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Electrophysiological effects of adenosine and adenosine triphosphate on sheep Purkinje fibres under normal and simulated ischaemic conditions

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1 The electrophysiological effects of adenosine and adenosine triphosphate (ATP) were examined in sheep Purkinje fibres, superfused *in vitro* with either a normal or a hypoxic, hyperkalaemic and acidotic physiological salt solution (PSS). The ability of adenosine to modify the effects of noradrenaline on action potential characteristics was also investigated.

2 The only statistically significant effects of adenosine (10^{-6}–10^{-4} M) and of ATP (10^{-6}–10^{-4} M) on normal action potential characteristics were a slight dose-dependent shortening of the action potential by adenosine and a depolarization by ATP, 10^{-4} M.

3 Superfusion with a hypoxic, hyperkalaemic and acidotic PSS caused marked reductions in resting membrane potential, upstroke and duration of the action potential.

4 Both adenosine and ATP attenuated the reduction in the rate of rise of the upstroke and the amplitude of the action potential caused by the modified PSS.

5 Adenosine did not alter the noradrenaline-induced effects on automaticity or on action potentials of normal or depressed Purkinje fibres.

6 Adenosine and ATP had electrophysiological effects on Purkinje fibres, exposed to conditions *in vitro* that mimic mild myocardial ischaemia, that were different from those observed on normally polarized fibres.

Introduction

It has been known for many years that adenosine and adenosine triphosphate (ATP) exert pronounced electrophysiological effects on the normal mammalian heart (Drury & Szent-Gyorgi, 1929). These effects include slowing of atrioventricular conduction (Urthaler & James, 1972), shortening of atrial action potential duration (Hollander & Webb, 1957) and modulation of the cardiac electrophysiological effects of catecholamines on Purkinje and ventricular tissue (Belardinelli *et al.*, 1982; Rardon & Bailey, 1984). Furthermore, in experimental animals, both adenosine (Fagbemi & Parratt, 1984; Parratt & Wainwright, 1985) and ATP (G. Boachie-Ansah, unpublished observations) protect the heart against ischaemia-induced ventricular arrhythmias. The mechanism(s) underlying this antiarrhythmic action of adenosine and ATP is not known, yet only one study appears to have examined the electrophysiological actions of adenosine on ischaemic cardiac tissue (Rosen *et al.*, 1983).

The aim of this work was to investigate the electrophysiological effects of adenosine and of ATP on sheep cardiac Purkinje cells exposed *in vitro* to conditions that mimic mild myocardial ischaemia. It has been shown that, during mild myocardial ischaemia *in vivo*, depressed Na^+-dependent action potentials occur (Downar *et al.*, 1977) and that these can be simulated *in vitro* by superfusion with a hypoxic, hyperkalaemic and acidotic physiological salt solution (Gilmour & Zipes, 1980). Therefore, the effects of adenosine and ATP on action potential characteristics of sheep Purkinje fibres superfused with such a modified PSS were examined and compared with those on normal fibres. In addition, the ability of adenosine to modify the cardiac electrophysiological effects of noradrenaline (Rardon & Bailey, 1984) was examined both under normal and simulated 'ischaemic' conditions. A preliminary account of these findings has been published (Boachie-Ansah *et al.*, 1986).

Methods

Action potential recording

Sheep hearts were obtained from a local abattoir and delivered in physiological salt solution (PSS) to the laboratory within 30 min of excision. Purkinje

fibres were pinned to the silastic base of the recording chamber and superfused at a rate of 5 ml min^{-1} with a normal PSS equilibrated with 95% O_2:5% CO_2. An equilibration period of about 1 h in normal PSS was allowed before beginning the experimental protocol. The composition of the normal PSS was as follows (mM): NaCl 125, $NaHCO_3$ 25, NaH_2PO_4 1.2, $MgCl_2$ 1.0, KCl 5.4, $CaCl_2$ 1.8 and glucose 5.5. That of the modified solution was as follows (mM): NaCl 141.5, $NaHCO_3$ 8.5, NaH_2PO_4 1.2, $MgCl_2$ 1.0, KCl 8.0, $CaCl_2$ 1.8, and glucose 5.5. The modified PSS was gassed with 95% N_2:5% CO_2, and this yielded a Po_2 and a pH in the organ bath of 33.9 ± 1 mmHg and 6.8 ± 0.01 u, respectively, compared with values in normal PSS of 419 ± 31 mmHg and 7.31 ± 0.02 u. The bath temperature was maintained at 36.5 ± 0.5°C.

The preparations were stimulated at a frequency of 1.5 Hz by rectangular pulses, 1 ms in duration and twice threshold voltage, delivered through a bipolar silver electrode. Transmembrane action potentials were recorded by conventional microelectrode techniques. The variables measured were as follows: resting membrane potential (RMP); action potential amplitude (APA); the maximum rate of depolarization of phase 0 (MRD), which was determined by an electronic differentiating circuit; and the action potential duration at 50 and 90% repolarization levels (APD_{50} and APD_{90}).

Experimental protocol

To observe drug effects on normal preparations, 8–10 action potentials were recorded before and 30–40 min after cumulative addition of the drug, dissolved in reservoirs of gassed normal PSS to obtain final bath concentrations detailed below. In a different set of fibres, the effects of modified PSS alone were measured; multiple action potentials were recorded before and at 30, 60, 90 and 120 min following exposure to the modified PSS. Drug-induced effects in the presence of modified PSS were examined following two protocols. In one, action potentials were recorded before and 30 min after superfusion with modified PSS alone and subsequently following the administration of two cumulative concentrations of the drug dissolved in modified PSS. Action potentials were again recorded 30 min after the addition of each concentration of the drug. In another series of experiments, drugs were administered prior to the introduction of the modified PSS. In this case, action potentials were recorded before and 30 min after the addition of one concentration of the drug dissolved in normal PSS and subsequently at 30 min intervals, over a 2 h period, following a changeover to the modified PSS containing the same concentration of the drug.

In order to study any adenosine-noradrenaline interaction, noradrenaline (10^{-5} M) was superfused for 30 min under normal or 'ischaemic' conditions followed by superfusion of adenosine (10^{-4} M) in the continued presence of noradrenaline. Action potential recordings were taken before, in the presence of noradrenaline and in the presence of both adenosine and noradrenaline. The frequency of spontaneous action potential initiation in the preparations was also measured in these experiments.

Drugs and concentrations

The following were used: adenosine (Sigma) 10^{-6}, 10^{-5} and 10^{-4} M; adenosine 5'-triphosphate (vanadium free) (ATP) (Sigma), 10^{-6}, 10^{-5} and 10^{-4} M; (−)-noradrenaline bitartrate (Sigma) 10^{-5} M.

Analysis of data

The mean values of measurements from each set of 8–10 multiple impalements were obtained and used to represent the data from each preparation. Data are reported either as mean values ± s.e.mean or mean percentage change from control values ± s.e.mean and are derived from 4–7 experiments. Multiple treatment and control mean values were analysed by a one-way Analysis of Variance and where the F-value permitted further analysis, individual treatment means were compared with respective control values by a Modified t test. For all other comparisons a two-tailed Student's t test was employed. $P < 0.05$ was considered to be statistically significant.

Results

Effects of adenosine and of ATP on normal action potential characteristics of sheep Purkinje cells

In the five preparations subjected to adenosine, control measurements of the variables were as follows: resting membrane potential −86.9 ± 0.3 mV, action potential height 109 ± 1.5 mV, maximum rate of depolarization of phase 0 (MRD) 427 ± 23 V s^{-1}, APD_{50} 155.2 ± 10.1 ms and APD_{90} 232.0 ± 9.3 ms. Adenosine (10^{-6}, 10^{-5} and 10^{-4} M) caused no statistically significant changes in resting membrane potential, action potential amplitude or in MRD. A slight concentration-dependent shortening of action potential duration, measured at both 50 and 90% repolarization, was observed following exposure to adenosine. The respective percentage reductions from the control APD_{90} were 3 ± 0.8, 6 ± 1.2 and 11 ± 0.5 ($P < 0.05$) following adenosine 10^{-6}, 10^{-5} and 10^{-4} M. Similar percentage reductions in APD_{50} were observed.

Table 1 The effects of the 'ischaemic' (i.e. hypoxic, hyperkalaemic, and acidotic) physiological salt solution on action potential characteristics of sheep Purkinje fibres

	Control	*Time post-ischaemia* (min)			
		30	60	90	120
RMP (mV)	−87.2 ± 0.8	−78.3 ± 0.4*	−78.1 ± 0.5*	−78.1 ± 0.3*	−77.5 ± 0.4*
MRD (Vs^{-1})	523 ± 51	261 ± 27*	231 ± 17*	196 ± 16*	195 ± 18*
APA (mV)	119.4 ± 1.4	86.6 ± 3.8*	82.4 ± 3.4*	79.9 ± 3.8*	80.0 ± 3.9*
APD_{50} (ms)	158.0 ± 10.6	106.1 ± 11.5*	98.1 ± 12.5*	96.8 ± 11.6*	97.6 ± 11.4*
APD_{90} (ms)	234.0 ± 15.6	170.1 ± 13.7*	165.5 ± 14.5*	164.3 ± 14.9*	165.2 ± 14.7*

*$P < 0.05$ significantly different from control value; $n = 7$
RMP, resting membrane potential; MRD, maximum rate of depolarization of phase 0; APA, action potential amplitude; APD, action potential duration at 50% repolarization level; APD_{90}, action potential duration at 90%, repolarization level

In five preparations exposed to ATP, control measurements similar to those detailed above were obtained. The only statistically significant effect observed of ATP (10^{-6}–10^{-4} M) was a slight depolarization which was significant at 10^{-4} M ATP (−84.4 ± 0.4 mV compared with a control value of −87.9 ± 0.4 mV). ATP-induced effects on action potential duration were inconsistent in that in 3/5 preparations a slight shortening was observed but in the other 2, ATP marginally prolonged APD.

Effects of altered physiological salt solution alone

Table 1 summarises the effects of the modified PSS on action potential characteristics of sheep Purkinje cells measured over a 2 h exposure period. By 30 min of exposure, the hypoxic, hyperkalaemic and acidotic PSS had reduced the resting membrane potential and there were concomitant reductions in action potential height and in MRD. The action potential duration was also markedly reduced. Although resting membrane potential was stable throughout the remainder of the observation period, MRD and action potential height continued to fall slightly between 30 and 120 min of exposure to the modified PSS.

The excitability of the preparations was depressed by the conditions that mimicked ischaemia. Two out of nine fibres exposed to the modified PSS became completely inexcitable and in 50% of the remainder the stimulation voltage had to be increased by approximately two fold.

Effects of adenosine, given during or before exposure to modified PSS

Figure 1 shows the effects of adenosine (10^{-5} and 10^{-4} M) when given after the first 30 min of exposure to the modified PSS. In contrast to its effects on normal Purkinje action potentials, adenosine significantly modified MRD and action potential amplitude. Thus the 'ischaemia'-induced fall in both of these variables was attenuated by adenosine, 10^{-5}

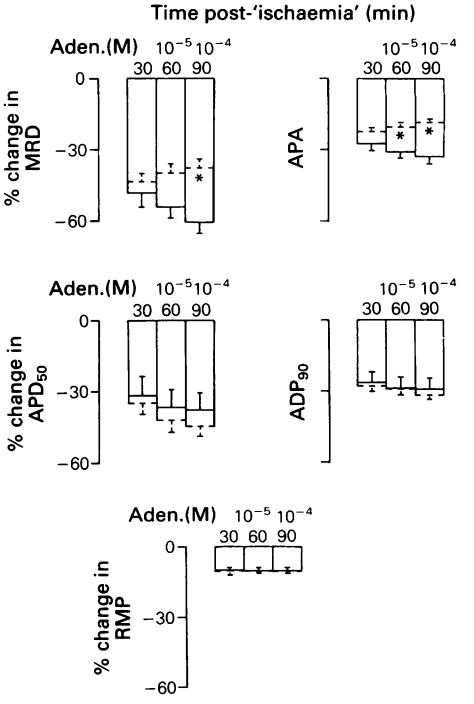

Figure 1 Percentage changes from control values in sheep Purkinje action potentials induced by the modified PSS alone (unbroken line) and modified PSS plus adenosine, 10^{-5} and 10^{-4} M (broken line). *$P < 0.05$ significantly different from value in modified PSS alone. $n = 6$–7. For abbreviations see text.

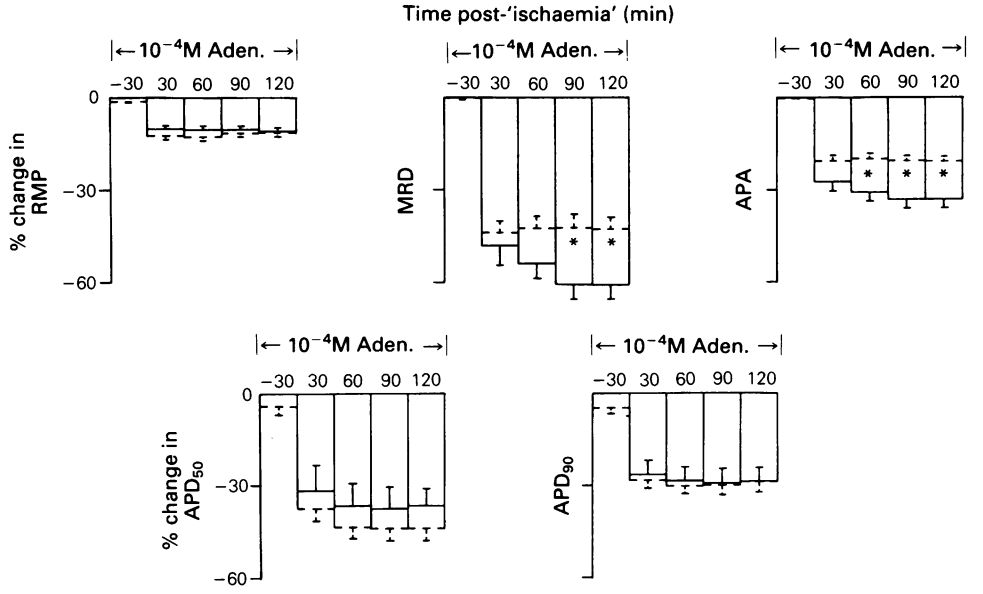

Figure 2 Percentage changes from control values in sheep Purkinje action potential characteristics induced by modified PSS alone (unbroken line) and adenosine before (−30 min) and during superfusion with modified PSS (broken line). *$P < 0.05$ significantly different from value in modified PSS alone. $n = 6$–7. For abbreviations see text.

and 10^{-4} M, without any concomitant effect on the resting membrane potential. Action potential duration was not significantly different in fibres exposed to the modified PSS alone and that containing adenosine.

When adenosine (10^{-4} M) was given prior to (for 30 min) and throughout exposure to the modified PSS, it did not modify the marked and rapid (within 30 min) changes observed in all of the variables (Figure 2). However, adenosine did abolish the further fall in MRD and in action potential amplitude that occurred between 30 and 120 min of superfusion with the modified PSS. The adenosine-induced shortening of APD_{50}, but not that of APD_{90}, persisted throughout the exposure to the 'ischaemic' solution.

Table 2 The effects of noradrenaline (NA) and noradrenaline together with adenosine (Aden) on action potential characteristics of sheep Purkinje fibres exposed to 'ischaemic' conditions

	RMP (mV)	*MRD* (Vs^{-1})	*APA* (mV)	APD_{50} (ms)	APD_{90} (ms)
Control	−83 ± 0.6	488 ± 31	113.4 ± 1.8	137.8 ± 11.9	234.7 ± 11.2
Modified PSS	−75.4 ± 0.4*	178 ± 45*	80.2 ± 1.5*	102.9 ± 9.1*	181.2 ± 9.3*
NA 10^{-5} M in mod. PSS	−76.2 ± 0.3*	161 ± 39*	82.6 ± 2.6*	173.7 ± 10.2†	269.4 ± 15.7†
NA 10^{-5} M + Aden 10^{-4} M in mod. PSS	−75.6 ± 0.7*	175 ± 40*	85.7 ± 2.5*	163.1 ± 11.0†	259.7 ± 13.2†

* $P < 0.05$ significantly different from control
† $P < 0.05$ significantly different from modified PSS alone. $n = 4$

Effects of adenosine on noradrenaline-induced electrophysiological actions

A detailed description of the effects of noradrenaline on sheep Purkinje cells superfused with normal or modified PSS is given by Boachie-Ansah *et al.* (1989). Noradrenaline caused a prolongation of action potential duration which was particularly marked under conditions that simulate myocardial ischaemia (Table 2). On normal action potentials, adenosine (10^{-4} M) administered concurrently with 10^{-5} M noradrenaline slightly but significantly attenuated the noradrenaline-induced prolongation of action potential duration at both 50% and 90% repolarization (by $21.0 \pm 3.5\%$ and $22.1 \pm 7.8\%$ reductions in APD_{50} and in APD_{90}, respectively). However, no suppression of noradrenaline-induced automaticity by adenosine (10^{-4} M) was observed. The spontaneous rate of beating of preparations exposed to noradrenaline and noradrenaline plus adenosine was 32 ± 8 and 32 ± 9 beats min^{-1}, respectively.

In modified PSS, a similar response was observed in that adenosine slightly reduced the prolongation of action potential duration induced by noradrenaline (Table 2). The % reductions in APD_{50} and APD_{90} induced by adenosine were 16.5 ± 6.7 and 10.7 ± 2.4, respectively. Noradrenaline did not induce automaticity in the presence of the modified PSS.

Effects of ATP, given during and before exposure to modified PSS

Figure 3 illustrates the effects of ATP, 10^{-5} and 10^{-4} M, (given 30 min after the start of superfusion with modified PSS) on depressed Purkinje action potentials. In contrast to its effects on normal Purkinje fibres and in a similar (but less marked) manner to adenosine, ATP significantly attenuated the fall in MRD and in action potential amplitude observed with the longer periods of exposure to the modified PSS. The changes in the other measured variables were not altered significantly by the presence of ATP, although action potential duration tended to be longer.

When ATP (10^{-4} M) was given before (30 min) and during exposure to modified PSS, it failed to modify the early (within 30 min) changes in action potential characteristics induced by the solution mimicking ischaemia. However, ATP tended to ablate the gradual subsequent fall in MRD and in action potential amplitude. Thus the % reductions in MRD and in action potential amplitude under ischaemic conditions were 60.6 ± 4.8 and 33.1 ± 3.0 respectively, compared with % reductions of 53.1 ± 4.0 and 25.8 ± 0.8 in the presence of ATP (10^{-4} M). ATP did not modify resting membrane potential of the depressed action potentials and once again although it tended to lengthen the action potential duration, this effect was not statistically significant.

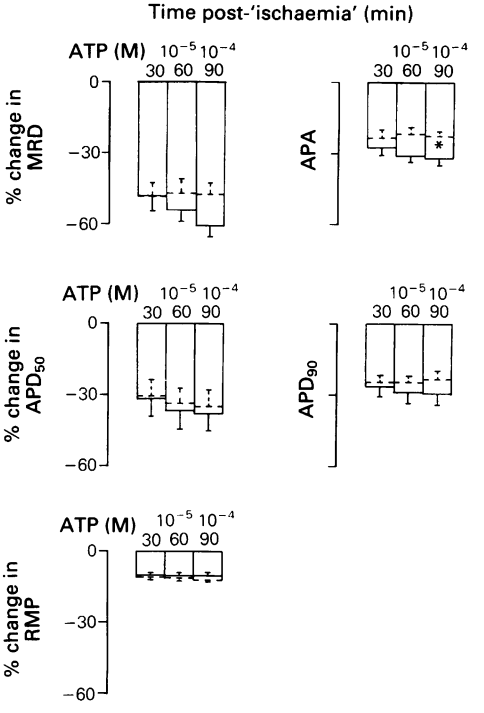

Figure 3 Percentage changes from control values in sheep Purkinje action potential characteristics induced by the modified PSS alone (unbroken line) and modified PSS plus ATP (broken line). * Significantly different from value in modified PSS alone. $n = 5$–7. For abbreviations see text.

Discussion

On normal paced sheep Purkinje fibres, adenosine (10^{-6} to 10^{-4} M) caused a concentration-dependent shortening of action potential duration without influencing any of the other measured variables. These results agree with the recent findings of Wesley & Belardinelli (1985) on guinea-pig isolated Purkinje cells but differ from those on canine Purkinje cells (Rosen *et al.*, 1983; Rardon & Bailey, 1984) in which adenosine was shown to have no effect on action potential duration. This suggests

that the action of adenosine on action potential duration is species-dependent. On atrial muscle, the adenosine-induced abbreviation of the action potential can be attributed either to an increase in K^+ conductance (Belardinelli & Isenberg, 1983; Nawrath & Jochem, 1983) or to a decrease in the inward Ca^{2+} current (Grossman & Furchgott, 1964; Schrader *et al.*, 1975). However, the mechanism underlying this effect of adenosine in Purkinje cells has not been studied. No consistent effect of ATP on the duration of the normal sheep Purkinje action potential was observed, which is in agreement with results obtained on canine Purkinje cells, using a stable analogue of ATP (Rosen *et al.*, 1983).

In contrast to their actions on normal Purkinje tissue, both adenosine and ATP, under conditions that mimic mild myocardial ischaemia, modified the upstroke of the action potential. The 'ischaemia'-induced reductions in the action potential amplitude and in the rate of rise of phase zero that occurred between 30 and 120 min of exposure to the modified PSS were attenuated by adenosine and to a lesser extent by ATP. Neither of the adenyl compounds, when given before exposure to the modified PSS, influenced the fall in resting membrane potential, amplitude and rate of rise of the action potential that occurred within the first 30 min of exposure.

The ionic mechanism underlying the protective effects of adenosine and ATP on the amplitude and rate of rise of the action potential during the 30–120 min post-ischaemic period remain unknown. Since these effects were not associated with any changes in the resting membrane potential, it would appear that the adenyl compounds may protect against the fall in the inward current that underlies phase 0 depolarization. Experiments carried out in this laboratory have indicated that the reduction in the resting membrane potential and the concommitant decreases in the amplitude and rate of rise of the action potential induced by the modified PSS within the first 30 min of exposure are mainly a consequence of the elevation in extracellular K^+ concentration (Boachie-Ansah *et al.*, 1989) whereas the continued decline in the upstroke and rate of rise is hypoxia and/or acidosis-mediated (D. Pacini, unpublished observations). The ionic mechanisms responsible for this effect of hypoxia and acidosis are also unknown but they may possibly involve induced increases in cytosolic Na^+ as a result of partial inhibition of the Na^+/K^+ pump and/or an induced shift in the membrane responsiveness curve (i.e., the relationship between membrane potential and amplitude of the inward Na^+ current) to more negative potentials. Adenosine and ATP may, in turn, attenuate these effects but such suggestions are purely conjectural and emphasise the need for further electrophysiological analysis of the effects of these drugs under ischaemic conditions. It would also be of interest to ascertain if the adenyl compounds are acting extra- or intra-cellularly and, furthermore, if the action of ATP depends upon enzymatic breakdown to adenosine. It is possible that this attenuation of the ischaemia-induced depression of the upstroke of the action potential by the adenyl compounds may, if it occurs *in vivo*, improve conduction and eliminate areas of unidirectional block. Such an action might explain the ability of adenosine and ATP to protect against ischaemia-induced arrhythmias in experimental animals (Fagbemi & Parratt, 1984; Parratt & Wainwright 1985; G. Boachie-Ansah, unpublished observations).

Neither adenosine nor ATP had a marked effect on APD under conditions that simulate mild myocardial ischaemia. Thus ATP, given extracellularly, is unable to protect against the abbreviation of the action potential that is, in part, induced by hypoxia (D. Pacini, unpublished work). Since it has been shown that intracellular injection of ATP does attenuate the action potential shortening caused by metabolic inhibitors (Taniguchi *et al.*, 1983), it is most likely that our results reflect the rapid breakdown of ATP and lack of uptake into the cytoplasm.

In addition to its direct electrophysiological effects, adenosine has been shown to modulate the effects of catecholamines on Purkinje tissue. For instance, adenosine reduces adrenaline-induced automaticity (Rosen *et al.*, 1983) and antagonizes the action potential shortening induced by isoprenaline (Rardon & Bailey, 1984). In our experiments, there was no evidence of any interaction between the effects of adenosine and noradrenaline, the neurotransmitter at sympathetic nerve endings. On normal sheep Purkinje fibres, adenosine did not modify noradrenaline-induced automaticity. It did reduce the noradrenaline-mediated prolongation of APD in both normal and 'ischaemic' fibres, but this effect could be explained by the ability of adenosine alone to shorten the action potential. The reason for this lack of interaction between adenosine and noradrenaline may be that adenosine specifically antagonises β- but not α-adrenoceptor-mediated catecholamine effects (Endoh & Yamashita, 1980; Hattori & Levi, 1984). At least part of the electrophysiological effects of noradrenaline on sheep Purkinje fibres is α-adrenoceptor-mediated (Boachie-Ansah *et al.*, 1989).

In summary, adenosine caused a concentration-dependent shortening of the action potential duration in normally-polarized sheep Purkinje fibres. Both adenosine and ATP attenuated the fall in the maximum rate of rise of phase 0 and the action potential amplitude in fibres exposed to combined hypoxia, hyperkalaemia and acidosis. No interaction between the effects of adenosine and noradrenaline

on action potential characteristics of normal or 'ischaemic' sheep Purkinje fibres was observed.

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