Effects of low [K+]o on the electrical activity of human cardiac ventricular and Purkinje cells

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Effects of low $[K^+]_o$, on the electrical activity of human cardiac ventricular and Purkinje cells

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SUMMARY Ventricular and Purkinje action potentials were recorded with a microelectrode in a strip of human papillary muscle. Lowering the K-content of the superfusing solution from 5.9 to 0.5 mmol.litre^{-1} at 37°C hyperpolarised ventricular diastolic potential steadily as long as $[K^+]_o$ was low (up to 70 min tested). Ventricular action potentials were transiently lengthened and then shortened. A positive inotropic effect was noted and attributed to Na-K pump inhibition since it was reversed by the addition of 2 mmol.litre^{-1} thallous chloride to the low $[K^+]_o$ solution. Beyond 40 min, transient depolarisations and after-contractions were found. During the first minutes in low $[K^+]_o$, Purkinje diastolic potential was hyperpolarised and the action potential was lengthened at all levels of repolarisation. Afterwards, the Purkinje diastolic potential suddenly depolarised by 30 mV. Restoration of the control solution caused a slow repolarisation and then a sudden return of the diastolic potential to near control value. This was reproduced during drive (38 stim.min^{-1}) and at rest. At the depolarised level of potential, stimulation elicited slow action potentials with diastolic slow depolarisation and spontaneous oscillations of potential appeared at rest. In Purkinje cells, increasing concentrations of tetrodotoxin from 10^{-7} to 8 X 10^{-6} mol.litre^{-1} in the control solution shifted the diastolic potential in negative direction by a few mV and shortened the action potential duration at all levels of repolarisation. The possible implications of these phenomena in the genesis of some cardiac arrhythmias are discussed.

Purkinje fibres differ in their electrophysiological properties from ventricular myocardial fibres in mammals, [1] including man. [2] Particularly, the duration of the action potential and the effective refractory period are longer in Purkinje than in ventricular myocardial fibres. These are longest in distal Purkinje fibres which act as a “gate” [3] preventing the propagation of premature impulses. This phenomenon was termed the “gating mechanism” by Myerburg et al. [3] The two tissues respond differently to several agents. When $[K^+]_o$ is lowered from about 5 to about 2 mmol.litre^{-1} , the duration of the action potential and the effective refractory period are lengthened in Purkinje fibres but shortened in ventricular myocardium.[1] Purkinje fibres may even be dramatically depolarised when $[K^+]_o$ is further lowered [4 5] while ventricular fibres are not. [6 7] However, Na-K pump blockade by digitalis or low $[K^+]_o$ induces transient depolarisations of both mammalian Purkinje [8] and ventricular fibres. [9] Agents that depress the sodium current (ie tetrodotoxin and local anaesthetics) also depress the steady-state (“window”) sodium current [10 11] and shorten Purkinje action potentials considerably more than ventricular myocardial ones. [12 13] All these phenomena have been involved in the explanation of cardiac arrhythmias. [6]

During a study of inotropic and arrhythmogenic effects of Na-K pump blockade by low $[K^+]_o$, we recorded strikingly different effects between myocardial and Purkinje fibres in a strip of human ventricle. Our results are quite similar to well established data from mammals and may be relevant to the mechanism of cardiac arrhythmias due to Na-K pump poisoning.

Methods

During surgical insertion of a valvular prosthesis, part of a papillary muscle was removed from the right ventricle of a 6 year-old male patient with a tetralogy of Fallot. This excision was part of the operative procedure, the excised part being discarded if unused for experiments. The patient had been receiving 8 $mg.kg^{-1}$ (body weight) digoxin daily for 5 years.

Upon excision, the sample was dropped into a solution at room temperature (20°C) having the following salt composition ($mmol.litre^{-1}$): NaCl 117, KCl 3.1, KH_2PO_4 1.2, $NaHCO_3$ 24.8, $CaCl_2$ 2.5, $MgS0_4$ 1.2, glucose 11.2 in distilled water and continuously gassed with CO_2 5% in O_2. [14] This was the control solution used in the experiments.

Transport to the laboratory took 10 min. A strip (2 x 1 x 1 mm) was then cut with scissors and pinned onto the sylgard-covered bottom of a 1.5 cm^3 perspex bath. Superfusion was started at a constant rate of 3 $cm^3.min^{-1}$ (*) for 30 min (peristaltic pump LKB 2115)) the temperature being elevated to 37°C within the first 10 min.

(*) Absolute delay from switch to input of bath and times needed for 99% rise and decay of bath $[Na^+]$ between 0 and 140 $mmol.litre^{-1}$ were evaluated to be 1.7 min, 6.5 min and 10 min respectively.

During another 30 min stabilisation period and during experiments, stimulations at 1.5 times threshold magnitude (dc square pulses 20 mA, 1.5 ms) were applied through an isolating unit between a large electrode in the bath and a punctate one near one end of the preparation. Both were silver-silver chloride electrodes. Unless otherwise stated, the driving frequency was 38 $stim.min^{-1}$.

Microelectrodes (resistance 30 to 50 MΩ) were filled with 3 $mol.litre^{-1}$ potassium chloride acidified to pH 2 with added HCl in order to reduce the tip potential. [15] The mean tip potential value in five microelectrodes was 5±1 mV. A microelectrode was mounted in a semi-floating fashion [16] on a Narishige micromanipulator. It was brought near the preparation, but kept away from damaged regions, and a stable impalement was searched for. The results shown below were obtained during impalements which remained stable during a control period of at least 10 min and for at least 15 min after changing the solution. Each figure was constructed from records obtained during a single impalement, unless otherwise stated.

Potentials were recorded through a high input impedance follower amplifier built with operational amplifiers (input impedance 10^{12} Ω, input current 10 pA) with a gain of 10. The reference electrode in the bath and the connection to the microelectrode were both silver-silver chloride electrodes.

Chart records (Gould Brush 2400) and pictures of the oscilloscopic display were taken (Tektronix RM 565 oscilloscope and Nihon-Kohden PC2A camera).

The modified K-depleted solution was prepared by uncompensated omission of all the KC1 and replacement of the appropriate amount of KH_2P0_4 by NaH_2PO_4. Tetrodotoxin (TTX) (Crystalline 3X Sankyo) was diluted into an aliquot of the control solution to 10^{-5} $mol.litre^{-1}$. Appropriate amounts of this solution were added to the control solution from the same batch to give the final concentrations given in the text.

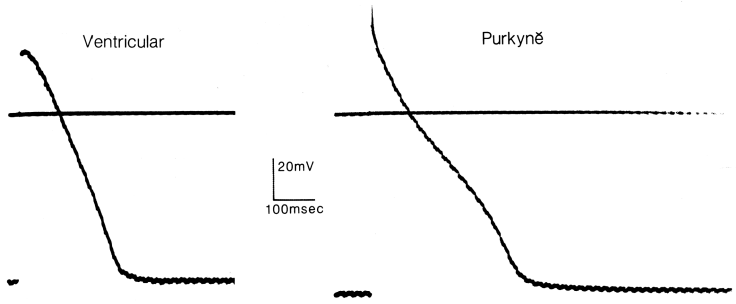

FIG 1: Oscilloscope records of ventricular and Purkinje action potentials in a strip of human papillary muscle. Note the triangular shape of the ventricular action potential and faster initial repolarisation, low plateau and longer duration of the Purkinje action potential.

Results

Two different action potential configurations were found in the preparation in control solution (fig 1). Some action potentials were very similar to the ones recorded by others in ventricular myocardial cells of adult humans. [2 7 17] Purkinje action potentials were also recorded. They showed initial rapid repolarisation and a low plateau (fig 1) and looked similar to action potentials recorded in Purkinje cells of adult man [2] and monkey. [18] Their duration at the late repolarisation phase was about 20% longer than in ventricular action potentials (fig 1).

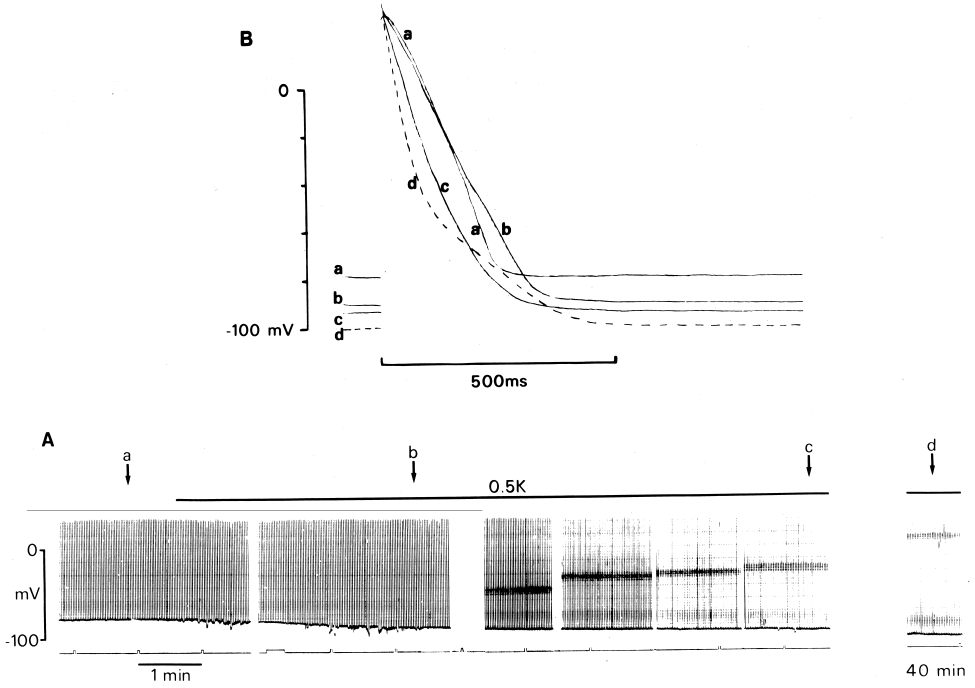

FIG 2 Modifications of ventricular myocardial action potential during exposure to low $[K^+]_o$.
***A**: Slow chart record. Note progressive hyperpolarisation of the diastolic membrane during the first 10 min. A slower hyperpolarisation followed at later times (eg at 40 min). **B**: Superimposed action potentials (redrawn from film images of the oscilloscope screen) obtained at times shown by the arrows labelled "a" to "d" in A. Note shortening of plateau duration and transient lengthening of final repolarisation at 4 min (b), and uniform shortening of action potential duration at 9 min (c). Beyond 30 min (eg (d) at 40 min), the late repolarisation phase was lengthened.*

DIASTOLIC AND ACTION POTENTIAL CHANGES IN LOW $[K^+]_o$

Myocardial ventricular cells

Within 10 min in low $[K^+]_o$, the diastolic potential was progressively shifted in a negative direction (fig 2A). This hyperpolarisation developed slowly, which is accounted for by the slow time course of changes in the bath concentration. Beyond 10 min the diastolic

potential shifted more slowly in a negative direction and reached -100 mV after 40 min in low $[K^+]_o$, (fig 2A).

During the first minutes in low $[K^+]_o$, the action potential duration was shortened at the plateau level and transiently lengthened at the late repolarisation phase (fig 2Bb). At intermediate times (eg 10 min, fig 2Bc), the action potential duration was shortened to half that of control both at plateau and late repolarisation phases. At later times, a slow phase of final repolarisation appeared (fig 2Bd).

These effects are in agreement with reported ones in ventricular myocardial preparations from mammals (eg [1 19]) including man [7] and amphibian. [20]

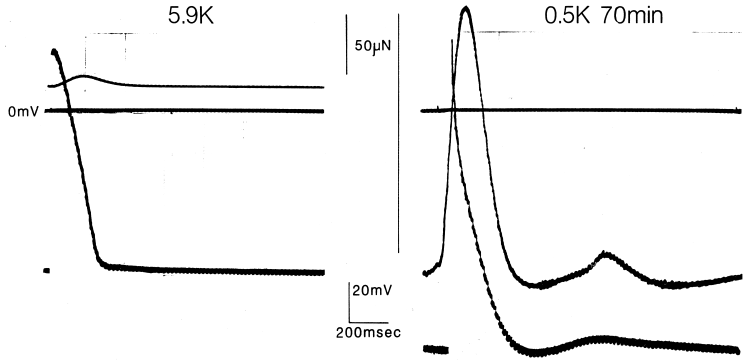

FIG 3 Ventricular myocardial membrane potential (lower trace) and tension (upper trace). The horizontal thick line shows the null potential. Left panel: at equilibrium in control solution. Right panel: after 70 min in low $[K^+]_o$. Note change in tension scale from left to right.

Positive inotropy developed in low $[K^+]_o$ which can be seen in fig 3 as an increase in peak twitch amplitude. Beyond 40 min, transient depolarisations appeared following the action potential, together with aftercontractions (fig 3). Such inotropic and arrhythmogenic effects can be attributed to inhibition of the Na-K pump in low $[K^+]_o$.[9 21 23] Thallous ions (2 mmol.litre^{-1} thallous chloride) were added to the 0.5 mmol.litre^{-1} solution in order to replace 3 mmol.litre^{-1} K^+ ions for the activation of the Na-K pump and to reverse related effects of low $[K^+]_o$. [22] Fig 4A shows that, in another strip of human ventricle, the positive inotropy was reversed by thallous ions in the same way as after returning to control $[K^+]_o$. Fig 4B shows that low $[K^+]_o$ -induced after-contractions in a human atrial trabecule were quickly reversed by thallous ions.

Purkinje cells

During the first minutes in low $[K^+]_o$, Purkinje diastolic potential became more negative by about 10 mV (fig 5A and B) along a time course similar to that in ventricular cells. The action potential was lengthened in duration at all levels of repolarisation (fig 5B). After longer times in low $[K^+]_o$ (ie 6 min in fig 5), the diastolic potential was suddenly shifted by about 30 mV in a depolarising direction and remained at this level as long as $[K^+]_o$ was kept low. At this depolarised level of potential, slowly rising action potentials were elicited upon stimulation and were followed by a slow diastolic depolarisation (fig 5C).
After returning to the control solution (5.9 mmol.litre^{-1} K^+), a slow repolarisation of the diastolic potential began. The diastolic potential then suddenly shifted to a value 5 mV more negative than the initial control value. Afterwards the diastolic potential went slowly back to the control level (fig 5A).

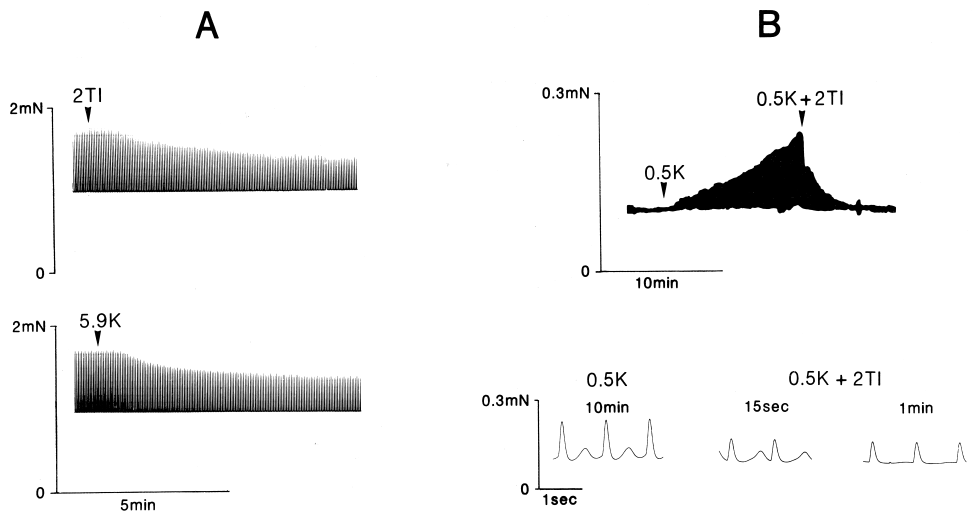

FIG 4 **A**: *Slow chart records of twitch amplitude in a strip of human ventricle. Upper trace: after 130 min in 0.5 mmol.litre^{-1} $[K^+]_o$, 2 mmol.litre^{-1} thallous chloride were added (arrow). Lower trace: after a subsequent 30 min equilibration period in 0.5 mmol.litre^{-1} $[K^+]_o$, control solution was switched on (arrow). Preparation issuing from a 4 months-old female with mitral stenosis.*

***B**: Human atrial preparation. Upper trace: after 10 min in 0.5 mmol.litre^{-1} $[K^+]_o$, 2 mmol.litre^{-1} thallous chloride were added. Note fast return to control twitch amplitude. Lower trace: expanded time scale record showing that after-contractions were rapidly abolished by thallous ions. Preparation from a 7 years-old female with aortic stricture.*

In A and B driving rate: 20 stim.min^{-1}.

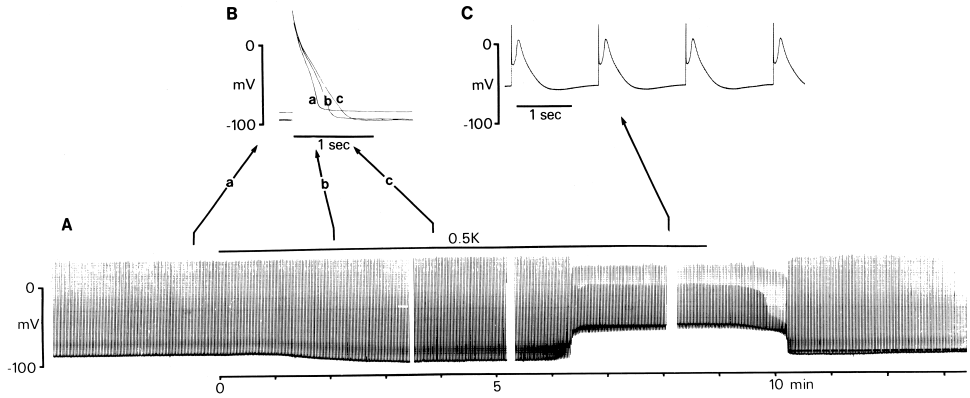

FIG 5 *Driven human Purkinje cell. **A**: Slow chart record of the diastolic potential during exposure to low $[K^+]_o$, and after returning to control solution. **B**: Superimposed action potentials (redrawn from film images of the oscilloscope screen). a: in control solution; b and c: in low $[K^+]_o$ at times shown by the foot of each oblique arrow in A. **C**: Fast chart record of action potentials triggered at the low level of potential. Note the slow rising phase and the diastolic depolarisation. Initial sharp spike corresponds to stimulation artefact.*

When the preparation was not stimulated, fairly similar phenomena were recorded (fig 6). The resting potential was stable in the control solution at about -80 mV. Superfusion with the K-depleted solution (fig 6) caused a progressive hyperpolarisation as when stimulated. After a few minutes, a depolarisation started which gave rise to an action potential seen as a sharp spike in fig 6. The repolarisation phase of this action potential was not complete: after a downward “notch”, the potential stabilised around -45 mV. In one case, spontaneous oscillations appeared with a proper frequency around 1 Hz (see insert to trace B of fig 6). Restitution of the control solution caused a slow repolarisation to -60 mV in 1 min and then the resting potential suddenly shifted to a value slightly more negative than the control value. Afterwards it slowly returned to control.

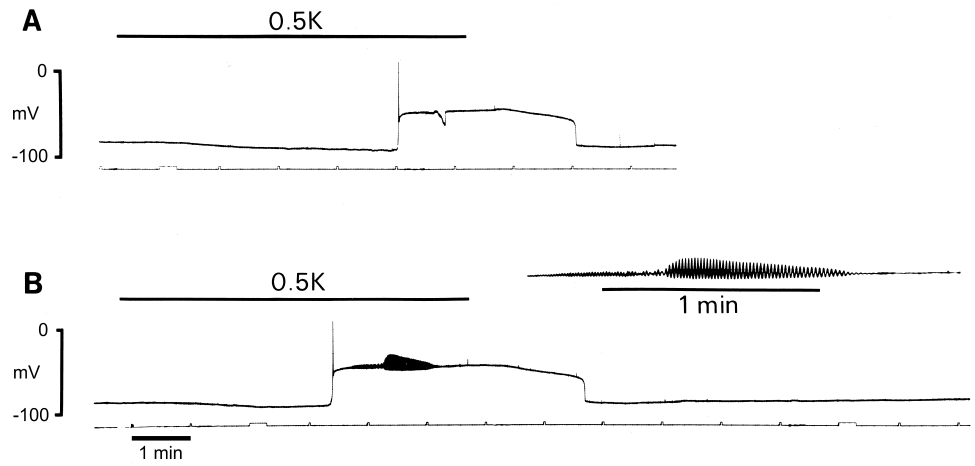

FIG 6 *Effects of lowering $[K^+]_o$, and of returning to control solution on the resting potential of two human cardiac Pukinje cells. In A and B, the upper thick line denotes time of low $[K^+]_o$, superfusion, middle trace is the membrane potential and the lower thin line shows clock pulses: short every minute, long every 10 min.*

EFFECTS OF TTX ON PURKINJE ACTION POTENTIAL

The preparation was exposed to gradually increasing concentrations of TTX from 10^{-7} to 8 x 10^{-6} mol.litre^{-1} by successive additions of stock TTX solution to the control solution within a 40 min period. The microelectrode remained impaled in the same Purkinje cell during the whole experiment. Fig 7 shows that TTX shifted the diastolic potential in a negative direction. The hyperpolarisation was maximal with 5 x 10^{-6} mol.litre^{-1} TTX and for higher concentrations it slightly decreased. TTX also decreased action potential duration at all levels of repolarisation. The higher the concentration, the greater the shortening.

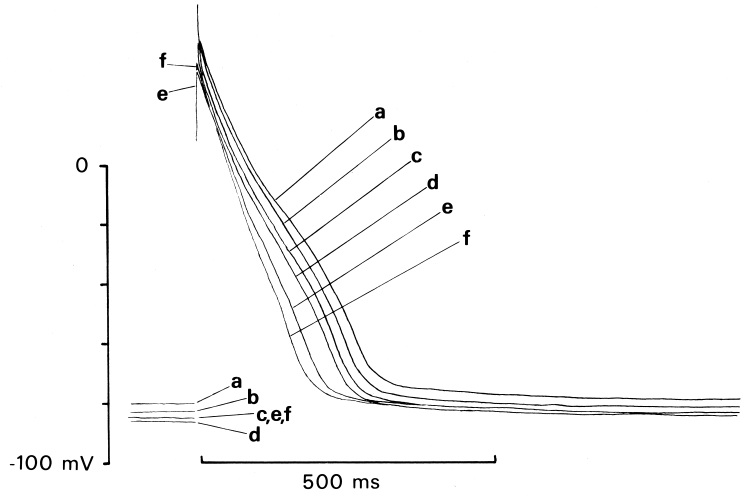

FIG 7 Effects of TTX on the electrical activity of a human cardiac Purkinje cell. Note the shortening of the action potential duration and negative shift of the diastolic potential. a: control: b: 10^{-7} ,c: 6 x 10^{-7}, d and e: 2 and 4 min in 5 x 10^{-6}, f: 8 x 10^{-6} mmol.litre^{-1} TTX.

Discussion

The shape of ventricular action potentials recorded in the present preparation and the value of the diastolic potential in the control solution (eg fig 1) were similar to the ones reported from adult human ventricular myocardium. [2 7 17] The preparation thus behaved as normal healthy myocardium, and seemed undisturbed by the previous digoxin treatment.

The finding of Purkinje action potentials (fig 1) in a papillary muscle may be attributed to subendocardial conductive cells. [24 25]

DIASTOLIC POTENTIAL CHANGES IN LOW $[K^+]_o$

In ventricular cells, low $[K^+]_o$ hyperpolarised the membrane, even after long durations (fig 2). This is in agreement with previous results of papillary muscles from adult human [7] and guinea-pig [19] hearts subjected to long exposures to K-free solution. This hyperpolarisation may be attributed to a shift of the equilibrium potential for potassium ions in a negative direction.

In Purkinje cells, a similar effect was observed only during the first minutes in low $[K^+]_o$. The large and sudden depolarisation observed at later times and the sudden repolarisation when returning to control $[K^+]_o$ were peculiar to Purkinje cells. Such de- and repolarisations have been studied in dog and sheep cardiac Purkinje fibres [4 5 26-29] (see also [30] for review) and were noted in fetal human ventricular myocardium. [31]

From the similarity of our results in human Purkinje cells to those from dog and sheep, we may infer that human Purkinje cells have a N-shaped steady-state membrane current-voltage (I(V)) relation with a region of negative slope-conductance. [26] Lowering $[K^+]_o$ shifts the I(V) relation in the inward direction. [32] (see fig 1 of [30]) Thus the region of negative slope- conductance crosses and goes below the voltage axis. This accounts for a small and slow depolarisation followed by a large regenerative one [26] (fig 5A). The reverse occurs on returning to control $[K^+]_o$. [26 28] These effects have been attributed to a fall in membrane permeability to K^+ ions, [33] at least partly due to a decreased gK1. [34] Such large depolarisations were also found in response to lowered temperature [35 37 38] or to Na-K pump blockade by dihydroouabain or lithium ions. [39] Thus, $[K^+]_o$-related changes in Na-K pump current [35] may also contribute. [26 36] In the present preparation, thallous ions could have been used to disclose the part played by Na-K pump blockade (as in the experiments of fig 4). This was not done, due to the limited time available for the experiment.

The fact that TTX hyperpolarises the membrane of Purkinje cells (fig 7 and 13) indicates that a steady sodium current flows, which could be partly responsible for the depolarisation once outward currents are sufficiently decreased. [10 26]

Such depolarisations are not found in ventricular muscle fibres, [6 7] which may indicate that the Na-K pump current [40] and/or the steady sodium current [26] play a larger part in resting potential generation in Purkinje fibres.

LOW $[K^+]_o$-INDUCED MODIFICATIONS OF MYOCARDIAL AND PURKINJE ACTION POTENTIALS

Ventricular action potentials were shortened at the level of the plateau and lengthened at the final repolarisation phase, during the first minutes in low $[K^+]_o$ (fig 2Bb). Thus low $[K^+]_o$ might increase the outward current at potentials. positive to about -20 mV and decrease it at potentials between the resting potential and -20 mV. [41] After longer periods, the inhibition of the Na-K pump [36] and the resulting increase in $[Na^+]_i$ would cause the Na-Ca exchange to extrude less Ca^{2+}. Increase in $[Ca^{2+}]_i$ can activate outward K^+-currents [42 43] and induce premature repolarisation of the action potential. Indeed signs of Ca-overload were observed in the preparation: ie transient depolarisations and after-contractions as seen in fig 3. [9 21] These oscillations in membrane potential and mechanical tension were related to oscillations of $[Ca^{2+}]_i$ [44 45] and seen to pre-exist in the control state. [44] They are enhanced by Ca-overload. [44 45] A slow phase of late repolarisation appeared beyond 30 min in low $[K^+]_o$ (fig 2Bd). This phenomenon has already been observed in papillary muscles of man [7] and guinea-pig. [9 19 23] It occurred simultaneously with the twitch [7 9 19 23] (see also fig 3)and might be considered as a plateau caused by a transient inward current such as that which developed in Purkinje fibres poisoned with strophanthidin. [8]

In Purkinje action potentials, a lengthening was observed at all levels of repolarisation (fig 5B).This may be accounted for by a depression of the electrogenic Na-K

pump current. [36] A direct depression of the membrane K^+ permeability [33] could also be involved.

Ventricular action potentials were shortened in low $[K^+]$,while Purkinje ones were lengthened (figs 2B and 5B). A similar effect was found in simultaneously recorded ventricular and Purkinje action potentials of the perfused pig moderator band by Gettes and Surawicz. [1] In their study, the effective refractory periods were altered in the same way. This means that low $[K^+]$,may increase the efficiency of the "gating mechanism" taking place in distal Purkinje fibres. [3 18]

ROLE OF A TTX-SENSITIVE STEADY-STATE SODIUM CURRENT IN PURKINJE RESTING AND ACTION POTENTIAL GENERATION

The shortening of the action potential duration and the hyperpolarisation described above in low concentrations of TTX are similar to those described in dog cardiac Purkinje fibres. [12 13] Both effects may be attributed to a steady-state TTX-sensitive sodium current [10] that flows at potentials between -10 mV and the diastolic potential. [10 11 13 46]

IMPLICATIONS FOR EXCITATION AND CONDUCTION

The severe extracellular K^+ depletion seen in this study does not occur clinically but may be considered as a means of blocking the Na-K pump [36] as in digitalis intoxication alone or combined with acute hypokalaemia. [6]

Spontaneous activity may occur in Purkinje fibres at a low membrane potential, [6 47] which may account for some ventricular tachyarrhythmias observed in man with digitalis intoxication and/or acute hypokalaemia. [6]

An increased difference between the durations of ventricular and Purkinje action potentials (as in low $[K^+]_o$) might improve the gating mechanism. However the earlier repolarisation of ventricular cells enables them to be prematurely re-excited. [6]

Transient depolarisations in low $[K^+]_o$ were not described in the human ventricle. They were recently found in human Purkinje fibres in the presence of adrenaline or ouabain. [2] When such transient depolarisations become suprathreshold, they trigger extrasystoles. This may account for the facilitator effect of hypokalaemia and digitalis on extrasystoles. [6 24]

The steady sodium current takes part in the maintenance of the gating mechanism. [13] Agents that decrease that current (eg local anaesthetics) may in turn depress the gating mechanism.

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