Arachidonic acid (AA) is a 20-carbon unsaturated fatty acid synthesised from cell membrane glycerophospholipids via activation of phospholipase A2. AA may subsequently be further metabolised to form additional chemical mediators. Examples of metabolic modification of AA are oxidation via the cyclo-oxygenase pathway to form prostanoids, action of the 5-lypoxygenase pathway to form leukotrienes and oxidation by the cytochrome P450 mono-oxygenase (epoxygenase) system to form a variety of metabolites including epoxyeicosatrienoic acids and their respective diols.

A variety of inotropic actions of AA have been described: a decrease in contraction has been reported for hamster papillary muscle (Finkel et al. 1992) and increases in contraction have been observed in rat papillary muscle and isolated ventricular myocytes (Otani et al. 1988; Damron et al. 1995). These inotropic actions of AA are thought to be mediated in part through changes in the amplitude of cytosolic calcium transients. In the case of rat myocytes exposed to AA, both an enhancement (Damron & Summers, 1997) and a reduction in the amplitude of calcium transients has been described in adult cells (Damron & Bond, 1993), and a reduction in response to AA has been reported for neonatal rat myocytes (Hoffmann et al. 1995). These changes appeared not to be mediated through cyclo-oxygenase, lypoxygenase or epoxygenase metabolites of AA.

Changes in the amplitude of cytosolic calcium transients following exposure to AA may occur in part as a consequence of corresponding changes in calcium entry through L-type calcium channels. An inhibition of L-type calcium currents has been reported in guinea-pig atrial (Cohen et al. 1990) and frog ventricular myocytes (Petit-Jaques & Hartzell, 1996). In contrast, Hoffmann et al. (1995) reported that the reduction in the amplitude of cytosolic calcium transients in neonatal rat ventricular myocytes observed following treatment with AA was not associated with changes in calcium entry. In addition, an enhancement in calcium entry through L-type calcium channels has also been reported in guinea-pig isolated ventricular myocytes (Huang et al. 1992).

There is thus conflicting evidence concerning whether the amplitude of calcium transients and contractions are increased or decreased by AA. Previously published investigations of calcium transients have all been carried out in rat cells which are known to show action potentials with a ‘plateau’ which is very negative compared to most other mammalian species, with associated consequences for calcium handling.

The present study is the first to combine investigations of AA on action potentials, calcium transients, contraction and ionic currents in mammalian myocytes from the guinea-pig, a species which, like human, has an action potential with a plateau at positive potentials.
In addition to the actions of AA on calcium transients and contractions accompanying stimulated action potentials, we also present evidence of the effects of AA under conditions where spontaneous electrical activity associated with SR calcium overload occurs. This may lead to further insights into the antiarrhythmic mechanisms of AA and its metabolites observed during myocardial ischaemic episodes (Mest et al. 1987; Parrat et al. 1987).

METHODS

Myocyte isolation

Experiments were performed on single cardiac ventricular myocytes isolated from guinea-pigs. The animals were stunned and then killed by cervical dislocation. Hearts were rapidly excised and single cells were enzymatically isolated as described previously (Powell et al. 1980; Mitchell et al. 1984). All experiments were carried out on rod-shaped intact myocytes with visible cross-striations.

Solutions and drugs

Cells were superfused with a solution of composition (mM): NaCl 118.5, NaHCO3 14.5, KCl 4.2, KH2PO4 1.18, MgSO4 1.18, CaCl2 2.5 and glucose 11.1; gassed with 95% O2-5% CO2 to maintain a pH of 7.2. All experiments were carried out at 36°C. Electrical recordings were made with single barreled microelectrodes containing 1 M KCH3SO4 and 10 mM KCl. Arachidonic acid and ETYA (5,8,11,14-eicosatetraynoic acid) were obtained from Sigma, and were dissolved in 50% DMSO to form 20 mM stock solutions which were stored at _20°C until required. Trihydroindomethacin was dissolved in ethanol to form a 10 mM stock solution, and stored at 0°C. Ro31-8220 was a kind gift from Roche Pharmaceuticals, and was dissolved in DMSO to form a 10 mM stock solution, and was also stored at 0°C until required.

Cell length measurements

Cell length was measured from the video image of cells viewed microscopically using an edge detection system (PCX video acquisition card, and software for cell length measurement, developed by Brian Reece Scientific, Newbury, UK). The interlacing of two fields for each video frame (video rate of 25 frames s^-1) allowed a temporal resolution of 20 ms per point.

Electrophysiology

Action potentials were stimulated by current pulses (2 ms in duration, 2–5 nA in amplitude) applied via an intracellular microelectrode at a frequency of 1 Hz (Axoclamp-2A micro-electrode system, bridge mode (Axon Instruments). Voltage-clamp experiments were also carried out using a discontinuous single electrode voltage-clamp system, in which the function of the electrode is rapidly switched from a current passing to a voltage recording mode at a frequency of 3–5 kHz (Wilson & Goldner, 1975).

L-type calcium currents were activated by step depolarisations from a holding potential of −40 mV to potentials between −40 and +40 mV for 200 ms at 0.3 Hz. Delayed rectifier potassium currents (Ik) were activated by step depolarisations from −40 to +40 mV for 10–800 ms and were measured as outward tails upon repolarisation to −40 mV (with 50 mM BAPTA in the electrode solution to suppress cytosolic calcium transients). Ik was measured as the peak tail current that deactivated on repolarisation; measurement of currents during the depolarising pulses was avoided (see Heath & Terrar, 1996) since these may be contaminated by several additional currents, including the sodium–calcium exchange current, a calcium-activated non-specific current and a calcium-activated chloride current (Ehara et al. 1988; Beukelmann & Weir, 1989; Terrar & White, 1989b; Zygmut & Gibbons, 1991; Sipido et al. 1993).

Calcium transients were constructed from the magnitude of calcium-activated tail currents following interruption of the action potential by a voltage clamp to −70 mV at time intervals between 10 and 200 ms after the upstroke of the action potential (Terrar & White, 1989a,b; Egan et al. 1989; White & Terrar, 1990a,b). These calcium-activated tail currents are believed to be carried predominantly by electrogenic Na⁺–Ca²⁺ exchange operating in calcium extrusion mode although a contribution from calcium-activated non-specific cation conductances may also be present. The magnitude of the peak of this current is believed to reflect the level of cytosolic calcium at the time of application of the voltage clamp, and interruption of the action potential at varying times after its initiation therefore allows the construction of a calcium transient from the magnitudes of these calcium-activated currents. At least 10 full action potentials were allowed between abbreviated action potentials to ensure adequate reloading of sarcoplasmic reticulum (SR) stores since interruption of an action potential by a voltage clamp to −70 mV would shift the balance of calcium-sequestering systems away from uptake into the SR and towards extrusion via Na⁺–Ca²⁺ exchange and hence lead to a depletion of calcium from the SR (see Terrar & White, 1989a).

In cells stimulated to fire action potentials at a rate of 1 Hz, spontaneous activity was induced by application of 40 nM isoprenaline, which caused delayed after-depolarisations and in most cells additional spontaneous action potentials. Spontaneous activity was also investigated under voltage-clamp conditions in cells held at −70 mV with trains of 15 depolarisations to +40 mV applied at an inter-pulse interval of 300 ms and cycle time of 10 s. Using this protocol, spontaneous activity was also provoked by exposure of cells to the Na⁺ pump inhibitor ouabain (1 µM; applied for 3–6 min and then washed away). This procedure was chosen since it was found that continuous superfusion with 1 µM ouabain throughout the course of the experiment did not produce 'stable' oscillatory behaviour using this voltage-clamp protocol, but caused the generation of oscillations which increased in severity with time, resulting in the eventual deterioration of the cell. In contrast, oscillations induced by short exposure to ouabain, were found to be stable for at least 10 min following washout of ouabain in the absence of AA.

Statistical analysis

Results are presented as means ± s.e.m. Student’s paired t test was used to assess statistical significance (P < 0.05 was considered to be significant).

RESULTS

Influence of arachidonic acid on contraction amplitude and action potential duration

In the absence of any drugs, myocyte contraction amplitude and action potential duration was well maintained over a period of 10 min. Contraction amplitude was 101 ± 2% (n = 7 cells) and the action potential duration at 20% repolarisation (APD20) was 103 ± 4% and at 90% repolarisation (APD90) was 102 ± 2% of the initial values. None of the changes were significant (P > 0.05).
When myocytes were exposed to AA at a concentration of 1–50 µM for 10 min, there was a dose-dependent reduction in the amplitude of the contraction (Fig. 1A). At 50 µM, AA abolished the contraction within 5–7 min and extensive ‘blebbing’ of the cells was observed. AA (10 µM) reduced myocyte contraction amplitude by 39 ± 6% after 10 min (n = 6; P < 0.05; Fig. 1B) without visible signs of cell deterioration. A dose of 10 µM AA was therefore used for all subsequent experiments in the present study. Figure 1C shows records from an individual cell, and it can be seen that the duration of the action potential associated with the contraction was also significantly reduced. In the six cells investigated, APD20 was reduced by 12 ± 2% and APD90 by 15 ± 2% of control (P < 0.05).

In approximately 50% of the cells investigated, a second rapid phase of action potential shortening was observed between 9 and 15 min after exposure to AA in which APD90 was reduced by more than 90%, and often action potentials could not be stimulated to fire, even with an increase in stimulus amplitude (Fig. 1D). This may be related to the activation of a large conductance potassium current (approximately 150 pS) by arachidonic acid (IK,AA) as previously described in rat atrial (Kim & Clapham, 1989) and ventricular (Kim & Duff, 1990) myocytes, although it was not further investigated in the present study. In all subsequent experiments, action potential duration and contraction amplitude were measured before this second rapid phase of action potential shortening was observed.

Effects of arachidonic acid on calcium transient amplitude

Figure 2 illustrates the effects of 10 µM AA on the amplitude of calcium transients measured from the magnitude of calcium-activated tail currents (see Methods). Exposure of
myocytes to 10 µM AA was associated with a decline in calcium transient amplitude, as shown in Fig. 2A. In these experiments, peak calcium transient amplitude was reduced by 38 ± 4% (n = 8; P < 0.05) after 10 min exposure to AA. In contrast, Fig. 2B illustrates that in the absence of AA, the amplitude of cytosolic calcium transients was well maintained over a similar period of 10 min (peak calcium transient amplitude fell by 1 ± 2% of control after 10 min exposure to AA, n = 6, P > 0.05).

### Influence of AA on contraction and action potential duration in the presence of trihydroindomethacin

To investigate whether these negative inotropic actions of AA were mediated through its cyclo-oxygenase metabolites, the influence of AA on the myocyte contraction amplitude was investigated in the presence of trihydroindomethacin, an inhibitor of cyclo-oxygenase. An example of myocyte contraction and accompanying action potentials is illustrated in Fig. 3A, which shows that exposure to 10 µM AA in the presence of 10 µM trihydroindomethacin was again associated with a reduction in myocyte contraction amplitude and action potential shortening. These changes were observed consistently and in a series of similar experiments the mean contraction amplitude was reduced by 42 ± 4% (n = 6; P < 0.05) while APD$_{20}$ was reduced by 14 ± 3%, and APD$_{90}$ by 13 ± 4% of control. None of these changes was significantly different from those observed in the presence of AA alone (P > 0.05).

### Influence of AA on contraction and action potential duration in the presence of ETYA

Inotropic actions of AA were also investigated in the presence of 10 µM ETYA (5,8,11,14-eicosatetraynoic acid), a false substrate for AA-metabolising enzymes which serves to inhibit their enzymatic activities (Capdevila et al. 1988). Figure 3B shows an example of a myocyte exposed to 10 µM AA in the presence of 10 µM ETYA with a reduction in contraction amplitude. A series of similar experiments showed consistent changes, and 10 min after application of AA, mean contraction amplitude was reduced by 46 ± 3%, mean APD$_{20}$ was reduced by 13 ± 3%, and APD$_{90}$ by 16 ± 3% of values in the absence of drugs (n = 5, P < 0.05 for all three changes). None of these effects of AA in the presence of 10 µM ETYA were significantly different from those observed with exposure to AA alone (P > 0.05 for all three changes).

### Influence of AA on L-type calcium currents

To determine whether an influence of AA on calcium entry through L-type calcium channels might contribute to the effects reported above, L-type calcium currents ($I_{Ca}$) were...
recorded under voltage-clamp conditions. Figure 4A illustrates an example of an L-type calcium current recorded before and after exposure to 10 \( \mu M \) AA in a typical cell. It can be seen that following treatment of myocytes with 10 \( \mu M \) AA, a reduction in \( I_{Ca} \) was observed. Similar effects were seen in a series of experiments and the mean changes at a range of potentials between -40 and +40 mV are shown in Fig. 4AI. The mean peak \( I_{Ca} \) at 0 mV was reduced by 38 \( \pm \) 7\% (\( n=5 \), \( P<0.05 \)). In contrast, L-type calcium currents were well maintained over a similar time period in the absence of AA, as shown in Fig. 4B and B1 (mean peak \( I_{Ca} \) after 10 min showed an apparent increase of 2 \( \pm \) 3\%, \( n=5 \); \( P>0.05 \)).

**Influence of AA on the delayed rectifier potassium current (\( I_K \))**

The possibility that changes in the delayed rectifier potassium current (\( I_K \)) might also contribute to the action potential shortening following exposure to AA was also investigated. Outward tail currents were recorded on repolarisation from step depolarisations to +40 mV and Fig. 4C shows examples of records before and after exposure to 10 \( \mu M \) AA. It can be seen that following exposure of myocytes to 10 \( \mu M \) AA, there was a small enhancement of \( I_K \). The mean data over a range of pulse durations from 10 to 800 ms are presented in Fig. 4CI, which shows a mean enhancement of outward tail current of 14 \( \pm \) 4\% for step depolarisations of 400 ms (\( n=7 \); \( P<0.05 \)) after a 10 min exposure to AA.

**Influence of AA on contraction and action potential duration in myocytes prestimulated with isoprenaline**

Since it has been reported that exposure of frog ventricular myocytes to AA caused a greater inhibition of \( I_{Ca} \) after prestimulation of the cells with isoprenaline (Petit-Jaques & Hartzell, 1996), we investigated the actions of AA in guinea-pig cells following \( \beta \)-adrenoceptor stimulation. Figure 5A and A1 illustrates the influence of AA on contraction amplitude and action potential duration, respectively, in myocytes prestimulated with 5 nM isoprenaline. It can be seen that the reduction of contraction amplitude and the shortening of action potential duration following application of AA were not greatly different in myocytes prestimulated with 5 nM isoprenaline from the changes observed in the absence of isoprenaline. The mean reduction of contraction caused by 10 \( \mu M \) AA in the presence of 5 nM isoprenaline was 39 \( \pm \) 5\% (\( n=7 \); \( P<0.05 \)), while APD\(_{20}\) was reduced by 16 \( \pm \) 3\%, and APD\(_{90}\) by 12 \( \pm \) 3\% of control (\( P<0.05 \)). None of the changes following exposure to AA under conditions of isoprenaline prestimulation was significantly different from that observed

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**Figure 3**

Effects of 10 \( \mu M \) AA in the presence of 10 \( \mu M \) trihydroindomethacin (\( A \)), 10 \( \mu M \) ETYA (\( B \)) and 1 \( \mu M \) Ro31-8220 (\( C \)) on contraction amplitude and action potential duration in cells stimulated to fire action potentials at 1 Hz over a period of 10 min. Continuous line, control; dashed line, following 10 min exposure to AA. Effects of 10 \( \mu M \) AA in the presence of 10 \( \mu M \) trihydroindomethacin (\( A1 \)), 10 \( \mu M \) ETYA (\( B1 \)) and 1 \( \mu M \) Ro31-8220 (\( C1 \)) on action potential configuration in representative cells. ○, control action potential; ●, control action potential following application of AA for 10 min.
under basal conditions \((P > 0.05)\). In the absence of AA, contraction amplitude and action potential duration of myocytes prestimulated with 5 nM isoprenaline were well maintained over a similar period of time (after 10 min, contraction amplitude was 102 ± 5 % of control, \(n = 6, P > 0.05\); \(\text{APD}_{20}\) was 98 ± 4 % and \(\text{APD}_{90}\) 97 ± 3 % of control, \(n = 7, P > 0.05\)).

**Influence of AA on contraction amplitude and action potential duration in myocytes prestimulated with 8-Br cAMP**

In the following experiments we have investigated whether the negative inotropic actions of AA observed under conditions of isoprenaline prestimulation are mediated through an influence of AA on intracellular cAMP levels. Consequently, we have investigated the inotropic actions of AA in cells prestimulated with 8-Br cAMP, a non-hydrolysable analogue of cAMP. Figure 5B illustrates the influence of 10 \(\mu\)M AA on contraction amplitude and action potential duration in myocytes prestimulated with 8-Br cAMP, present in the electrode solution at a concentration of 1 mM. Exposure of myocytes prestimulated with 8-Br cAMP to 10 \(\mu\)M AA was associated with a reduction in contraction amplitude as shown in Fig. 5B, after 10 min, contraction amplitude was reduced by 33 ± 5 % (\(n = 6, P < 0.05\)). This was not significantly different from the reduction observed under conditions of isoprenaline prestimulation (\(P > 0.05\)). Furthermore, Fig. 5B1 illustrates that in the six cells investigated, action potential duration was reduced (\(\text{APD}_{20}\) to 86 ± 3 %, and \(\text{APD}_{90}\) to 85 ± 4 % of control; \(P < 0.05\)).

In contrast, in the absence of AA, contraction amplitude and action potential duration of myocytes prestimulated with 8-Br cAMP was well maintained over a similar period of time (after 10 min contraction amplitude was 103 ± 2 % of control, \(n = 6, P > 0.05\); \(\text{APD}_{20}\) was 105 ± 4 % and \(\text{APD}_{90}\) 97 ± 6 % of control).

**Figure 4**

Effects of 10 \(\mu\)M AA (A) and control solution (B) on typical L-type calcium currents activated by 200 ms step depolarisation from −40 to 0 mV. A1 and B1, mean data from five cells, \(I_{\text{Ca}}\) activated by step depolarisations from a holding potential of −40 mV to a range of potentials from −40 to +40 mV for 200 ms, following exposure to 10 \(\mu\)M AA (A1) and control solution (B1). Circles, peak inward current during pulse; diamonds, current at end of pulse. Filled symbols, control; open symbols, following a 10 min exposure to 10 \(\mu\)M AA. Peak inward current was significantly reduced from −20 to +40 mV (\(P < 0.05\)) following exposure to 10 \(\mu\)M AA. C, typical effects of 10 \(\mu\)M AA on \(I_{\text{K}}\) current activated by a 400 ms step depolarisation from −40 to +40 mV. C1, mean data from six cells, tail currents activated in the range 80–700 ms were significantly enhanced following exposure to AA (\(P < 0.05\)).
Influence of AA on calcium transient amplitude in myocytes prestimulated with isoprenaline

Figure 6 illustrates the effects of AA on the amplitude of calcium transients constructed from the magnitude of calcium-activated tail currents in cells prestimulated with 5 nM isoprenaline. The data presented in Fig. 6A illustrate that exposure of myocytes prestimulated with 5 nM isoprenaline to 10 µM AA was associated with a suppression of calcium transient amplitude; after 10 min peak calcium transient amplitude was reduced by 32 ± 5% (n = 4; P < 0.05). In comparison, Fig. 6B illustrates that calcium transients from cells prestimulated with 5 nM isoprenaline were well maintained over a period of 10 min in the absence of AA; after 10 min, peak calcium transient amplitude was 105 ± 3% of control (n = 5, P > 0.05).

Influence of AA on isoprenaline-prestimulated L-type calcium currents

Figure 6C and D illustrates the influence of 10 µM AA on L-type calcium currents (I_{Ca}) in cells prestimulated with 5 nM isoprenaline. It can be observed in Fig. 6C that following prestimulation with isoprenaline, exposure of a typical cell to 10 µM AA was associated with a reduction in I_{Ca}. Mean data are shown in Fig. 6D; a 10 min exposure to AA significantly reduced the peak amplitude of I_{Ca} in isoprenaline-prestimulated cells by 49 ± 7% (n = 5, P < 0.05). This was not significantly different from the reduction in the peak amplitude of I_{Ca} observed under control conditions following exposure to AA (P > 0.05).

Effects of AA on isoprenaline-induced oscillations in cells stimulated to fire action potentials

Since AA was observed to influence the amplitude of calcium transients under the conditions of our experiments, the possibility that AA might influence spontaneous calcium oscillations was also investigated. In the first series of experiments SR calcium overload and consequent oscillations were induced by exposure to high concentrations of the β-adrenergic agonist isoprenaline. Figure 7 illustrates the effects of 10 µM AA on spontaneous electrical activity recorded in a representative cell exposed to 40 nM isoprenaline and stimulated to fire action potentials at 1 Hz. In the absence of any drugs, only stimulated action potentials were recorded and no spontaneous electrical activity was observed. Subsequent exposure to 40 nM isoprenaline resulted in the appearance of spontaneous action potentials between
the stimulated action potentials, as shown in Fig. 7A. The data presented in Fig. 7A illustrate that exposure of this cell to 10 µM AA completely abolished spontaneous electrical activity within 7 min, whereas the generation of stimulated action potentials was not affected. Similar experiments were conducted in a total of nine cells, and in six of these cells spontaneous electrical activity induced by 40 nM isoprenaline was completely abolished within 3–10 min following exposure to AA. In the remaining three cells, spontaneous activity was markedly suppressed, so that relatively infrequent after-depolarisations remained after treatment with AA for 10 min.

In comparison, Fig. 7B illustrates that the spontaneous electrical activity provoked by 40 nM isoprenaline was well maintained over a period of 10 min in the absence of AA. In six such cells little or no change in spontaneous electrical activity was observed over a period of 10 min in the absence of AA.
The effects of $10\,\mu M$ AA on the frequency of occurrence of isoprenaline-induced spontaneous electrical activity may be summarised as follows: within 10 min of exposure to AA, the number of spontaneous events recorded over a 5 s period was significantly reduced from $4.7 \pm 0.1$ to $0.4 \pm 0.2$ ($P < 0.05, n = 9$). In contrast, in the absence of AA, the number of spontaneous events recorded over a 5 s period was well maintained over a period of 10 min ($4.4 \pm 0.2$ to $4.7 \pm 0.2; P > 0.05, n = 6$).

**Effects of AA on ouabain-induced oscillations under voltage-clamp conditions**

In a further series of experiments, the influence of AA on ouabain-induced oscillations was investigated under voltage-clamp conditions. Cells were voltage-clamped at $-70\,mV$ and trains of 15 depolarisations to $+40\,mV$ were applied, with an inter-pulse interval of 300 ms and a cycle time of 10 s. Figure 8 illustrates the effects of $10\,\mu M$ AA on spontaneous electrical activity induced by $1\,\mu M$ ouabain using this protocol. In all cells studied, superfusion of cells with $1\,\mu M$ ouabain for 3–5 min resulted in the appearance of a transient inward current during the rest period, as illustrated by the representative cell presented in Fig. 8A. These transient inward currents observed following administration of $1\,\mu M$ ouabain are believed to occur as a consequence of spontaneous release of calcium from the Ca$^{2+}$-overloaded SR, and are thought to be carried predominantly by sarcolemmal Na$^+$–Ca$^{2+}$ exchange (operating in calcium extrusion mode). Figure 8A1 illustrates that subsequent exposure of this cell to $10\,\mu M$ AA was associated with a complete suppression of this transient inward current within 5 min. A similar complete suppression of transient inward currents was observed within 5–10 min in all five cells investigated. In contrast, Fig. 8B illustrates that in the absence of AA, the transient inward currents induced by $1\,\mu M$ ouabain were well maintained over a period of 10 min. Similar experiments were carried out in a total of six cells in which little or no change in spontaneous electrical activity was observed.

The effects of $10\,\mu M$ AA on the frequency of occurrence of ouabain-induced transient inward currents may be summarised as follows: within 10 min of exposure to AA, the number of transient inward currents recorded between five trains of voltage-clamp pulses was significantly reduced from $5.2 \pm 0.1$ to $0.2$ ($P < 0.05, n = 5$). In contrast, in the absence of AA the number of transient inward currents recorded between five trains of voltage-clamp pulses was well maintained over a period of 10 min ($5.3 \pm 0.2$ to $5.2 \pm 0.2; P > 0.05, n = 6$).

**DISCUSSION**

The main findings of the present paper are that AA at a concentration of $10\,\mu M$ caused a shortening of guinea-pig action potentials, accompanied by a reduction in calcium currents, calcium transients and contraction. We have also observed suppression of spontaneous electrical activity induced by ouabain or isoprenaline. To our knowledge, this is the first report of the inotropic actions of arachidonic acid in guinea-pig isolated ventricular myocytes and the first to combine investigations of AA on action potentials, calcium transients, contraction and ionic currents in mammalian myocytes from the guinea-pig, a species which, like human, shows an action potential plateau at positive potentials.

Under the conditions of our experiments, these negative inotropic actions of AA appeared not to be mediated by its cyclo-oxygenase, lypoxygenase or epoxygenase metabolites since the inotropic actions of AA were not significantly altered in the presence of the cyclo-oxygenase inhibitor trihydroindomethacin or ETYA, a false substrate for AA-metabolising enzymes which serves to inhibit their function. It has previously been reported that AA may mediate some of its biological actions through a direct activation of protein kinase C (PKC) (Linden & Routtenberg, 1989; Damron & Bond, 1993). However, it seems unlikely that AA mediates its inotropic actions through the activation of PKC under the conditions of our experiments since the presence of the...
selective PKC inhibitor Ro31-8220 did not significantly influence the response to AA.

Our results confirm the negative inotropic actions of AA reported in hamster papillary muscles (Finkel et al. 1992). In contrast, increases in the amplitude of contraction have been observed in rat papillary muscle and isolated ventricular myocytes (Otani et al. 1988; Damron et al. 1995; Damron & Summers, 1997). These differences may represent a genuine species difference between the actions of AA in rat ventricular myocytes and guinea-pig ventricular myocytes. Damron & Summers (1997) reported an enhancement in calcium transient amplitude in rat ventricular myocytes following exposure to AA, but reductions in calcium transients, similar to the observations presented in the present investigation, have also been reported in other studies in rat cells (Damron & Bond, 1993; Hoffmann et al. 1995). These effects were similarly insensitive to inhibitors of the cyclo-oxygenase, lypoxygenase and epoxygenase pathways in both adult (Damron & Bond, 1993) and neonatal (Hoffmann et al. 1995) rat ventricular myocytes.

In the present study we have used calcium-activated tail currents to monitor changes in intracellular calcium during the course of a cardiac action potential (Terrar & White, 1989a, b; White & Terrar, 1990a, b; Rakovic et al. 1996). Ca²⁺-activated tail currents are believed to be carried predominantly by Na⁺–Ca²⁺ exchange in calcium extrusion mode (Terrar & White, 1989a, b) and it is important to consider the possibility that the reduction in the Ca²⁺-activated tail current amplitude observed following exposure to AA may occur as a consequence of a direct inhibition of Na⁺–Ca²⁺ exchange by AA. However, such a hypothesis does not seem likely since it has been previously reported that AA enhances Na⁺–Ca²⁺ exchange (Philipson & Ward, 1985), which would, if anything, lead to an underestimation of the observed reduction in amplitude of the cytosolic calcium transient constructed from the magnitudes of calcium-activated tail currents (carried predominantly by Na⁺–Ca²⁺ exchange).

We have observed a shortening in action potential duration following exposure to AA which appeared to be mediated in part through a reduction in the L-type calcium current (I_{Ca,L}), and possibly through a small but significant enhancement in the delayed rectifier potassium current. This is the first report regarding the actions of AA on the delayed rectifier potassium current. In contrast, Bogdanov et al. (1998) demonstrated a prolongation of action potential duration following exposure to AA in rat ventricular myocytes mediated predominantly through an inhibition of the transient outward current (I_{to}). It will be interesting to test in future studies whether this species difference between rat and guinea-pig might contribute to the positive inotropic effect and increases in calcium transients which have been observed in some experiments on rat myocytes (Otani et al. 1988; Damron et al. 1995; Damron & Summers, 1997).

A similar reduction in I_{Ca,L} to that reported in the present study has been previously described in guinea-pig atrial myocytes (Cohen et al. 1990) and frog ventricular myocytes (Petit-Jaques & Hartzell, 1996) following exposure to AA, which was not mediated through its metabolites. Petit-Jaques & Hartzell (1996) demonstrated that this inhibition of I_{Ca,L} by AA was mediated in part by the AA-dependent activation of a protein phosphatase, leading to a channel dephosphorylation. It is interesting to note that Petit-Jaques & Hartzell (1996) observed that the degree of inhibition of I_{Ca,L} by AA was much greater after prestimulation with isoprenaline or cAMP than under basal conditions. Channels would be in a more...
phosphorylated state following prestimulation with isoprenaline or cAMP, hence channel dephosphorylation by activation of a phosphatase may be expected to have greater functional consequences on \( I_{\text{Ca}} \) after prestimulation with isoprenaline or cAMP. In contrast, we have observed an approximately similar degree of inhibition of \( I_{\text{Ca}} \) following exposure to AA in control and isoprenaline-stimulated cells, which may appear surprising if activation of a phosphatase was the main mechanism of AA-mediated regulation of the L-type calcium current in guinea-pig ventricle. Consequently, the importance of this mechanism requires further investigation.

AA may also reduce L-type calcium currents through a direct action on the channel protein, and indeed Finkel et al. (1992) demonstrated that AA competed with the radiolabelled carrier, and so it would be of interest to investigate whether this would significantly alter the response to AA.

During the later stages of exposure to AA (between 9 and 15 min), a second rapid phase of action potential shortening was observed in which APD\(_{90}\) was reduced by more than 90\%, and action potentials could not be stimulated to fire, even with an increased stimulus, in approximately 50\% of the cells investigated. This phase of action potential shortening was still observed in the presence of inhibitors of AA metabolism and protein kinase C. Although we have not investigated the mechanisms which underlie this rapid phase of action potential shortening, an interesting hypothesis is that it may be related to a direct activation of a large conductance potassium current (150 pS) by AA (\( I_{\text{K,AA}} \)) as previously described in rat atrial (Kim & Clapham, 1989) and ventricular (Kim & Duff, 1990) myocytes, although further investigations are required to address this issue.

The observed reduction in calcium entry through L-type calcium channels would be expected to contribute to the reduction in calcium transient amplitude observed following exposure to AA by reducing the release of calcium from SR stores as a consequence of the reduced amplitude of the calcium trigger and a reduced calcium loading of SR stores. AA may also influence the amplitude of cytosolic calcium transients through direct actions on SR calcium stores: for example Hoffmann et al. (1995) have demonstrated a reduction in the calcium content of SR stores in neonatal rat ventricular myocytes following treatment with AA, which is independent of changes in calcium entry. Furthermore, AA has been demonstrated to inhibit the activity of the SR Ca\(^{2+}\)-ATPase pump (Swanson et al. 1989), which may contribute to such a reduced calcium content of SR stores. In addition, using \(^{45}\)Ca\(^{2+}\)-loaded canine SR microsomes, Dettbarn & Palade (1993) demonstrated that AA enhances Ca\(^{2+}\) efflux from the SR, which was partially inhibited by the SR ryanodine receptor blocker rutenium red. Indeed Uehara et al. (1996) have demonstrated that AA binds close to the ryanodine binding site of the ryanodine receptor.

It has also been demonstrated that AA inhibits the voltage-sensitive sodium current (\( I_{\text{Na}} \)) in neonatal rat cardiac myocytes (Xiao et al. 1995) by directly binding to the sodium channel (Kang & Leaf, 1996). Such an inhibition of \( I_{\text{Na}} \) would lead to a secondary reduction in intracellular sodium activity, and thereby enhance the extrusion of calcium via sarcolemmal Na\(^{+}\)-Ca\(^{2+}\) exchange. This would shift the balance from Ca\(^{2+}\) uptake into the SR to Ca\(^{2+}\) extrusion across the sarcolemma via Na\(^{+}\)-Ca\(^{2+}\) exchange, which may also contribute to the reduction in the Ca\(^{2+}\) content of SR stores observed following exposure to AA.

Under conditions of \( \beta \)-adrenergic prestimulation with isoprenaline, we have also observed a reduction in myocyte contraction amplitude which was associated with a decline in amplitude of the cytosolic calcium transient and the L-type calcium current following exposure to AA. The inotropic actions of AA observed after prestimulation with isoprenaline are unlikely to be mediated at the level of the \( \beta \)-receptor, G-protein or adenylate cyclase since a negative inotropic effect of AA was also observed in myocytes prestimulated with 8-Br cAMP. In addition, it is unlikely that AA mediates its negative inotropic actions in isoprenaline-prestimulated myocytes through a mechanism which involves a reduction in intracellular cAMP (i.e. by activation of a phosphodiesterase) since the negative inotropic effect of AA was observed in myocytes prestimulated with 8-Br cAMP, a non-hydrolysable analogue of cAMP.

Exposure to AA abolished the spontaneous activity associated with exposure to ouabain and isoprenaline under the conditions of our experiments. This is the first report investigating the influence of AA on spontaneous electrical activity in guinea-pig ventricular myocytes. The reduction in calcium entry through L-type calcium channels that we have observed following exposure to AA would reduce calcium loading of SR stores and so decrease the gain of calcium-induced calcium release, hence reducing the probability of spontaneous calcium release from the SR under conditions of calcium overload. Such a mechanism may contribute to the observed suppression of ouabain or isoprenaline-induced spontaneous activity following exposure to AA under the conditions of our experiments. However, additional direct actions of AA on SR calcium loading mediated through a reduced calcium uptake (Swanson et al. 1989) and/or an enhanced calcium efflux (Dettbarn & Pallade, 1993) cannot be ruled out.

In summary, we have observed a reduction in amplitude of both contraction and calcium transients following exposure of myocytes to AA, which appeared not to be mediated through the metabolites of AA under the conditions of our experiments. These changes may occur in part as a consequence of a reduced calcium entry through L-type calcium channels, although direct actions on SR stores such as a reduced calcium uptake and/or an enhanced calcium efflux from stores remains possible.
In addition, we have observed that AA suppresses spontaneous electrical activity recorded under conditions of calcium overload, induced by ouabain or isoprenaline. This may be related to a reduced calcium loading of SR stores occurring as a consequence of a reduced calcium entry, or possibly through a direct action of AA on SR stores, such as a reduced uptake and/or enhanced leak of calcium.


