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Characterisation of the Na, K pump current in atrial cells

from patients with and without chronic atrial fibrillation

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Abstract

Antony J Workman, Kathleen A Kane, Andrew C Rankin. Characterisation of the Na, K pump current in atrial cells from patients with and without chronic atrial fibrillation.

Objective: To assess the contribution of the Na, K pump current (I_p) to the action potential duration (APD) and effective refractory period (ERP) in human atrial cells, and to investigate whether I_p contributes to the changes in APD and ERP associated with chronic atrial fibrillation (AF). **Methods:** Action potentials and ion currents were recorded by whole-cell patch clamp in atrial myocytes isolated from consenting patients undergoing cardiac surgery, who were in sinus rhythm (SR) or AF (>3 months). **Results:** In cells from patients in SR, the I_p blocker, ouabain (10 µM) significantly depolarised the membrane potential, V_m, from -80±2 (mean±SE) to -73±2 mV, and lengthened both the APD (174±17 *vs* 197±23 ms at 90% repolarisation) and ERP (198±22 vs 266±14 ms; *P*<0.05 for each, Student's *t*-test, *n*=7 cells, 5 patients). With an elevated pipette $[Na^+]$ of 30 mM, I_p was measured by increasing extracellular $[K^+]$ ($[K^+]_o$) from 0 to 5.4 mM. This produced an outward shift in holding current at -40 mV, abolished by 10 µM ouabain. K^+- and ouabain-sensitive current densities were similar, at 0.99±0.13 and 1.12±0.11 pA/pF, respectively (*P*>0.05; *n*=9 cells), confirming the K^+-induced current as I_p. I_p increased linearly with increasing V_m between -120 and +60 mV (*n*=25 cells). Stepwise increments in $[K^+]_o$ (between 0 and 10 mM) increased I_p in a concentration-dependent manner (maximum response, E_{max}=1.19±0.09 pA/pF; EC_{50}=1.71±0.15 mM; *n*=27 cells, 9 patients). In cells from patients in AF, the sensitivity of I_p to both V_m and $[K^+]_o$ (E_{max}=1.02±0.05 pA/pF, EC_{50}=1.54±0.11 mM; *n*=44 cells, 9 patients) was not significantly different from that in cells from patients in SR. Within the group of patients in AF, long-term digoxin therapy (*n*=5 patients) was associated with a small, but significant, reduction in E_{max} (0.92±0.07 pA/pF) and EC_{50} (1.35±0.15 mM) compared with non-treatment (E_{max}=1.13±0.08 pA/pF, EC_{50}=1.76±0.14 mM; *P*<0.05 for each, *n*=4 patients). In cells from non-digoxin-treated patients in AF, the voltage- and $[K^+]_o$-sensitivity (E_{max} and EC_{50}) were similar to those in cells from patients in SR. **Conclusions:** The Na, K pump current contributes to the human atrial cell V_m, action potential shape and ERP. However, the similarity in I_p sensitivity to both $[K^+]_o$ and V_m between atrial cells from patients with and without chronic AF indicates that I_p is not involved in AF-induced electrophysiological remodelling in patients.
Keywords: Na/K-pump; Membrane currents; Atrial function; Arrhythmia (mechanisms); Remodelling.

Introduction

The Na, K pump (Na, K-ATPase) functions to regulate the intracellular Na^+ concentration, $[Na^+]_i$, by actively extruding 3 Na^+ in exchange for 2 K^+ [1]. Na, K-ATPase is therefore electrogenic, exerting an outward, hyperpolarising current, I_p, which is known to influence the resting potential, V_m and repolarisation [2]. The Na, K pump current has been measured directly in various cardiac tissues and myocytes isolated from sheep [3], guinea pigs [4], rabbits [5,6] and dogs [7]. However, despite its potential to influence, or be influenced by, cardiac arrhythmias, I_p has not yet been measured directly in cells or tissues from the human heart.

Chronic atrial fibrillation (AF) shortens the atrial action potential duration (APD) and effective refractory period (ERP), thus contributing to the stabilisation of the arrhythmia [8-13]. The pattern of changes in ionic currents responsible for this is not fully understood. The density of atrial L-type Ca^{2+} (I_{CaL}) and transient outward K^+ (I_{TO}) currents is consistently and markedly reduced in AF or rapid atrial pacing, in dogs [10] and humans [11-13]. However, recent work from our laboratory in human atrial cells [12] suggests that the shortening of APD and ERP by chronic AF cannot be explained by changes in these currents alone, consistent with a mathematical model [14], and data on the main alternatives are presently either unavailable, or in some cases, conflicting [13].

The involvement or otherwise of I_p or Na,K-ATPase in AF-induced atrial electrophysiological remodelling is presently unclear [13]. In sheep, short episodes of rapid atrial pacing increased the expression of atrial Na, K-ATPase [15], although there was no effect on its activity [16]. In the goat model of AF, treatment with the I_p blocker, digoxin, had either no effect on AF-induced changes in atrial ERP and AF inducibility [17], or delayed their reversal [18]. However, I_p was not measured directly in any of these studies, and has not yet, to our knowledge, been recorded in any model of rapid atrial pacing or AF.

It is conceivable that the shortening of atrial APD and ERP by chronic AF in humans might involve an increase in atrial I_p. The aim was to test this hypothesis, by: 1) assessing the contribution of I_p to human atrial cell APD and ERP; 2) measuring I_p directly in atrial cells from patients in sinus rhythm (SR) and AF; 3) comparing the density, voltage- and extracellular $[K^+]$ ($[K^+]_o$)-sensitivity of I_p between these patient groups.

Methods

The tip of the right atrial appendage was obtained from 25 consenting patients undergoing cardiac surgery. Procedures approved by the institutional research ethics committee were followed. The investigation conforms with the principles outlined in the Declaration of Helsinki [19]. Atrial cells were isolated by enzymatic dissociation and mechanical disaggregation, using protease (Type XXIV, Sigma) and collagenase (Type 1, Worthington), as described in detail previously [12].

Action potentials and ion currents were recorded using the whole cell patch clamp technique. Microelectrodes were pulled (Narishige PP-83) from filamented borosilicate glass tubes (Clark Electromedical) and heat polished to resistances of 3-7 MΩ. Cells were superfused at 35-37°C, at 1.5-2 ml/min in a 200 μl perfusion chamber (RC-24E, Warner). An Axopatch-1D amplifier (Axon Instruments) and "WinWCP" software (donated by J Dempster, Strathclyde University) was used to stimulate and record electrical activity. Capacitative transients were compensated electronically prior to recording. Signals were low-pass filtered at 5 kHz and digitised (Digidata 1200 A-D converter, Axon) prior to storage on magnetic and compact discs.

Action potentials were recorded using an extracellular solution containing (mM): NaCl (130), KCl (4), CaCl$_2$ (2), MgCl$_2$ (1), glucose (10) and HEPES (10), and a pipette solution containing: K-aspartate (110), KCl (20), MgCl$_2$ (1), EGTA (0.15), Na$_2$ATP (4), Na$_2$GTP (0.4) and HEPES (5). A liquid junction potential of +7$\pm$0.3 mV (*n*=6) was measured and compensated prior to seal formation. Action potentials were stimulated using 5 ms duration current pulses of 1.2x threshold strength, with an 8-pulse (S$_1$) conditioning train (75 beats/min). All cells were current-clamped (with hyperpolarising current of <150 pA) initially to a maximum diastolic potential (MDP) of ~-80 mV (measured from the 7th S$_1$ response) and the holding current was kept constant in each cell thereafter. The APD was calculated as the interval between the action potential upstroke and repolarisation to the level of 50, 75 and 90% (APD$_{50}$, APD$_{75}$ and APD$_{90}$, respectively). Action potential restitution was measured by introducing a progressively premature test pulse (S$_2$) after the S$_1$ trains, with S$_1$ and S$_2$ of equal magnitude. From this, the cell's ERP was measured, as previously [12,20], as the longest S$_1$-S$_2$ interval failing to elicit an S$_2$ response of amplitude >80% [21] of the preceding S$_1$ action potential. The action potential maximum upstroke velocity (V$_{max}$) was measured by automatically scanning phase 0 at high time resolution for the maximum slope between two adjacent voltage samples. Action potentials were recorded before and after superfusion with ouabain (10 μM) for 120 s, and again following

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its removal. This concentration was chosen to avoid "rundown" of currents during the experimental

protocols, since complete I_p block by 1 μM ouabain required 6-10 min superfusion, whilst 10 μM ouabain

required only 40-80 s (eg: see Fig 2).

I_p was recorded over a wide voltage range, using solutions selected to minimise contamination from

other currents [4,6]. The extracellular solution contained (mM): NaCl (145), CsCl (2), $MgCl_2$ (0.5), glucose

(10), HEPES (5), $NiCl_2$ (2), $BaCl_2$ (1). Cs^+ was used to block I_f, I_{K1} and I_{KACh}; Ni^{2+} to block $I_{Na/Ca}$, and Ba^{2+} to

block I_{KP} [22]. $[Ca^{2+}]_o$ was kept at zero to avoid I_{CaL}. $[K^+]_o$ was initially kept at zero to record currents in the

absence of I_p, prior to its activation. I_{Na} was inactivated with a holding potential of -40 mV. The pipette

solution contained (mM): Aspartic acid (100), CsCl (20), $MgCl_2$ (2), HEPES (5), MgATP (5), EGTA (10),

K-creatine phosphate (5), CsOH (90) and NaOH (30). K^+ was replaced by Cs^+ to block outward K^+ currents.

A high degree of intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$)-buffering minimised Ca^{2+}-activated currents, and high $[Na^+]_i$

was used to record I_p at close to full saturation [4,6]. A liquid junction potential (+7±0 mV, *n*=3) was

compensated. The holding current was measured before and after superfusion with 5.4 mM KCl, and

following its removal. One or more of the following protocols was then performed. The sensitivity to a

cardiac glycoside of the extracellular K^+ (K^+_o)-induced holding current shift was investigated, using ouabain

at 1 and 10 μM. The voltage-sensitivity of K^+- and glycoside-sensitive currents was determined using voltage

ramps (increasing from -120 to +60 mV, at 36 mV/s) or rectangular voltage pulses (300 ms duration,

increasing from -120 to +60 mV, at 0.1 Hz). Finally, the sensitivity of the current shift to $[K^+]_o$ was

determined with stepwise-incrementing $[K^+]_o$ between 0 and 10 mM.

Details of patients' clinical characteristics and drug treatments were obtained from the case notes and

stored in a database (Access, Microsoft). All currents were normalised to cell capacity. $[K^+]_o$-response data

were fitted iteratively in each cell with variable slope sigmoidal curves (Prism, GraphPad). Values are cell

means±SE. Differences between means were assessed using 2-tailed paired or unpaired Student's *t* tests, as

appropriate. Differences between incidences of patients receiving specified drugs were compared using a χ^2

test. *P*<0.05 was regarded as statistically significant.

Results

Patients' clinical characteristics

The patients' characteristics are shown in Table 1. The majority of patients underwent coronary artery bypass graft surgery (76%) and suffered from angina (84%). Of patients in AF at the time of surgery, only those in which AF had persisted for longer than 3 months were included. Half of these patients underwent mitral valve surgery, versus none of those in SR. The medication taken by the patients is detailed in Table 1 and it is noteworthy that none of the patients in SR were taking digoxin, in contrast to 60% of patients in chronic AF. All patients on digoxin had been receiving the drug for longer than 2 months and patients received their routine cardiac drugs on the day of surgery.

Effects of superfusion with ouabain on human atrial cell action potentials and refractoriness

The I_p blocker, ouabain (10 μM) significantly affected the single cell V_m, action potential shape and ERP, as shown in the representative recordings in Fig 1 and mean data in Table 2. Ouabain depolarised the MDP by ~7 mV (Fig 1A and Table 2), associated with a marked reduction in the action potential V_{max}, overshoot and amplitude and a significant prolongation of early (APD_{50}) and late (APD_{75} and APD_{90}) repolarisation. Fig 1B shows the action potential restitution characteristics and ERP measurement in the absence and presence of ouabain, in the same cell as Fig 1A. The ouabain-induced depolarisation was associated with slowing of the recovery of excitability, flattening of the restitution curve, and marked and significant lengthening of the cell ERP, from 198±22 to 266±14 ms ($P<0.05$; n=5 cells, 4 patients).

Activation of human atrial I_p by physiological $[K^+]_o$ was abolished by ouabain

The representative current trace shown in Fig 2A shows that, using an elevated pipette $[Na^+]$ of 30 mM, increasing $[K^+]_o$ from 0 mM to the physiological value of 5.4 mM produced an outward shift in the holding current at -40 mV, of approximately 1 pA/pF. This response was complete after approximately 60 s of K^+ application, and was fully reversed after K^+ removal. A second application of K^+ produced a current shift of similar magnitude. This was abolished by superfusion with ouabain (10 μM), with a similar time course of effect as that caused by the prior removal of K^+. The effect of 1 μM ouabain was also investigated for comparison, in a different cell (Fig 2B). The K^+-induced outward current was again abolished by ouabain,

but with a substantially longer time course than at 10 μM. A comparison between the mean current shift produced by the increase in $[K^+]_o$ and the reverse shift produced by ouabain, is shown in Fig 2C. The K^+- and ouabain-sensitive current densities were similar, at 0.99 ± 0.1 and 1.12 ± 0.113 pA/pF (for 10 μM ouabain), respectively ($P>0.05$; $n=9$ cells, 3 patients), indicating that the K^+-induced current was I_p.

Human atrial I_p was voltage-dependent

The steady-state voltage-dependency of I_p was examined using rectangular voltage pulses, in cells from 9 patients in SR. Fig 3A*i* shows representative currents recorded in the absence and presence of 5.4 mM K^+_o. All cells displayed steady-state responses between -120 and +20 mV (Fig 3A*i*). A small time-dependent component was observed at +60 mV in 59% of cells, possibly reflecting incomplete K^+ current suppression. The currents recorded prior to K^+ application were digitally subtracted from those recorded in its presence, as shown in the bottom panel of Fig 3A*i*. The subtraction currents were time-independent between -120 and +60 mV in all cells. The mean current-voltage (I-V) relationship of these currents, shown in Fig 3A*ii*, demonstrated that the K^+_o-sensitive current was voltage-dependent, increasing approximately linearly from 0.47 ± 0.10 pA/pF at -120 mV to 1.36 ± 0.22 pA/pF at +60 mV ($P<0.05$, $n=17$ cells). Voltage ramp pulses were also used, to examine and compare the pseudo-steady-state I-V relationships of both K^+- and 10 μM ouabain-sensitive currents. Fig 3B*i* shows the current responses to these ramps, prior to and following superfusion with 5.4 mM K^+ and after its removal, with the K^+-sensitive (subtraction) current in the lower panel. Mean I-V relationship data (Fig 3B*ii*), obtained in cells from 12 patients, confirmed that the voltage ramp-evoked K^+_o-sensitive current displayed a similar voltage-dependence to that of the steady-state current. The voltage-dependent characteristics of the ouabain-sensitive current (Fig 3C) were similar to those of both the steady-state (Fig 3A) and pseudo-steady-state (Fig 3B) currents, indicating that the voltage-dependent characteristics of the K^+_o-evoked current were those of I_p.

I_p was increased by K^+_o in a concentration-dependent manner

The amplitude of I_p increased in response to increasing $[K^+]_o$, as shown in Fig 4. I_p was measured from a holding potential of -40 mV, and $[K^+]_o$ was increased in a stepwise manner between 0 and 10 mM. I_p was near maximal at 5.4 mM K^+_o. The 10 mM K^+_o-induced current was abolished by the washout of K^+. The concentration-response relationship for the effect of K^+_o on I_p was examined in cells from 9 patients in SR. In

each cell, I_p density was fitted iteratively to logarithmic $[K^+]_o$ with a variable slope sigmoidal curve, using

the Hill equation: $Y=E_{min}+[E_{max}-E_{min}]/[1+(x/EC_{50})^P]$, where $Y=I_p$ density (pA/pF), $E_{min}=I_p$ at 0 mM (set to 0

pA/pF), E_{max}=maximum I_p response elicitable by K^+_o (pA/pF), $x=[K^+]_o$ (mM), $EC_{50}=[K^+]_o$ producing 50% of

E_{max} (mM) and P=Hill coefficient. Fig 5A (open circles) shows the mean I_p density recorded at each $[K^+]_o$ on

a linear scale, with a single hyperbolic curve fitted to these data points using the same procedure as for the

individual cells. The mean E_{max} and EC_{50}, calculated from the individual cells' curves, were 1.19±0.09 pA/pF

and 1.71±0.15 mM, respectively (*n*=27 cells).

Atrial fibrillation was not associated with a significant alteration of I_p $[K^+]_o$- or voltage-sensitivity

The $[K^+]_o$-dependency of I_p was examined in 9 patients who were in chronic AF, and compared with

that of cells from the 9 patients in SR. Fig 5A (filled circles) shows the mean I_p density at different $[K^+]_o$ (0-

10 mM) in the cells from patients in AF, with a single hyperbolic curve fitted, using the Hill equation, to the

mean data points. The I_p $[K^+]_o$-response in cells from the patients in AF was similar to that in cells from the

patients in SR (Fig 5A, open circles). The mean I_p at 5.4 and 10 mM K^+_o was not significantly different in the

cells from patients in SR, at 0.97±0.06 and 1.05±0.08 pA/pF, respectively (*n*=27 cells) from those in the cells

from patients in AF, at 0.85±0.04 and 0.91±0.05 pA/pF, respectively (*P*>0.05 for each; *n*=44 cells).

Furthermore, the mean EC_{50} and E_{max} values were not significantly different in the patients in chronic AF (at

1.54±0.11 mM and 1.02±0.05 pA/pF, respectively) from those measured in the patients in SR, as shown in

Fig 5B. The 5.4 mM K^+_o-induced I_p steady-state I-V relationship was examined in cells from 8 patients in

chronic AF, and compared with that obtained in cells from 9 patients in SR, as shown in Fig 5C. There was

no significant difference in the voltage-dependency of I_p between the two patient groups, with both

displaying an approximately linear increase in I_p with increasing voltage.

Long-term digoxin therapy in patients with AF was associated with a small reduction in I_p

Sixty percent of the patients who were in chronic AF were treated pre-operatively with digoxin, versus

none of the patients in SR (Table 1) (*P*<0.05, χ^2 test). Since digoxin therapy may be expected to reduce I_p in

human atrial tissues [23], the group of patients in chronic AF was sub-divided into those who were treated

with digoxin and those who were not. The $[K^+]_o$-dependency of the atrial cell I_p was compared between these

sub-groups, with the mean I_p values and $[K^+]_o$-response curves shown in Fig 6A*i*, and the mean EC_{50} and

E_{max} in Fig 6A*ii*. Digoxin therapy was associated with a small, but significant, reduction in mean E_{max}, by 19%, from 1.13±0.08 pA/pF (*n*=23 cells, 5 patients) to 0.92±0.07 pA/pF (*P*<0.05; *n*=21 cells, 4 patients). Digoxin therapy was also associated with a small, but significant, reduction in the mean EC_{50}, by 23%, from 1.76±0.14 to 1.35±0.15 mM (*P*<0.05). The potential influence on I_p of angiotensin converting enzyme (ACE) inhibition was also examined, since I_p was increased in rabbits by captopril treatment [24]. In cells from patients treated with ACE inhibitors, the I_p $[K^+]_o$-response E_{max} and EC_{50} were 1.29±0.11 pA/pF and 1.98±0.25 mM, respectively (*n*=11 cells, 4 patients), not significantly different from that in cells from non-treated patients, at 1.12±0.13 pA/pF and 1.51±0.17 mM, respectively (*n*=16 cells, 5 patients; *P*>0.05 for each).

Atrial I_p was similar in the non-digoxin-treated patients in AF to that of the patients in SR

The concentration-response relationship for the effect of K^+_o on I_p was compared in cells from the patients who were in chronic AF but not treated with digoxin, to that obtained in cells from those patients in SR, none of whom were taking digoxin. Fig 6B shows that chronic AF, in the absence of the influence of digoxin therapy, was not associated with a significant change in the density or K^+_o-sensitivity of atrial I_p, with similar mean I_p $[K^+]_o$-response curves (Fig 6B*i*) and similar mean EC_{50} and E_{max} values (Fig 6B*ii*) between the two patient groups.

Discussion

The Na, K pump current, I_p, was measured directly for the first time to our knowledge in human myocardium, and was shown to have similar characteristics to those recorded in several other species, including the responses to extracellular potassium, voltage and ouabain [3-7]. The half-maximal response to K^+_o was within the reported range (1.0-2.8 mM), and the I_p-voltage relationship was linear, in line with the majority of studies, although this may have been influenced by incomplete K^+ current suppression, since I_p saturation was reported at 0 mV [6]. In addition, evidence has been provided, novel in any species, of a lack of change in atrial I_p associated with chronic AF, and also of a reduction in I_p associated with long-term digoxin therapy.

In cells from patients in chronic AF, I_p displayed a similar density, voltage- and $[K^+]_o$-sensitivity to that of cells from patients in SR, indicating that a change in neither the density nor functional properties of atrial I_p is involved in human AF-induced atrial electrophysiological remodelling. This is in line with the reported absence of change in atrial Na, K-ATPase activity following 2 hours of AF in sheep [16], and with the lack of effect of digoxin on atrial electrophysiological remodelling produced by 24 hours of AF in goats [17], although in the latter model, digoxin delayed the reversal of such remodelling [18]. However, the magnitude of I_p *in-vivo* may increase in patients during AF, since the rapid rate would increase the time spent in depolarisation. This may increase both $[Na^+]_i$ and $[K^+]_o$, each of which increases I_p [3,4,6,7], as confirmed by the present data, which would contribute to the shortening of atrial APD and ERP during AF. However, I_p cannot be the major factor since atrial electrophysiological remodelling is observed in human atrial isolated cells even when the ionic concentrations are controlled [10-13,25].

Long-term digoxin therapy was associated with a small reduction in atrial I_p. This was consistent with the reported reduction by digoxin therapy in the hyperpolarisation caused by rewarming of human atrial tissue considered due to reactivation of the Na,K pump following $[Na^+]_i$-loading [23]. The present magnitude of I_p reduction, of ~20%, corresponded with the reported percentage occupancy of digitalis receptors in chronically digitalised failing human ventricle, measured after protracted washing of tissues in anti-digoxin antibodies [26]. Alternatively, the observed reduction in I_p may represent an adaptive change analogous to the pharmacological remodelling of human atrial ion currents by chronic β-blockade [20]. Such a process would be consistent with the reduction in the number of Na, K-ATPase sites in HeLa cells exposed to ouabain [27]. It is not known whether the I_p reduction was influenced mainly by residual receptor occupancy

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or by pharmacological remodelling. Since digoxin increased the sensitivity of I_p to K^+_o, an effect on the Na,K-ATPase α-2 isoform is a possibility, since its activity is strongly dependent on $[K^+]_o$ [28]. Long-term ACE inhibition did not affect human atrial I_p, in agreement with the reported lack of effect on human atrial cell action potentials and ERP [20]. There have been no previous reports of effects of ACE inhibitors on human atrial I_p, although ventricular I_p was increased in captopril-treated rabbits, due to a reduction in interstitial and/or intracellular concentrations of angiotensin II, and not to a change in Na, K-ATPase expression [24].

A change in atrial I_p, whether by digoxin therapy as observed here, or by altered voltage and/or ionic conditions during AF in the absence of an electrophysiologically-remodelled Na,K pump would affect the APD and ERP, particularly with the high input resistance of human atrial cells [12,20]. Our results with ouabain suggested the potential for such an effect. It should be noted, however, that although ouabain completely blocked I_p and prolonged the APD and ERP in cells *in-vitro*, the degree of block *in-vivo* in patients treated with digoxin would be less (and possibly as low as the observed 19% reduction in E_{max}), and the consequent effect on the APD and ERP may also, therefore, be predicted to be less than that observed *in-vitro*. No reports of effects of cardiac glycosides on action potentials in human atrial cells were found, but a small prolongation in atrial ERP was reported in patients administered ouabain [29] or digoxin [30]. However, acute digoxin had no effect on the ERP in goats [17,18] and APD-shortening by digitalis has also been reported [31,32]. In human atrial fibres [31], APD-shortening was secondary to vagal stimulation, but in guinea pig ventricular myocytes [32], it was due to a secondary ionic effect of I_p block, namely attenuation of the normally inward Na^+/Ca^{2+} exchanger current ($I_{Na/Ca}$) by ouabain-induced $[Na^+]_i$-loading. The effects of digitalis on the APD and ERP *in-vivo* therefore result from a complex interaction between direct and secondary effects of I_p blockade and effects of vagal stimulation. It is also the case that the measurement of ERP that is made in single cells is not identical to that made *in-vivo*, since ERP is conventionally measured in terms of propagation failure, which cannot be measured in single cells. Nevertheless, action potentials of amplitude >80% of normal, as used here to define the cell ERP, have been shown to propagate, with graded responses occurring at lower amplitudes [21]. Additionally, 10 μM ouabain may affect action potentials via currents additional to I_p, such as I_{CaL} [33]. However, since I_{CaL} blockade with nifedipine had no effect on V_m or ERP in human atrial cells [12], this suggests that 10 μM ouabain largely affected these measurements by blocking I_p, supported by a mathematical model [5].

Digoxin is used clinically as an inotropic drug to improve haemodynamic function, and also for ventricular rate control during AF, through central and peripheral augmentation of vagal tone to prolong the AV nodal ERP. Long-term digoxin treatment is positively inotropic by increasing $[Ca^{2+}]_i$, secondary to $[Na^+]_i$-loading by I_p blockade, although therapeutic serum concentrations (1-2 nM) are at least 50-fold lower than the minimum 0.1 μM required for acute I_p block [34]. $[Na^+]_i$-loading may contribute to APD-shortening in the atrium *in-vivo*, via a reduced $I_{Na/Ca}$, and $[Ca^{2+}]_i$-loading may also shorten the APD, via electrophysiological remodelling of I_{CaL} and I_{TO} [13]. Either or both of these influences on the APD may explain the reported delay by digoxin of recovery from AF-induced atrial electrophysiological remodelling in goats [18], and may also contribute to the lack of efficacy of digoxin in converting human AF [35]. Of note, in previous studies of human atrial cellular electrophysiological remodelling, the majority of patients in AF were taking digoxin [11,12,25]. However, any influence on the APD of $[Na^+]_i$-loading would have been removed by the controlled $[Na^+]_i$ in each of these studies. Moreover, in the presence of residually-bound or down-regulated digoxin receptors, a reduction in the hyperpolarising and repolarising influences of I_p would contribute a direct APD-lengthening effect, as observed here with ouabain. Thus, the reported APD-shortening associated with chronic AF in human atrial cells [11,12] may have been underestimated.

In conclusion, the present data indicate that chronic AF in humans, in the absence of the influence of digoxin therapy, was not associated with a significant change in the density of atrial I_p, or in its sensitivity to voltage or extracellular K^+.

Acknowledgements

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Tables and Fig legends

Table 1. Patients' characteristics.

Values are numbers of patients (*n* and % of total, respectively) with selected clinical characteristics, except

for age (mean±SE). CCB=calcium channel blocker, CABG=coronary artery bypass graft surgery,

AVR=aortic valve replacement, MVR=mitral valve replacement, LV=left ventricular, MI=myocardial

infarction.

Table 2. Effects of ouabain on human atrial cell action potentials and refractory period.

MDP=maximum diastolic potential; V_{max}=action potential maximum upstroke velocity; APD_x=action

potential duration at x% repolarisation; ERP=effective refractory period. Values are means±SE (*n*=7 cells, 5

patients, except for ERP: 5 cells, 4 patients). All patients were in SR. Stimulation rate=75 beats/min.

Asterisks=*P<0.05 vs* control).

Figure 1.

Effects of ouabain on human atrial cell action potentials and refractoriness.

Original action potential recordings from a single atrial cell from a patient in SR, in the absence (O) and

presence (●) of ouabain (10 μM). *A*, Superimposed action potentials stimulated by the 7th of a train of

conditioning current pulses, S_1 (rate: 75 beats/min) showing the effects of ouabain on V_m and action potential

configuration. *B*, Superimposed action potentials elicited, in the same cell, by the 7th and 8th S_1 pulses,

followed by responses to an increasingly premature test pulse, S_2, showing the effects of ouabain on action

potential restitution. The cell ERP (solid bars) was calculated as the longest S_1-S_2 interval failing to elicit an

S_2 response of amplitude >80% of the preceding S_1 action potential. In each case, the S_2 response used to

measure this interval is labelled (↘).

Figure 2.

Identification of human atrial Na, K pump current, I_p.

Whole-cell current changes evoked by K^+_o and/or ouabain in atrial myocytes from patients in SR. Traces

show A, activation of an outward current (I) by increasing $[K^+]_o$, current inhibition after decreasing $[K^+]_o$,

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and abolition of K^+_o-induced current by ouabain; B, non-reversible and relatively slow abolition of I_p by 1

μM ouabain. Bars show concentration and time-course of K^+ and ouabain application. C, Histogram

comparing mean K^+_o- and ouabain-sensitive current densities, calculated as peak K^+_o-induced current minus

corresponding current in absence of K^+_o (■: *n*=17 cells, 4 patients) and in presence of ouabain at 1 μM (▨:

n=9 cells, 3 patients) and 10 μM (▨: *n*=9 cells, 3 patients). All patients were in SR. Values are means±SE,

NS=*P*>0.05 *vs* K^+_o-sensitive current.

Figure 3.

Voltage-dependency of human atrial I_p.

Current-voltage (I-V) relationships for A, steady-state K^+_o-sensitive currents (*n*=17 cells, 9 patients); B,

pseudo steady-state K^+_o-sensitive currents (*n*=25 cells, 12 patients); C, pseudo steady-state ouabain-sensitive

currents (*n*=4 cells, 2 patients) in atrial cells from patients in SR. Upper panels (*i*) show representative

whole-cell current traces and lower panels (*ii*) mean±SE I-V relationships at baseline (○), in the presence of

5.4 mM K^+_o (△) and following either washout of K^+ (□) or addition of 10 μM ouabain (◇), with digital

subtraction of baseline currents from those recorded in the presence of K^+ and ouabain indicated by ▲ and

◆, respectively.

Figure 4.

Extracellular $[K^+]_o$-sensitivity of human atrial I_p.

Original recording of whole cell current at a holding potential of -40 mV in a cell from a patient in SR,

showing the effects on I_p amplitude of stepwise increments in $[K^+]_o$ between 0 and 10 mM.

Figure 5.

Comparison of atrial I_p characteristics between patients in SR and chronic AF.

I_p $[K^+]_o$-response curves (A) and characteristics (B) in cells from patients in SR (open symbols; *n*=27 cells, 9

patients) and chronic AF (filled symbols; *n*=44 cells, 9 patients). I_p is expressed relative to that at 0 mM K^+_o.

Mean data points were fitted by the Hill equation (see Results). Mean E_{max} (maximal I_p response to K^+_o) and

EC_{50} ($[K^+]_o$ producing 50% of E_{max}) were calculated from the individual cell curve-fit data. NS=*P*>0.05

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between patient groups. C, Steady-state I_p I-V relationship in cells from patients in SR ($\bigcirc$: *n*=17 cells, 9 patients) and chronic AF ($\bullet$: *n*=24 cells, 8 patients). All values are means±SE.

Figure 6.

Effects of digoxin therapy on atrial I_p, and comparison between non-digoxin-treated patients in SR and AF

A, Comparison of the I_p $[K^+]_o$-response between cells from patients in chronic AF treated and not treated with digoxin. B, Comparison of the I_p $[K^+]_o$-response between patients in AF not treated with digoxin and those in SR. Concentration-response curves (*i*) and characteristics (*ii*) are shown. Values are means±SE. $\blacktriangledown$ and $\equiv$=AF, not treated with digoxin (*n*=21 cells, 4 patients); $\blacktriangle$ and ▥=AF, treated with digoxin (*n*=23 cells, 5 patients); open symbols=SR (*n*=27 cells, 9 patients). Asterisks=*P*<0.05 and NS=*P*>0.05 between patient sub-groups.

Table 1

		Sinus rhythm		Atrial fibrillation	
		n	*%*	*n*	*%*
Patient details	Male/female	7/8	47/53	6/4	60/40
	Age (years)	60±3	-	65±3	-
Drug treatments	Lipid lowering	12	80	5	50
	β-blocker	10	67	5	50
	Nitrate	12	80	3	30
	CCB	7	47	3	30
	ACE inhibitor	7	47	6	60
	Diuretic	5	33	6	70
	Digoxin	0	0	6	60
Operation type	CABG	13	87	4	40
	AVR	2	13	1	10
	MVR	0	0	3	30
	CABG + MVR	0	0	2	20
LV function	Normal	7	47	5	50
	Mild/moderate	8	53	2	20
	Severe	0	0	3	30
Disease	Angina	15	100	6	60
	Hyperlipidaemia	12	80	4	40
	Hypertension	7	47	2	20
	Previous MI	8	53	3	30
	Diabetes	2	13	1	10

Table 2

	Control	Ouabain (10 μM)
MDP (mV)	-80 ± 2	-73 ± 2 *
V_{max} (V/s)	171 ± 16	72 ± 15 *
Overshoot (mV)	47 ± 4	11 ± 5 *
Amplitude (mV)	127 ± 6	84 ± 6 *
APD_{50} (ms)	10.1 ± 1.6	19.9 ± 4.1 *
APD_{75} (ms)	80 ± 7	101 ± 10 *
APD_{90} (ms)	174 ± 17	197 ± 23 *
ERP (ms)	198 ± 22	266 ± 14 *

Figure 1

Figure 2

A

1 min

100 pA

I (pA)

[K$^+$]$_o$ (mM)   0   5.4   0   5.4

[ouab]$_o$ (μM)   0   10

B
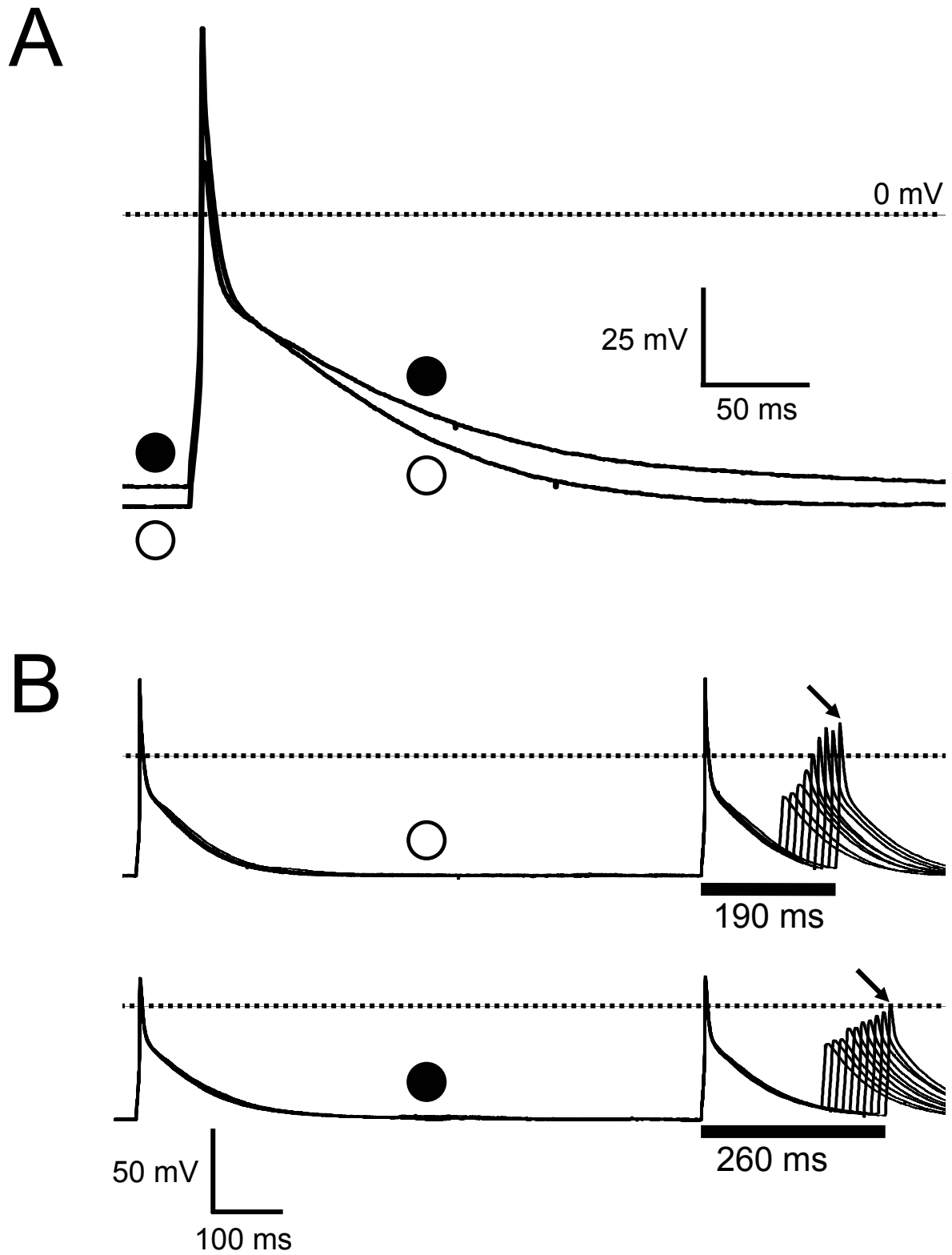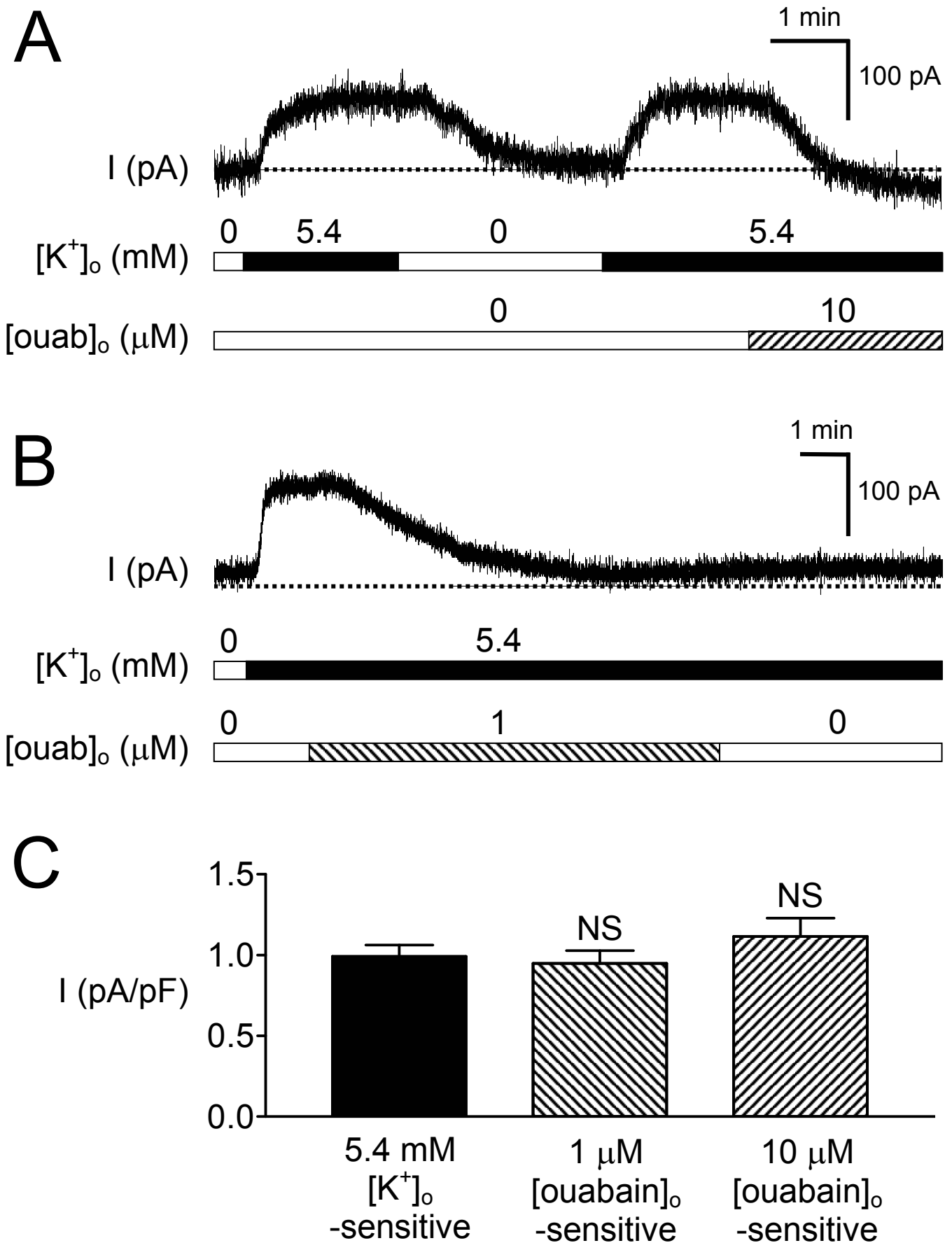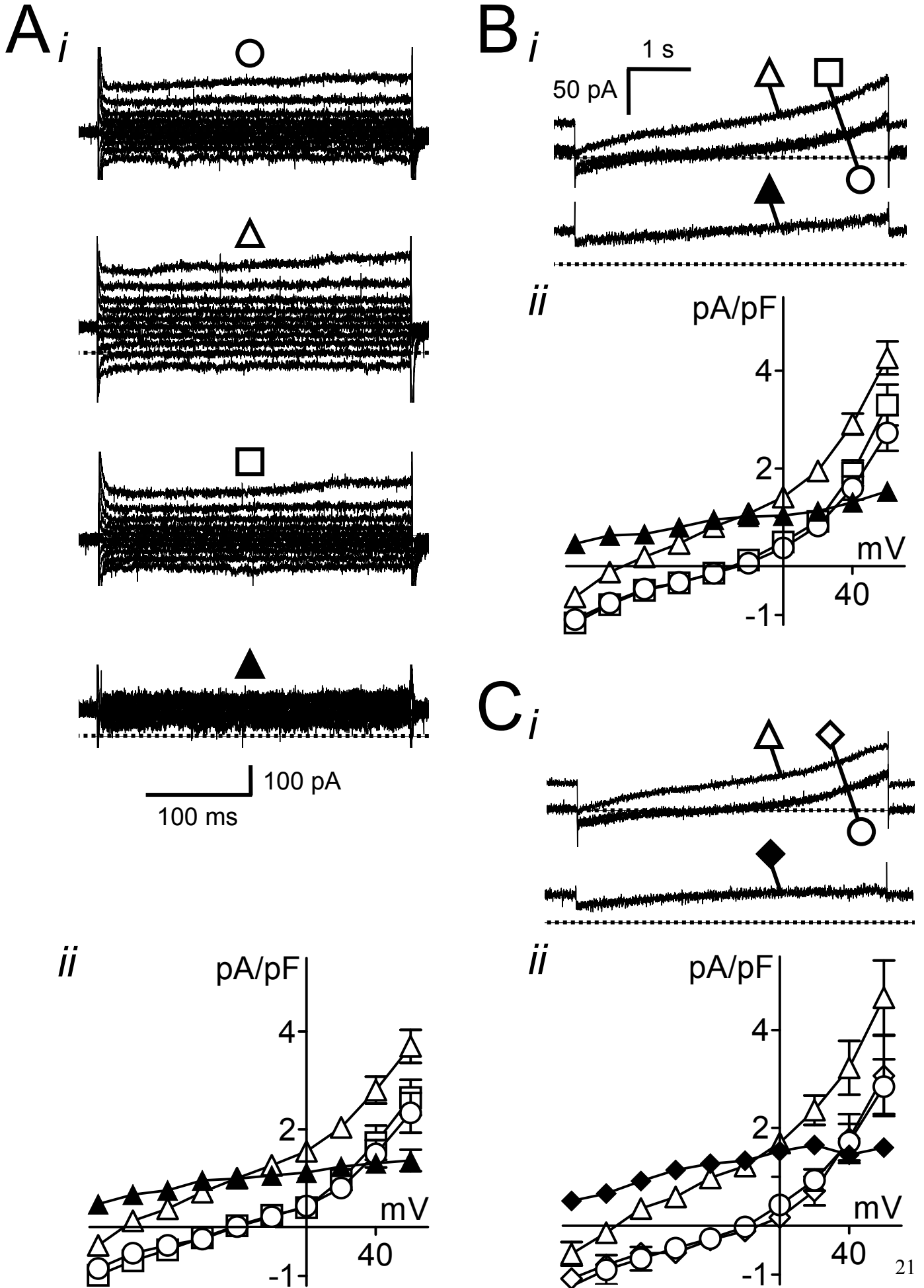
1 min

100 pA

I (pA)

[K$^+$]$_o$ (mM)   0   5.4

[ouab]$_o$ (μM)   0   1   0

C

Figure 3

Figure 4

Figure 5

Figure 6