Acute effects of diuretics on potassium exchange, mechanical function and the action potential in rabbit myocardium

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Summary

1. We have studied the acute effects of frusemide, triamterene and amiloride on potassium exchange, the action potential and mechanical function of isolated rabbit myocardium.

2. Potassium exchange in the myocardium was unaltered by these diuretics.

3. Frusemide and amiloride did not affect the action potential of rabbit papillary muscles. Triamterene caused a transient shortening of the action potential.

4. Frusemide and triamterene did not alter myocardial mechanical function in rabbit papillary muscles or the interventricular septum. Amiloride caused a reduction of about 5% in developed tension in two out of three papillary muscles.

Key words: action potential, amiloride, diuretics, frusemide, mechanical function, myocardium, potassium exchange, triamterene.

Introduction

It has been suggested that diuretics have a direct effect on potassium balance in heart muscle (Seller, Banach, Namey, Neff & Swartz, 1975) and potassium-sparing diuretics have been advocated in the treatment of cardiac failure (Donaldson, Patrick, Sivapragasm, Woo Ming & Alleyne, 1976) and digitalis intoxication (Sellar, Greco, Banach & Seth, 1975; Weber, 1972) because they may diminish intracellular potassium depletion. Myocardial potassium depletion is important in the pathogenesis of dysrhythmias (Harris, Bisteni, Russell, Brigham & Firestone, 1954; Cherbakoff, Toyama & Hamilton, 1957; Elharrar & Zipes, 1977) and is well documented in reversible myocardial ischaemia (Case, Nasser & Crampton, 1969; Parker, Chiong, West & Case, 1970), infarction (Russell, Crafoord & Harris, 1961; Jennings, Sommers, Kaltenbach & West, 1964), digitalis toxicity (Neff, Mendelsohn, Kim, Banach, Swartz & Seller, 1972), tachycardia (Sarnoff, Gilmore, Mitchell & Remensnyder, 1963) and congestive cardiac failure (Calhoun, Cullen & Clarke, 1930; Iseri, Alexander, McLaughhey, Boyle & Myers, 1952). This study was designed to establish whether diuretics alone altered potassium fluxes or net potassium balance in the isolated myocardium.

Methods

The experimental preparation was the isolated but arterially perfused interventricular septum from the rabbit heart. The method has been previously described (Poole-Wilson & Langer, 1975). Male rabbits (2–4 kg) were injected with heparin and anaesthetized with pentobarbitone. The heart was removed within 2–4 min and a small cannula inserted into the artery which supplies the interventricular septum. Tissue which did not contract or change colour on perfusion was excised. The triangular piece of muscle remaining weighed between 0.7 and 1.2 g. A tension transducer (UC 4; Statham,
Oxnard, California, U.S.A.) was attached to the apex. The stimulation frequency was 0.7 Hz and temperature 28°C. Radioactivity was recorded with a counter (J. and P. Engineering, Reading, Berks., U.K.) and a Geiger–Müller probe placed 1 cm in front of the muscle.

The perfusate contained (mmol/l): Na⁺, 142; K⁺, 5.0; Ca²⁺, 1.8; Mg²⁺, 1.0; Cl⁻, 124; H₂PO₄⁻, 0.4; HCO₃⁻, 28; glucose, 5.6. The solution was equilibrated with humidified CO₂/O₂ (5:95). ⁴²K⁺ (PES. 1P; The Radiochemical Centre, Amersham, Bucks., U.K.) was made up in a calculated amount of potassium-free perfusate in order to maintain the final potassium concentration at 5.0 mmol/l.

Drugs were added directly to the perfusate in these concentrations: frusemide, 400 mg/l (1.2 x 10⁻³ mol/l); triamterene, 30 mg/l (1.2 x 10⁻⁴ mol/l); amiloride, 4 mg/l (1.3 x 10⁻⁵ mol/l). These doses were chosen because they are substantially above the highest therapeutic concentrations found in human plasma (Pruitt, Dayton & Steinhorst, 1974; Weiss, Hersey, Dujovne & Bianchine, 1969). As triamterene is relatively insoluble, a concentrated solution containing particles of the drug was added to the perfusate, when the drug dissolved completely.

The septa were allowed to equilibrate with the control perfusate for 2 h (Poole-Wilson & Langer, 1975). The muscle was then labelled for 45 min with ⁴²K⁺ at a concentration of up to 2.5 mCi/l. The washout of isotope was begun by switching to perfusate containing no radioisotope. Tissue radioactivity was recorded by the Geiger–Müller probe over periods of 1 min. Approximately every 30 s during the washout three effluent drops were collected in glass vials and the exact time was noted. The time for the three drops to form was also recorded. After addition of 10 ml of water the radioactivity in the effluent was measured by Cerenkov counting (Garrahan & Olyn, 1966) in a liquid-scintillation counter (Tricarb, Packard). The radioactive count rate (c.p.m.), after background subtraction and correction for decay, was multiplied by 60 and divided by the time in seconds taken for the drops to form. The radioactivity was then expressed as counts min⁻¹ min⁻¹ (Fig. 1). This procedure accounts for any small changes that might occur in the time for three effluent drops to form, despite the constant rate of perfusion (Poole-Wilson & Langer, 1975). After washout for 20 min the perfusate was switched to one containing the drugs for a period of 20 min, followed by the control perfusate for a further 20 min. This method allows small changes in the efflux of potassium to be detected.

In a further series of experiments septa were equilibrated with ⁴²K⁺ for at least 2 h, by which time the tissue radioactivity count rate was constant. The perfusate was changed for 20 min to one containing drugs and having an identical specific radioactivity of ⁴²K⁺, so as to detect net changes in tissue potassium.

In a separate series of experiments intracellular action potentials and isometric tension were recorded from rabbit right ventricular papillary muscles of diameter less than 1 mm. The muscles were suspended in a small open trough and bathed with the same solutions as the septa. One end of the muscle was attached to a transducer (UC 4; Statham, Oxnard, California, U.S.A.) to record tension. The muscle was stretched until developed tension was maximal. Intracellular action potentials

![Graph](image)

**Fig. 1.** Washout of ⁴²K⁺ from the cardiac septum of a rabbit (at 28°C, rate 42 beats/min). Frusemide has no effect on the efflux of ⁴²K⁺. See the text for details.
were recorded from glass micro-electrodes filled with KCl (3 mol/l) and with a resistance of 10–20 mohm. The input impedance was greater than $10^{11}$ ohm and the response flat to greater than 80 KHz. The signal was displayed on a storage oscilloscope (Textronix, 5111). The temperature was maintained at 33°C and the stimulation frequency was 1 Hz (Bassingthwaighte, Fry & McGuigan, 1976).

Results are given as mean value ± SEM.

Results

Fig. 1 shows the washout of $^{42}$K$^+$ from the septum. The points marked as ‘probe’ represent the tissue radioactivity recorded each minute by the Geiger–Müller probe and are expressed as counts/min. The points marked as ‘effluent’ represent the radioactivity in the effluent drops and are expressed as counts min$^{-1}$ min$^{-1}$ of perfusate flow. The initial fall in both sets of points is due to the efflux of $^{42}$K$^+$ from the vascular and extracellular space. After 10 min the two lines are straight and parallel, indicating that the efflux of $^{42}$K$^+$ follows single-order kinetics and originates from a homogeneous compartment in the tissue. Frusemide (1.2 × 10$^{-3}$ mol/l) added to the perfusate after 20 min had no effect on the efflux of $^{42}$K$^+$. Similarly, triamterene (1.2 × 10$^{-4}$ mol/l) and amiloride (1.3 × 10$^{-5}$ mol/l) did not alter $^{42}$K$^+$ efflux. The experiment was repeated in four separate septa for each diuretic.

The addition of diuretics to the perfusate when septa were equilibrated with $^{42}$K$^+$ showed no net gain or loss of $^{42}$K$^+$ in four separate septa with each diuretic (Fig. 2).

Under control conditions developed tension of septa was 13.8 ± 1.0 g ($n = 11$) and resting tension was 6.2 ± 0.5 g. The diuretics had no effects on mechanical function in the interventricular septum.

The isometric tension and action potential were recorded in six rabbit right ventricular papillary muscles. Their mean weight was 2.2 ± 0.4 mg ($n = 6$) and under control conditions developed tension was 3.0 ± 0.4 kN/kg ($n = 6$). The resting membrane potential was 78 ± 1 mV ($n = 6$) and the time to 95% repolarization was 233 ± 11 ms ($n = 6$). The effect of each diuretic was studied in three different papillary muscles. Frusemide and triamterene had no effect on developed tension or maximum rate of rise of tension but in two out of three experiments amiloride caused a small (less than 5%) decline in peak tension. All three diuretics had no effect on the resting membrane potential. Frusemide and amiloride had no effect on the configuration or the duration of the action potential throughout the exposure to the diuretic. During the initial 5–10 min of exposure to triamterene action potential duration decreased to 73, 75 and 79% of the control value. After 20–30 min exposure the configuration and duration were similar to the control action potential.

Discussion

None of the diuretics we tested had any direct effect on myocardial potassium exchange. The concentrations of the drugs were substantially above the highest therapeutic plasma values (Pruitt et al., 1974; Weiss et al., 1969). Our method would detect a net myocardial potassium loss of the order of 0.2 mmol/kg wet weight of tissue, which represents less than 0.5% of the total tissue potassium. The diuretics had no effect on mechanical function of the myocardium except that amiloride caused a minimal reduction in tension in two out of three papillary muscles. Triamterene caused a transient shortening of the action potential but after 30 min exposure to any of the diuretics the action potential was unchanged.

Study of the effects of frusemide, amiloride and triamterene on myocardial microsomal Na$^+$–K$^+$-dependent ATPase (EC 3.6.1.3) shows inhibition
only at high concentrations above $10^{-3}\text{ mol/l}$ (Gibson & Harris, 1970; Erdmann & Krawietz, 1976). Ouabain binding to the Na$^+\text{--}K^+$-dependent ATPase receptor is only inhibited at similarly high concentrations of these three diuretics and triamterene binds to a different protein fraction from that which binds ouabain and possesses Na$^+\text{--}K^+$-dependent ATPase activity, suggesting that the diuretic effect is nonspecific (Erdmann & Krawietz, 1976). Amiloride reverses the electrophysiological changes produced by cardiac glycosides but only at high concentrations of $10^{-2}\text{ mol/l}$ (De Azevedo, Dreifus, Seller & Castel, 1973). Chronic administration of frusemide and ethacrynic acid to rats does not affect myocardial microsomal Na$^+\text{--}K^+$-dependent ATPase activity (Gibson & Harris, 1970), and chronic administration of frusemide (Hall & Cameron, 1977) or thiazide diuretics (Tobian, Janacek, Foker & Ferreira, 1962) does not result in myocardial potassium depletion. Thus diuretics affect Na$^+\text{--}K^+$-dependent ATPase activity only in concentrations substantially higher than those found in clinical practice.

Seller et al. (1975a, b) suggested that acute administration of frusemide, ethacrynic acid, triamterene and amiloride to anaesthetized dogs had a direct effect on potassium efflux from the myocardium. They showed that changes in the arterio-coronary sinus potassium difference induced by cardiac glycosides could be altered acutely by diuretics. Whether their results demonstrated an effect of diuretics on myocardial potassium exchange in the absence of glycosides has been disputed (Walter, 1976). It is difficult to draw confident conclusions about myocardial potassium release from arteriovenous differences when coronary flow is not measured and when arterial potassium is not held steady and changes by up to 2.6 mmol/l in 10 min. Nevertheless, triamterene can prevent and reverse glycoside toxicity (Weber, 1972; Seller et al., 1975b) in animals, but it is not proven that this effect results from direct inhibition of myocardial potassium efflux. Acid--base changes in the experiments of Weber (1972), or alterations of the plasma potassium (Seller et al., 1975a,b), may be the explanation.

Our results show that diuretics in concentrations just above therapeutic values have no important effects on mechanical function, potassium exchange or the action potential in the normal rabbit myocardium. Changes in intracellular electrolytes in patients with heart failure treated with triamterene (Donaldson et al., 1976) are probably secondary to alterations in plasma potassium. It remains possible that diuretics may exert a direct effect on potassium exchange in the failing or ischaemic myocardium.

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**References**


Diuretics and potassium in heart muscle


