 0.1–0.3 mg/l. The results are shown in Fig 5.

2. Propranolol: The effect of propranolol 0.1–0.3 mg/l on the response of Purkinje fibers to epinephrine was almost the same as that of ACC. Records from one experiment are shown in Fig 5, in which propranolol 0.3 mg/l was compared with ACC 0.3 mg/l on the same single Purkinje fiber. The results show the similarity of the effects of the 2 drugs.

DISCUSSION

ACC and propranolol both exhibited β receptor blocking effects on the response of Purkinje fibers to epinephrine in concentrations of 0.1–0.3 mg/l. The stimulatory effects of epinephrine on automaticity is mediated through β adrenergic receptor activation. ACC therefore has a potency on a weight base which is similar to propranolol.

However, at higher concentrations, the direct effects of ACC and propranolol on the transmembrane potential of normal Purkinje fibers are quite different. The predominant effect of propranolol is to accelerate repolarization and this is manifested by a marked decrease in the plateau phase of the action potential. The APD was also shortened. Propranolol>1 mg/l decreased the $\dot{V}_{max}$, OS and the APA. ERP was shortened. RP decreased when the concentration of propranolol was raised to 10 mg/l.

On the other hand, no significant changes in repolarization were noted with ACC 0.1–10.0 mg/l. Effects on $\dot{V}_{max}$, OS and APA occurred only when the concentration of ACC reached 3 mg/l or higher, concentrations much higher than those needed for β receptor blockade. At these concentrations of ACC, the membrane responsiveness curves were shifted to the right and down. These results suggest the possibility of a direct membrane effect of high concentrations of ACC to decrease gNa^+. The duration of ERP was slightly shortened, only when the concentration of ACC reached 10 mg/l. The so-called graded responses were not abolished as they were with propranolol. The direct membrane effects of ACC were therefore much weaker than that of propranolol and ACC will probably have no direct depressant effects on the action potential *in vivo*.

ACC also influenced the RP of Purkinje fibers differently than did propranolol. In the presence of propranolol 10 mg/l and above, the RP decreased. No significant decrease in RP were seen at ACC 0.1–10.0 mg/l. However, the RP slightly, yet significantly, increased in the presence of ACC 0.3 mg/l. At the same concentration, the membrane responsiveness curve was slightly shifted to the left and up. It is possible that the conduction velocity of Purkinje fibers may increase slightly at ACC 0.3 mg/l.

REFERENCES

1 Wolfson S, Robbins SI, Krasnow N. *Am Heart J* 1966; 72 : 177
2 Wit AL, Hoffman BF, Rosen MR. *ibid* 1975; 90 : 665
3 Friedman PL, Stewart JR, Fenoglio JJ Jr, Wit AL. *Circ Res* 1973; 33 : 597
4 Bigger JT Jr, Bassett AL, Hoffman BF. *ibid* 1968; 22 : 221
5 Snedecor GW, Cochran WG. *Statistical methods*. 7th ed, Ames Iowa, The Iowa State University Press, 1980 : 83
6 Temte JV, Davis LD. *Circ Res* 1967; 20 : 32
7 Davis LD, Temte JV. *ibid* 1968; 22 : 661
8 Weidmann S. *J Physiol (Lond)* 1955; 127 : 213
9 Hoffman BF, Kao CY, Suckling EE. *Am J Physiol* 1957; 190 : 473
10 Kao CY, Hoffman BF. *ibid* 1958; 194 : 187
11 Dangman KH, Hoffman BF. *J Pharmacol Exp Ther* 1981; 217 : 851

新 β 受体阻滞剂 ACC-9089 对犬心浦氏纤维跨膜电位的作用

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提要　ACC 与心得安 0.1 mg/l 对跨膜电位无影响，但可减弱肾上腺素诱发的自律性增高。ACC 0.3 mg/l 使 RP 加大，心得安的各种浓度（高至 10 mg/l）对 RP 无影响。心得安 0.3-10.0mg/l 使 2 相和 APD 缩短，ACC 无此作用。心得安 3 mg/l 使 ERP 显著缩短，ACC 至 10 mg/l 才轻度缩短 ERP。ACC 10 mg/l 使 $\dot{V}_{max}$、OS 和 APA 降低，但其降低程度不如心得安 3 mg/l。认为 ACC 是强大的 β 受体阻滞剂，其直接膜抑制作用很弱。

关键词　抗心律失常药；　ACC-9089；　心得安；　浦氏纤维；　舒张期去极化；　膜反应性；　有效不应期；　动作电位时程

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