Antiarrhythmic peptide has no direct cardiac actions

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Summary. The electrophysiologic, inotropic, and muscarinic effects of antiarrhythmic peptide (AAP) were examined in canine cardiac Purkinje fibers, ferret papillary muscle, and canine cardiac membranes, respectively. Aside from a prolongation of time to peak force in papillary muscle, no biologically significant effects of AAP could be determined in any preparation, suggesting that its antiarrhythmic effects are not mediated by direct membrane actions.
Key words. Cardiac Purkinje fiber; electrophysiology; ferret; dog; muscarinic; inotropic.

Antiarrhythmic peptide (AAP, Gly-Pro-Hyp-Gly-Ala-Gly), isolated from bovine atria [1], improves rhythmicity of cultured myocardial cells [2] and has a protective effect against chemically-induced arrhythmias in mice [3]. Whereas other antiarrhythmic agents have well-defined electrophysiological and inotropic effects, the cellular basis for the antiarrhythmic action of AAP has never been explored. In particular, the electrophysiological actions of AAP have never been reported. The purpose of this study was to determine the electrophysiologic actions of AAP in isolated canine Purkinje fibers. Since cholinergic agents can have antiarrhythmic activity, we also determined its affinity for muscarinic receptors in canine atrial membrane preparations. Finally, since inotropic actions reflect membrane effects of many antiarrhythmic agents, we investigated its inotropic actions in isolated ferret papillary muscles.

Methods

Four mongrel adult dogs (12–15 kg) unselected for sex were anesthetized with sodium pentobarbital (30 mg/kg; i.v.). Hearts were quickly removed and placed into cold (4 °C) oxygenated (95% O_2-5% CO_2) Tyrode's solution [4]. Free running Purkinje fiber strands were removed from both the right and left ventricle and equilibrated at 35 °C in a Tyrode's superfused recording chamber for standard intracellular microelectrode analysis. Preparations were stimulated with a 2-ms pulse at twice diastolic threshold with silver bipolar electrodes at a basic cycle length (BCL) of 1000, 500 and 300 ms. Signals were amplified, displayed on an oscilloscope (Tektronix 5111 A) and digitized (PDP-11/23) for on-line analysis of maximum diastolic potential (MDP), action potential amplitude (APA), maximum rate of phase zero depolarization (V_{max}), and action potential duration at 50 and 95% repolarization (APD 50 and APD 95, respectively). Preparations were exposed to increasing concentrations of AAP in the superfusate, and measurements were repeated after a 30-min equilibration with each concentration.

It is often difficult to obtain canine ventricular trabeculae of the appropriate size to study the inotropic actions of drugs. Therefore, we used the ferret papillary muscle for this phase of the study. Five ferrets, unselected for sex or weight, were anesthetized with pentobarbital (50 mg/kg, i.p.). The heart was excised and a papillary muscle was removed for study under isometric conditions at 35 °C and at optimal length [5]. Muscles were stimulated at 0.2 Hz at approximately 10% above threshold using platinum point electrodes. Peak developed force (F), time to peak force (TTP), the maximum rates of force development (df/dt) and relaxation (-df/dt) and the relative rate of relaxation (-df/dt/F) were determined after 15 min of exposure to each concentration of AAP.

The affinity of AAP for cardiac muscarinic receptors was assessed in a crude membrane fraction of dog atrium [6]. Membranes (0.4 mg protein) were incubated (60 min, 37 °C) with [^{3}H]-quinuclidinyl benzylate (QNB, 50 pM) and AAP (0.1–100 μM) in a total volume of 2.0 ml of sodium phosphate buffer. Reactions were terminated by dilution followed immediately by rapid vacuum filtration (Whatman GF/C filters) to separate bound complexes. The filters were air dried and counted for radioactivity in a liquid scintillation spectrometer. Nonspecific binding was defined as that binding which occurred in the presence of 10 μM atropine. Binding activity at each concentration of peptide was determined relative to control binding (i.e., in the absence of peptide) after subtracting nonspecific binding.

Data were analyzed by ANOVA with repeated measures and by t-test using Bonferroni's correction.

AAP was synthesized at Berlex Laboratories, Inc., by solid phase methodology and was shown to be identical in purity (> 95%) and structure to commercially available material.

Results and discussion

Each of four Purkinje fiber preparations was consecutively exposed to 0.01, 0.1, 1, 10 and 30 μM of AAP in the superfusate. No significant effects were observed at a BCL = 1000 ms on MDP, APA, V_{max}, APD 50 or APD 95 (table 1). Similarly, no rate-dependent changes were observed in any of the action potential parameters measured at the faster stimulation rates.

AAP had no significant inotropic effects in isolated ferret papillary muscle (table 2). High concentrations (1–

Table 1. Effect of antiarrhythmic peptide on the action potential of canine cardiac Purkinje fibers.

Conc. (μM)	Percent change MDP (mV)	APA (mV)	V_{max} (V/s)	ADP50 (ms)	ADP95 (ms)
0.01	−1.8 ± 1.9	−0.5 ± 0.7	1.5 ± 0.7	0.8 ± 1.4	1.25 ± 0.8
0.1	−0.3 ± 1.3	0.3 ± 1.0	−0.7 ± 1.2	−2.25 ± 1.9	2.25 ± 0.9
1	−0.8 ± 1.1	1.0 ± 2.7	−4.7 ± 0.8	1.0 ± 0.0	2.8 ± 0.9
10	2.0 ± 0.7	0.8 ± 1.7	−7.0 ± 4.0	3.0 ± 0.8	4.0 ± 1.1
30	0.5 ± 1.7	−3.3 ± 1.2	−6.7 ± 4.7	2.3 ± 2.7	5.8 ± 1.3

Mean ± SEM; n = 4 except V_{max} n = 3; BCL = 1000 ms. MDP: maximum diastolic potential; APA: action potential amplitude; V_{max}: maximum upstroke velocity, APD: action potential duration at 50 and 95% of repolarization.

Table 2. Inotropic effect of antiarrhythmic peptide in ferret papillary muscle

AAP (μM)	Force (g/mm^2)	df/dt ($g/mm^2/s$)	TTP (ms)	−df/dt/F (s^{-1})
0	1.5 ± 0.2	16.2 ± 1.5	166 ± 8	8.1 ± 0.4
0.01	1.6 ± 0.2	16.4 ± 1.5	171 ± 9	8.0 ± 0.5
0.1	1.5 ± 0.2	15.9 ± 1.4	171 ± 8	7.8 ± 0.3
1	1.6 ± 0.2	16.1 ± 1.5	174 ± 10*	7.6 ± 0.3
3	1.6 ± 0.2	16.8 ± 1.2	175 ± 9*	8.0 ± 0.7
10	1.6 ± 0.2	16.1 ± 1.1	175 ± 9*	7.7 ± 0.4

Mean ± SEM; n = 5; *$p < 0.05$; df/dt: maximum rate of force development; TTP: time to peak force; − df/dt/F: relative rate of relaxation.

10 μM) of AAP increased time to peak force (table 2), suggesting a decrease in the rate of calcium sequestration. Control specific binding of QNB was 97 ± 17 (Mean ± SEM, n = 4) fmol/mg protein. AAP did not displace the binding of QNB at any concentration tested. AAP improves rhythmicity and increases spreading in cultured neonatal rat myocardial cells [1, 2]. The effect on spreading is seen with concentrations as low as 10 nM and the effect on rhythm is seen with concentrations on the order of 10 μM. Quinidine also has an antiarrhythmic effect and increases spreading in these cells [2]. AAP (10–30 mg/kg, i.v.) reduces mortality in calcium-chloride or ouabain-induced arrhythmias, as does quinidine (15 mg/kg, i.v.), bretylium (15 mg/kg, i.v.), and verapamil (2 mg/kg, i.v.) in mice [3]. In addition, AAP (0.1 μM) reduces calcium uptake in isolated myocardial cells by approximately 50% [2]. These data suggest that AAP alters membrane properties (e.g., calcium permeability, intercellular coupling, etc.).

In rats, arrhythmias evoked by calcium chloride, aconitine, and epinephrine were associated with an increase in circulating levels of AAP from approximately 2 pmol/ml to approximately 7 pmol/ml [7]. These concentrations are clearly much lower than those having significant antiarrhythmic effects in vitro or in vivo.

In contrast to the well-defined electrophysiologic and inotropic actions of quinidine and verapamil, AAP had no significant electrophysiologic effects in canine cardiac Purkinje fibers or inotropic actions in isolated ferret papillary muscles. We also examined the effect of AAP on QNB binding as a measure of its affinity for the muscarinic receptor, which might account for its antiarrhythmic actions via indirect changes in heart rate. Again, AAP showed no activity. Thus, our data suggest that AAP does not exert its antiarrhythmic actions through direct electrophysiologic effects, and therefore, it must act via some other mechanism. AAP does have an effect on platelet aggregation [8], suggesting that it may exert its antiarrhythmic actions via other cellular mediators (e.g., by modulating the release of thromboxane A_2). As an alternative, and perhaps less likely, explanation, AAP may have effects on passive membrane properties (e.g., membrane capacitance, input resistance, etc.). Such changes could significantly alter conduction of premature beats in the intact heart but not significantly affect the action potential measured in a relatively small piece of Purkinje fiber. Such passive effects were not measured in this study. Regardless of the mechanism underlying the action of AAP, antiarrhythmic drugs typically have specific electrophysiological effects in mammalian myocardium [9], actions lacking in AAP. AAP is therefore unique among antiarrhythmic compounds in its lack of defined electrophysiologic effects.

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