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ELECTROMECHANICAL EFFECTS OF ANTHOPLEURIN-A (AP-A) ON RABBIT VENTRICULAR MUSCLE: INFLUENCE OF DRIVING FREQUENCY, CALCIUM ANTAGONISTS, TETRODOTOXIN, LIDOCAINE AND RYANODINE

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1 Anthopleurin-A (AP-A 5×10^{-9} M, 1×10^{-8} M) caused a prolongation of action potential duration (APD) and an increase of contractile force in rabbit isolated ventricular muscle preparations.

2 The prolongation of APD and the positive inotropic effect of AP-A (1×10^{-8} M) were augmented by lowering the driving frequency from 2.0 to 0.2 Hz, resulting in an apparent negative staircase of contractile force in this frequency range. When the preparation was driven at an extremely low frequency (0.017 Hz), AP-A did not increase the contractile force, but caused a considerable prolongation of APD.

3 Verapamil (1×10^{-6} M) and nifedipine (1×10^{-6} M) had no apparent influence on the APD prolongation by AP-A (5×10^{-9} M, 1×10^{-8} M). The positive inotropic effect of AP-A was also relatively well maintained even in the presence of these calcium antagonistic drugs when the preparation was driven at a lower frequency (0.2 Hz).

4 Tetrodotoxin (TTX 2×10^{-6} M) and lidocaine (1×10^{-4} M) markedly inhibited both the APD prolongation and the positive inotropic effect of AP-A (1×10^{-8} M).

5 In the presence of ryanodine (2×10^{-6} M), an agent which is known to interfere with calcium release from the intracellular activator pool, AP-A (1×10^{-8} M) failed to cause its positive inotropic effect in spite of the marked prolongation of APD.

6 These results suggest that the effects of AP-A on cardiac muscle are primarily mediated by the fast sodium inward current. Thus, delayed inactivation of sodium inward current may cause APD prolongation, and probably induces an alteration of intracellular calcium kinetics reflected by an increase of contractile force.

Introduction

Anthopleurin-A (AP-A) is a polypeptide of 49 amino acids isolated from the sea anemone *Anthopleura xanthogrammica* (Shibata, Dunn, Kuchii, Kashiwagi & Norton, 1974; Shibata, Norton, Izumi, Matsuo & Katsuki, 1976; Norton, Shibata, Kashiwagi & Bentley, 1976; Tanaka, Haniu, Yasunobu & Norton, 1977). Subsequent pharmacological studies (Shibata, Izumi, Seriguchi & Norton, 1978; Blair, Peterson & Bishop, 1978; Shimizu, Iwamura, Toyama, Yamada & Shibata, 1979; Scriabine, Van Arman, Morgan, Morris, Bennet & Bohidar, 1979; Kodama, Toyama, Shibata & Norton, 1980) have revealed that the polypeptide possesses several unique properties as a cardiotonic agent. Thus, AP-A causes *in vivo* a selective positive inotropic effect on the heart without affecting heart rate or blood pressure. The positive inotropic effect is not inhibited by reserpine treatment or by β-adrenoceptor blocking agents. *In vitro*, AP-A prolongs the action potential duration and the refractory period of mammalian cardiac muscle. AP-A does not affect the membrane Na^+-K^+ adenosine triphosphatase (ATPase) activity. The positive inotropic effect of AP-A is relatively resistant to temperature stress, extracellular calcium depletion, calcium antagonists or energy-depleted experimental conditions. These properties suggest that cardiac effects of AP-A are mediated by some mechanism which is quite different from those for other conventional cardiotonic drugs such as cardiac glycosides or catecholamines. However, much remains to be clarified as to the cellular mechanism of AP-A. In the present study, we investigated the electromechanical effects of AP-A on rabbit isolated ventricular muscle through the simultaneous recording of transmembrane action potential and mechanical tension at various driving frequencies and under the influence of drugs affecting (a) the slow calcium inward current; (b) the fast sodium

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inward current; or (c) the intracellular translocation of calcium. We then sought to elucidate the underlying mechanism for the action of this potent cardiotonic drug.

Methods

Experimental procedures were essentially similar to those reported previously (Kodama *et al.*, 1980). Right ventricular papillary muscle, 3 to 4 mm in length and less than 1 mm in diameter, was dissected from the heart of rabbits (1.5–2.0 kg) killed by a blow on the head. The preparation was fixed in a tissue bath and superfused at 30°C with Krebs-Ringer solution of the following composition (mM): NaCl 120.3, KCl 4.8, $CaCl_2$ 1.2, $MgSO_4.H_2O$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 24.2 and glucose 5.5. The solution was equilibrated with 95% O_2 and 5% CO_2, and the pH was 7.4. In 'low calcium' solution the $CaCl_2$ concentration was reduced to 0.3 mM.

The tendinous end of the muscle was connected to a force displacement transducer for recording the isometric tension. The resting tension (RT) applied to each preparation was adjusted to 0.40 g under control conditions. The preparation was stimulated at various driving frequencies (0.017 to 2.0 Hz) with pulses, 2 ms in duration and twice the diastolic threshold in intensity, delivered through contiguous silver wire bipolar electrodes. Transmembrane action potentials were recorded through glass microelectrodes filled with 3 M KCl. The action potentials and their first time derivatives obtained by electronic differentiation were displayed simultaneously with the signal from the force displacement transducer on a storage oscilloscope.

The following parameters were measured in each experiment to assess the electromechanical performance of ventricular muscles: peak developed tension (DT); maximum rate of force development (dT/dt_{max}); time to peak developed tension (tPT); resting tension (RT); maximum diastolic potential (MDP) and overshoot (OS) of action potential; maximum upstroke velocity of action potential (dV/dt_{max}); and duration of action potential from the upstroke to 30% and 80% repolarization (APD 30, APD 80).

Drugs used were AP-A supplied by T.R. Norton (University of Hawaii, Department of Pharmacology, Honolulu, Hawaii); verapamil, nifedipine, tetrodotoxin (TTX) (Sankyo, Tokyo, Japan); lidocaine and ryanodine (S.B. Penick Company, N.Y.). When these drugs were tested, the preparation was superfused with Krebs-Ringer solution containing them at a given concentration for 30 to 60 min.

Statistical analysis of measured parameters was performed by Student's t test, and P values of less than 0.05 were considered to indicate significant differences. More details on each procedure are given under Results.

Results

Influence of driving frequency

The influence of driving frequency on the electromechanical effects of AP-A was studied in eight muscles (Table 1). Steady state values of the parameters for contraction and transmembrane action potentials were determined in each preparation stimulated by different frequencies before and after application of AP-A at 1×10^{-8} M. A previous study indicated that this concentration of AP-A exerted a submaximal positive inotropic effect on rabbit ventricular muscles without any toxic manifestations (Kodama *et al.*, 1980). The driving frequency was altered by stepwise reduction. In the control preparations, reducing the frequency from 2.0 to 0.017 Hz caused a progressive decline of contractile force (positive staircase, Figure 1, 2). After 30 min contact with AP-A (1×10^{-8} M), the second measurements were made. As illustrated in Figures 1 and 2, the increase of contractile force induced by AP-A was

Table 1 Action potential duration (APD) at various driving frequencies

	APD 30 (ms)		*APD 80 (ms)*	
DF (Hz)	*Control*	*AP-A* 1×10^{-8} M	*Control*	*AP-A* 1×10^{-8} M
0.017	93 ± 8	2,499 ± 750*	135 ± 12	5,366 ± 2,138*
0.2	120 ± 12	796 ± 287*	166 ± 18	1,796 ± 546
0.5	145 ± 20	261 ± 101*	200 ± 28	781 ± 212*
1.0	145 ± 18	191 ± 34*	197 ± 22	340 ± 76*
2.0	117 ± 23	127 ± 23	165 ± 20	198 ± 26*

Each value indicates mean ± s.d. of eight preparations before and 30 min after addition of AP-A (1×10^{-8} M). DF: driving frequency; APD 30: action potential duration from the upstroke to 30% repolarization; APD 80: action potential duration from the upstroke to 80% repolarization. The driving frequency was altered by stepwise reduction from 2.0 Hz to 0.017 Hz, and measurements were performed at steady state with each driving frequency. *Significantly different from the control value at $P < 0.05$.

remarkably augmented by lowering the frequency from 2.0 to 0.2 Hz, but there was no significant increase at 0.017 Hz. When the preparation was driven at 2.0 Hz, the time to peak developed tension (*t*PT) was somewhat shortened after the AP-A treatment, but not at other frequencies tested. AP-A caused a marked prolongation of APD without affecting any other electrical parameters. The prolongation of APD by AP-A was almost linearly increased as the driving frequency was lowered (2.0 to 0.017 Hz). Thus, at the lowest frequency (0.017 Hz, interbeat interval of 60 s), although a positive inotropic effect of AP-A was virtually undetectable, APD was prolonged up to several seconds.

In tissues driven at a lower frequency than 0.5 Hz, AP-A often caused a secondary component of tension to develop (see Figure 1). As illustrated, an initial rapid development of tension (phasic tension) was followed by a period of incomplete relaxation and maintained tension (tonic tension); and the tissue completely relaxed only upon membrane repolarization. A similar contraction curve showing two distinct components was reported to be induced only when a very long depolarization (more than 2s) was applied to mammalian ventricular muscles by voltage-clamp techniques (Morad & Goldman, 1973).

Calcium antagonistic drugs and low calcium perfusate

The influence of calcium antagonistic drugs (verapamil, nifedipine) and of the low calcium perfusate on the electromechanical effects of AP-A was examined to elucidate the possible role of the slow calcium inward current for the prolongation of APD and for the increase of contractile force induced by this drug. The preparation was driven at 1.0 Hz or 0.2 Hz for the measurements to be made at steady state.

The results in experiments with verapamil are presented in Figure 3 and Table 2. After treatment with verapamil (1×10^{-6} M) for 60 min, AP-A (5×10^{-9} M or 1×10^{-8} M) was applied to the tissue for 30 min. Pretreatment with verapamil caused a marked decrease in contractile force as well as a slight shortening of APD at both driving frequencies, but the characteristic effect of AP-A on transmembrane action potentials was apparently not affected by the verapamil treatment (Figure 3 and Table 2). However, the positive inotropic effect of AP-A was markedly attenuated or nearly abolished when the preparation was driven at the higher frequency (1.0 Hz). On the other hand, at the lower frequency (0.2 Hz), AP-A was still able to cause a large positive inotropic effect (Figure 3).

Similar results were also obtained when AP-A was applied to the preparation pretreated with nifedipine (1×10^{-6} M) or in a low calcium medium. When AP-A was applied to the preparation superfused with Krebs-Ringer solution containing Ca^{2+} of 0.3 mM, it caused the usual marked prolongation of APD at both driving frequencies of 1.0 Hz and 0.2 Hz. The positive inotropic effect of AP-A was observed at both driving frequencies, although the effect was much greater at the lower frequency.

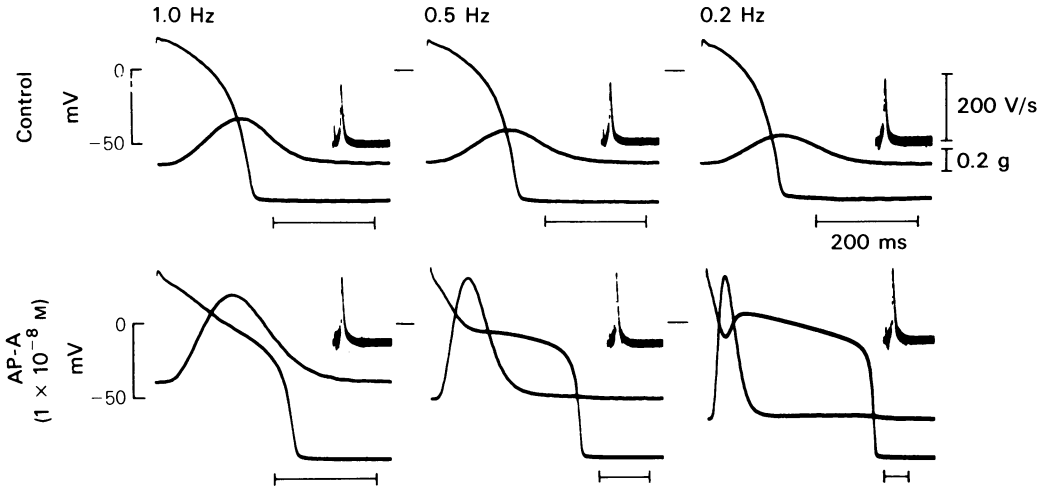

Figure 1 Influence of driving frequency on electromechanical effects of anthopleurin-A (AP-A). Upper panels show control records at driving frequencies of 1.0, 0.5 and 0.2 Hz. Lower panels are the corresponding records obtained 30 min after application of AP-A (1×10^{-8} M). The top trace shows the first derivative of the action potential. The middle trace is developed tension, and the bottom trace is a membrane action potential. Only the first derivative of action potential was recorded at a faster sweep velocity.

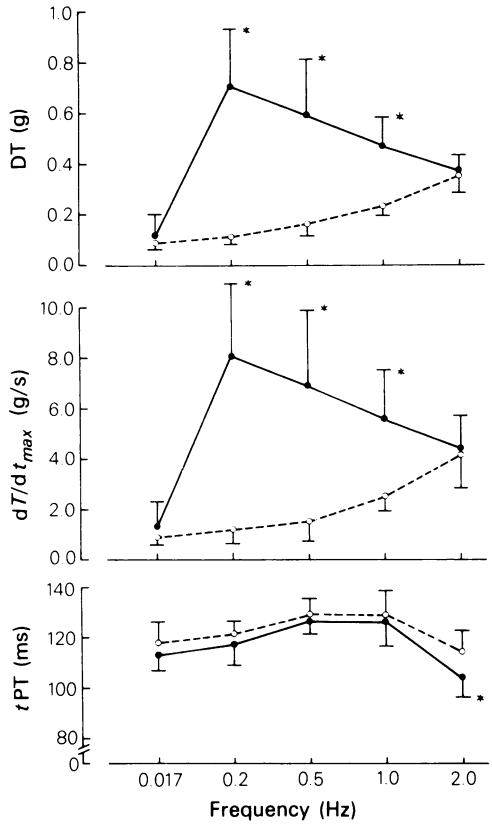

Figure 2 Influence of driving frequency on the positive inotropic effect of anthopleurin-A (AP-A). DT: peak developed tension; dT/dt_{max}: maximum rate of force development; tPT: time to peak developed tension. Mean values were obtained from eight preparations before (○) and 30 min after application of AP-A, 1×10^{-8} M (●). The driving frequency was altered by stepwise reduction from 2.0 Hz to 0.017 Hz, and measurements were performed at steady state with each driving frequency. Vertical lines indicate s.d.* Significantly different from the control values at $P < 0.05$.

Effects of tetrodotoxin (TTX) and lidocaine

The contribution of the fast sodium inward current to the action of AP-A was examined by using tetrodotoxin (TTX) and lidocaine. The measurements were performed at a steady state with a driving frequency of 1.0 Hz or 2.0 Hz.

Figure 4 and Table 3 summarize the results obtained in five experiments with TTX. After treatment with TTX (2×10^{-6} M) for 30 min, the contractile force of the preparation was slightly decreased and dV/dt_{max} of action potential was reduced to about a half of the control value. Other parameters of action potentials (MDP, OS, APD) were not affected by the treatment with TTX. In such preparations, AP-A (1×10^{-8} M) failed to cause a positive inotropic effect or prolongation of APD. This inhibitory effect of TTX on the electromechanical action of AP-A (1×10^{-8} M) occurred at each driving frequency (1.0 Hz and 0.2 Hz). However, when a much higher concentration of AP-A (1×10^{-7} M) was added (not shown) it then caused an apparent positive inotropic effect and prolongation of APD. In some other experiments, TTX (2×10^{-6} M) was added to the preparation, which had been equilibrated with AP-A (1×10^{-8} M) for 30 min. In these cases the increase in APD and contractile force evoked by the initial application of AP-A was almost completely reversed by TTX.

Lidocaine likewise exerted an antagonistic action against the electromechanical effects of AP-A. The prolongation of APD and the increase in contractile force induced by AP-A (1×10^{-8} M) were abolished within 10 min by the additional application of lidocaine (1×10^{-4} M).

Effects of ryanodine

The effects of ryanodine, which is known to interfere with the intracellular translocation of calcium (Sutko, Willerson, Templeton, Jones & Besch, 1979; Jones,

Table 2 Effects of anthopleurin-A (AP-A) on action potential duration in the presence of verapamil

	APD 30 (ms)		*APD 80 (ms)*	
	1.0 Hz	0.2 Hz	1.0 Hz	0.2 Hz
Control	152 ± 12	101 ± 20	210 ± 14	162 ± 22
Verapamil 1×10^{-6} M	130 ± 21*	90 ± 16*	185 ± 17*	143 ± 20*
Verapamil 1×10^{-6} M + AP-A 5×10^{-9} M	193 ± 40†	627 ± 107†	293 ± 49†	891 ± 131†
Verapamil 1×10^{-6} M + AP-A 1×10^{-8} M	278 ± 50†	1,381 ± 240†	473 ± 76†	2,195 ± 231†

Values indicate mean ± s.d. of six preparations under control conditions, 60 min after addition of verapamil (1×10^{-6} M), and 30 min after subsequent additional application of AP-A (5×10^{-9} M, and 1×10^{-8} M). Measurements were performed at steady state with a driving frequency of 1.0 Hz and 2.0 Hz. Abbreviations are the same as in Table 1. *Significantly different from the control value at $P < 0.05$. † Significantly different from the value obtained 60 min after addition of verapamil (1×10^{-6} M) at $P < 0.05$.

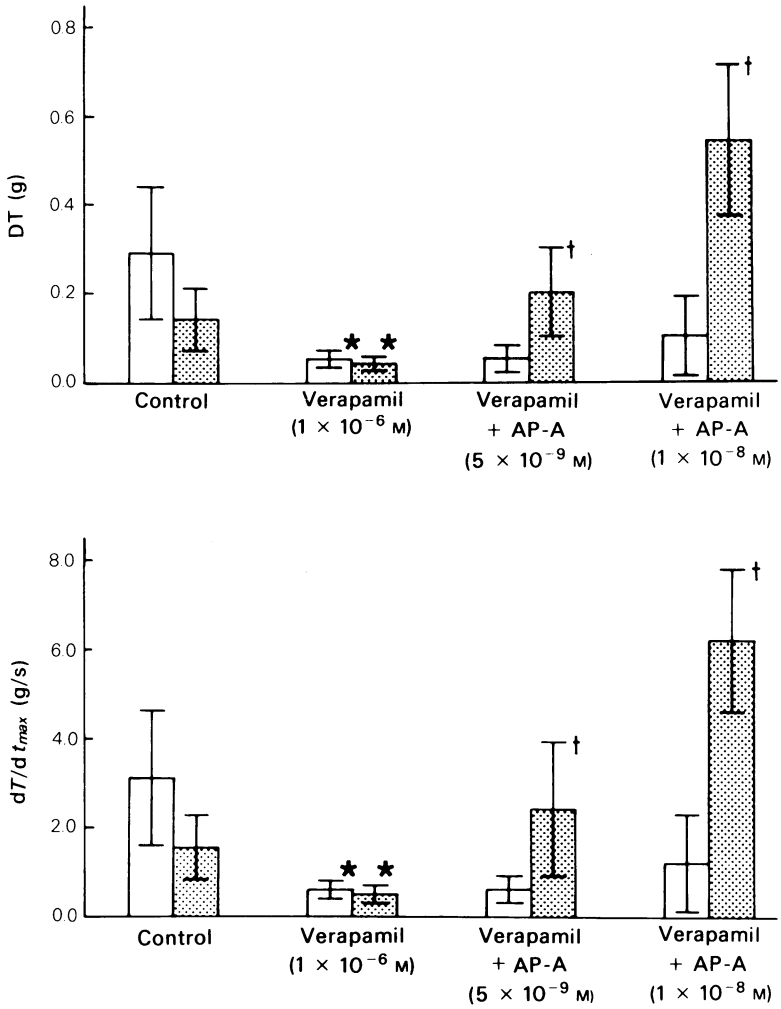

Figure 3 Influence of verapamil on the positive inotropic effect of anthopleurin-A (AP-A). DT: peak developed tension; dT/dt_{max}: maximum rate of force development. Mean values were obtained from six preparations when they were driven at 1.0 Hz (open columns) and 0.2 Hz (stippled columns), under control conditions 60 min after addition of verapamil (1×10^{-6} M), and 30 min after subsequent additional application of AP-A (5×10^{-9} M and 1×10^{-8} M), Vertical lines indicate s.d.* Significantly different from the values of control at $P < 0.05$;† significantly different from the values 60 min after addition of verapamil (1×10^{-6} M).

Besch, Sutko & Willerson, 1979) were examined on the electromechanical action of AP-A. The measurements were performed at a steady state with a driving frequency of 1.0 Hz or 0.2 Hz after exposure to ryanodine (2×10^{-6} M) for 30 min, and also 30 min after the additional application of AP-A (1×10^{-8} M). The results obtained are presented in Figure 5 and Table 4. The pretreatment with ryanodine caused a slight prolongation of APD at both driving frequencies, and a slight decrease in contractile force especially at 0.2 Hz. Other parameters of membrane action potential were not influenced. When AP-A (1×10^{-8} M) was applied for 30 min to the pretreated preparation, it failed to cause any positive inotropic effect at either driving frequency. In contrast, the usual electrical effect of AP-A (marked prolongation of APD) was not inhibited by the pretreatment with ryanodine, indicating an apparent dissociation of mechanical and electrical events.

Discussion

The effects of AP-A on cardiac muscle are character-

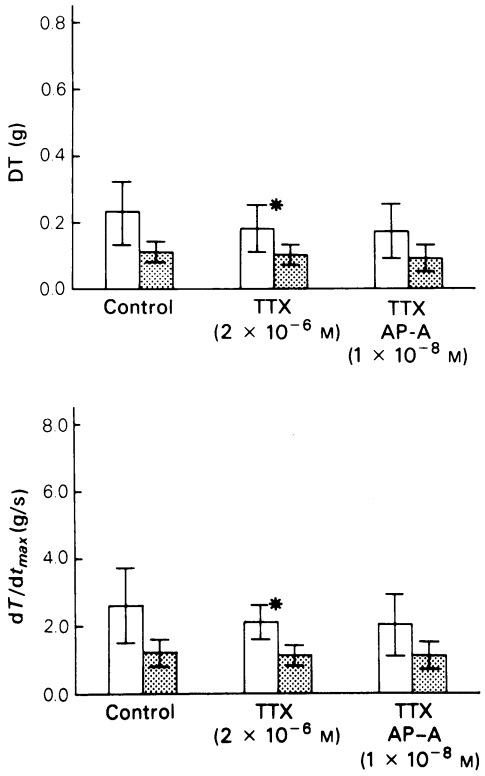

Figure 4 Influence of tetrodotoxin (TTX) on the positive inotropic effect of anthopleurin-A (AP-A). Mean values were obtained from five preparations when they were driven at 1.0 Hz (open columns) and 0.2 Hz (stippled columns), under control condition, 30 min after addition of TTX (2×10^{-6} M), and 30 min after subsequent additional application of AP-A (1×10^{-8} M). Vertical lines indicate s.d.* Significantly different from the control value at $P < 0.05$.

ized by a marked prolongation of APD and an increase of contractile force (Shibata *et al.*, 1974; 1976; 1978; Shimizu *et al.*, 1979; Kodama *et al.*, 1980). The former electrical effect has been attributed to several possible mechanisms such as delayed inactivation of the fast sodium inward current, increase in magnitude or delayed inactivation of the slow calcium inward current (Shimizu *et al.*, 1979; Kodama *et al.*, 1980), although none of them was previously confirmed.

The present study revealed that the prolongation of APD of ventricular muscles induced by AP-A was markedly inhibited by TTX or lidocaine, agents known to block the fast sodium channel in excitable membranes (Carmeliet & Vereecke, 1979). This finding leads to the speculation that the APD prolongation induced by AP-A is mediated by a mechanism similar to that proposed for the effects of veratrine (Horackova & Vassort, 1974), veratridine (Honerjager & Reiter, 1975) and toxin II, a polypeptide isolated from *Anemone sulcata* (Ravens, 1976). Thus, the delayed inactivation of the fast sodium inward current may play a primary role in the prolongation of APD. A recent report by Low, Wu & Narahashi (1979) lends support to this idea. Their voltage-clamp experiments revealed that the inactivation process of the fast sodium current of the crayfish giant axon was selectively inhibited by AP-A. In contrast, a contribution of the slow calcium inward current to the prolongation of APD in cardiac muscle is quite unlikely, since neither blocking agents of the slow inward current (verapamil, nifedipine) nor extracellular calcium depletion prevented the APD prolongation.

The prolongation of APD by AP-A was almost linearly increased as the driving frequency was lowered. Although at the present time we have no available data to explain the underlying mechanism, a similar augmentation of APD prolongation was also reported in the veratridine-treated ventricular muscle (Honerjager & Reiter, 1975).

In our previous study on rabbit ventricular muscle

Table 3 Effects of anthopleurin-A (AP-A) on membrane potential in the presence of tetrodotoxin (TTX)

	APD 30 (ms)		*APD 80 (ms)*		dV/dt_{max} (V/s)	
	1.0 Hz	0.2 Hz	1.0 Hz	0.2 Hz	1.0 Hz	0.2 Hz
Control	142±6	99±8	198±18	153±10	184±30	198±31
TTX (2×10^{-6} M)	145±10	110±15	197±22	150±17	107±74*	118±66*
TTX (2×10^{-6} M) + AP-A (1×10^{-8} M)	161±9	102±16	222±18†	160±26	118±74	132±65

Values are mean ± s.d. of five preparations, under the control condition, 30 min after addition of TTX (2×10^{-6} M), and 30 min after subsequent additional application of AP-A (1×10^{-8} M). Measurements were performed at steady state with a driving frequency of 1.0 Hz and 0.2 Hz. APD 30: action potential duration from the upstroke to 30% repolarization; APD 80: action potential duration from the upstroke to 80% repolarization; dV/dt_{max}: maximum upstroke velocity of action potential. *Significantly different from the control value at $P < 0.05$; †Significantly different from the value obtained 50 min after addition of TTX (2×10^{-6} M) at $P < 0.05$.

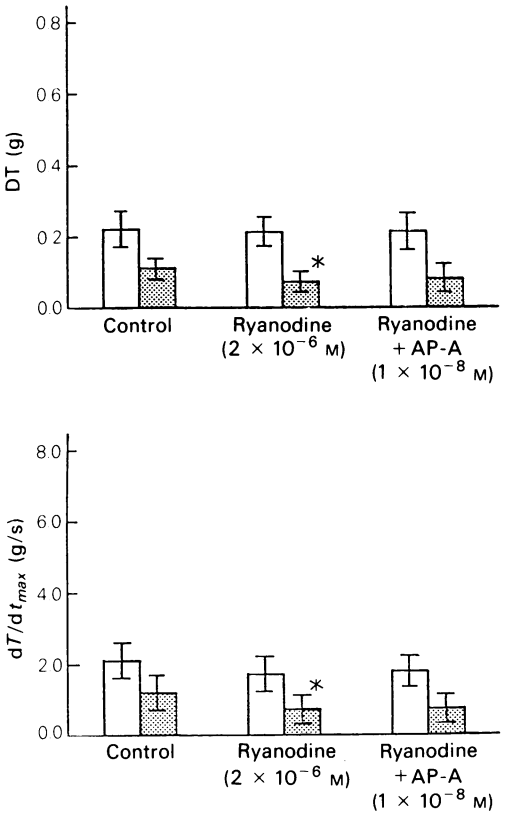

Figure 5 Influence of ryanodine on the positive inotropic effect of anthopleurin-A (AP-A). Mean values were obtained from four preparations when they were driven at 1.0 Hz (open columns) and 0.2 Hz (stippled columns), under control conditions, 30 min after addition of ryanodine (2×10^{-6} M), and 30 min after subsequent additional application of AP-A (1×10^{-8} M). Vertical lines indicate s.d.* Significantly different from the control values at $P < 0.05$.

(Kodama *et al.*, 1980), a positive inotropic effect of AP-A was always accompanied by a prolongation of APD, especially at the later stage or repolarization. Therefore, it was assumed that mechanisms delaying the repolarization process are correlated to the positive inotropic effect of AP-A. In the present study, supporting evidence for this assumption was provided by the experiments with TTX and lidocaine. Thus, these drugs, which effectively inhibited the APD prolongation induced by AP-A, likewise inhibited its positive inotropic effect. A high concentration (1×10^{-7} M) of AP-A was still able to cause an apparent increase both in contractile force and APD even in the presence of TTX (2×10^{-6} M). This may be explained by a fraction of sodium channels remaining unblocked by TTX sufficient to mediate the effects of the high concentration of AP-A.

From the findings discussed above it seems reasonable to suggest that the positive inotropic effect of AP-A is primarily mediated by altered kinetics (delayed inactivation) of the fast sodium channel in the cardiac cell membrane. Nevertheless, a further mechanism, translating the modified electrical and ionic events at the cell membrane (sarcolemma) into the positive inotropic response, remains to be elucidated. Three possibilities can be considered: (1) APD prolongation may induce an increase of calcium influx during the plateau phase of the action potential through the electrogenic slow inward current or through Ca^{2+}-K^+ exchange mechanism (Morad & Goldman, 1973; Carmeliet & Vereecke, 1979); (2) the delayed inactivation of the fast sodium inward current would increase the sodium influx and intracellular sodium concentration, which may in turn enhance the trans-sarcolemmal calcium influx through the Na^+-Ca^{2+} exchange mechanism (Reuter, 1974); (3) prolonged and increased sodium influx during excitation may induce a more effective release of calcium for contraction through some intracellular mechanism, such as Na^+-Ca^{2+} competition for their binding site (Vassort, 1973).

Among these possibilities the former two seem to be less likely for the following reasons. Firstly, APD

Table 4 Effects of anthopleurin-A (AP-A) on membrane action potential in the presence of ryanodine

	APD 30 (ms)		*APD 80 (ms)*		dV/dt_{max} (V/s)	
	1.0 Hz	0.2 Hz	1.0 Hz	0.2 Hz	1.0 Hz	0.2 Hz
Control	151 ± 33	116 ± 32	208 ± 28	172 ± 25	164 ± 15	170 ± 37
Ryanodine (2×10^{-6} M)	173 ± 43*	134 ± 18	227 ± 44*	178 ± 7	174 ± 34	180 ± 30
Ryanodine (2×10^{-6} M) + AP-A (1×10^{-8} M)	235 ± 37†	827 ± 302†	372 ± 91†	1984 ± 762†	168 ± 26	192 ± 45

Values are mean ± s.d. of four preparations, under control conditions, 30 min after addition of ryanodine (2×10^{-6} M), and 30 min after subsequent additional application of AP-A (1×10^{-8} M). Measurements were performed at steady state with driving frequency of 1.0 Hz and 0.2 Hz. Abbreviations are the same as in Table 3. *Significantly different from the control value at $P < 0.05$. †Significantly different from the value obtained 30 min after addition of ryanodine (2×10^{-6} M) at $P < 0.05$.

prolongation of ventricular muscle by AP-A was more marked at the late stage of repolarization, whereas APD at the early stage around 0 mV, where electrogenic calcium inward current was found to be most prominent (Carmeliet & Vereecke, 1979), was sometimes shortened by AP-A (Kodama *et al.*, 1980). Secondly, the positive inotropic effect of AP-A on atrial muscle of rabbit and guinea-pig was found to be relatively resistant to calcium antagonistic agents such as verapamil, nifedepine or lanthanum, or to extracellular calcium depletion (Shibata *et al.*, 1976; 1978). In the present study, the positive inotropic effect of AP-A on rabbit ventricular muscle was not inhibited by verapamil, nifedipine or extracellular calcium depletion, provided that the preparation was driven at a lower frequency (0.2 Hz). Thirdly, Bailey, Shibata, Seriguchi & Dresel (1980) found that AP-A had no detectable effect on the rate of calcium influx or the tissue content of calcium in kitten hearts. Thus, all the findings available up to this time suggest that the positive inotropic effect of AP-A does not depend on the increase of trans-sarcolemmal calcium influx either through electrogenic slow inward current or Ca^{2+}-K^+, Ca^{2+}-Na^+ exchange mechanism.

Shibata *et al.* (1978) and Bailey *et al.* (1980) showed that the positive inotropic effect of AP-A was considerably reduced by ryanodine or dantrolene, agents which are known to interfere selectively with the release of calcium from the sarcotubular system. The present findings with ryanodine are in agreement with theirs, and have also revealed that only the mechanical effect and not the electrical effect of AP-A is antagonized by ryanodine. These results suggest that intracellular translocation of calcium plays an important role in the positive inotropic effect of AP-A, and further, they provide some support for the third possibility.

The force-frequency relationship in the preparation treated by AP-A might also reflect on action of this drug on intracellular calcium kinetics. In mammalian cardiac muscle, the phasic contraction is elicited by calcium released mainly from the intracellular storage site (activator pool) (Morad & Goldman, 1973; Allen, Jewell & Wood, 1976). The replenishment of this activator pool is considered to occur partly by trans-sarcolemmal calcium influx during the action potential and partly by intracellular recirculation of calcium. The force-frequency relationship of cardiac muscle is, therefore, affected by the relative contribution of these two components for the replenishment of the activator pool. An increase in frequency will usually increase the duration of membrane depolarization per unit of time, which could lead to an increased calcium influx through the cell membrane resulting in a greater release of activator calcium for contraction (Morad & Goldman, 1973; Edman & Johannson, 1976). On the other hand, a faster frequency above some optimal level tends to inhibit the replenishment of the activator pool through the recirculation component, because a considerably longer time is required for the calcium in the cytosol to be sequestered and transported into the releasing site of the activator pool (Morad & Goldman, 1973; Edman & Johannson, 1976). Our finding that rabbit ventricular muscles treated by AP-A showed a negative staircase of contractile force at driving frequencies from 0.2 to 2.0 Hz could be explained by the increase of the recirculation component for replenishment of the activator pool, as in the case of rat ventricular muscles (Henderson, Brutsaert, Forman & Sonnenblick, 1974; Boden & Sonnenblick, 1975). The fact that AP-A failed to exert a positive inotropic effect at the lowest frequency (0.017 Hz) is also compatible with such as assumption, because the calcium stored in the activator pool is considered to be extruded almost completely into the extracellular space when the muscle is allowed to rest for a long interbeat interval such as 60 s (Allen *et al.*, 1976).

Thus, from the evidence presented here, we conclude that AP-A causes a prolonged and increased sodium influx through the delayed inactivation of fast sodium inward current; and this primary effect may elicit not only the prolongation of APD but also an alteration of intracellular calcium kinetics resulting in more effective release of calcium for contraction from the activator pool.

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