Actin microfilament disrupters enhance K_{ATP} channel opening in patches from guinea-pig cardiomyocytes

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1. To determine whether actin filament networks are associated with the regulation of ATP-sensitive K^+ (K_{ATP}) channel activity, single channel currents were measured in the inside-out configuration, and cytoskeletal disrupters applied to the internal side of patches excised from guinea-pig ventricular myocytes.

2. Treatment of patches with DNase I (10–200 μg ml^{-1}), which forms complexes with G-actin and prevents actin filament formation, antagonized the ATP-induced inhibition of K_{ATP} channels.

3. In the absence of ATP, DNase I did not increase K_{ATP} channel activity.

4. When denatured by boiling or co-incubated with purified actin subunits (200 μg ml^{-1}), DNase I (100 μg ml^{-1}) did not antagonize the ATP-induced inhibition of K_{ATP} channels.

5. The DNase I-induced decrease in the sensitivity of K_{ATP} channels towards ATP-induced inhibition was partially restored by addition of purified actin subunits (200 μg ml^{-1}).

6. Cytochalasin B (10 μM), another actin filament disrupter, but neither taxol nor nocodazole (30–100 μM), two antimicrotubule agents, enhanced K_{ATP} channel activity in the presence of ATP.

7. Hence, actin filament disrupters can attenuate the ATP-dependent inhibitory gating of K_{ATP} channels. This suggests that subsarcolemmal actin filament networks may be associated with the regulation of cardiac K_{ATP} channels.

ATP-sensitive K^+ (K_{ATP}) channels, a class of ion channels that provide a link between the metabolic status and electrical excitability of cells, have been directly associated with critical cellular functions such as hormone secretion, vasodilatation and cardioprotection (reviewed by Ashcroft & Ashcroft, 1990; Davies, Standen & Stanfield, 1991; Lazdunski, 1994). Although the defining property of K_{ATP} channels is their inhibition by intracellular ATP (ATP_i) (Noma, 1983), a reduction in $[ATP]_i$ appears not to be the sole mechanism responsible for channel opening (reviewed by Nichols & Lederer, 1991; Weiss & Venkatesh, 1993; Findlay, 1994; Terzic, Tung & Kurachi, 1994*c*).

Indeed, in membrane patches excised from a cardiac cell, half-maximal inhibition of K_{ATP} channel activity is usually produced by 20–30 μM ATP_i (see Nichols & Lederer, 1991; Terzic, Jahangir & Kurachi, 1995), whereas the concentration of ATP_i remains in the millimolar range in all but the most pathological of circumstances (Weiss & Venkatesh, 1993). Therefore, it appears that the mechanism responsible for the opening of K_{ATP} channels is complex (see Ashcroft & Ashcroft, 1990; Davies *et al.* 1991; Findlay, 1994; Terzic *et al.* 1994*c*). Major variations in the sensitivity of K_{ATP} channels towards ATP_i-induced channel inhibition have been reported following excision of a patch from a cardiac cell membrane, which cannot be explained by changes in the concentration of intracellular nucleotides and/or in the intrinsic properties of the channel protein (Findlay & Faivre, 1991; Terzic *et al.* 1994*c*). This observation may indicate that previously unrecognized mechanism(s) associated with the patch membrane could participate in the regulation of K_{ATP} channel gating in cardiac cells.

As integral membrane proteins, ion channels are surrounded by cytoskeletal strands, in particular actin filaments (Ruknudin, Song & Sachs, 1991; Horber, Mosbacher, Haberle, Ruppersberg & Sakmann, 1995). Structural, as well as functional, interactions between several epithelial Na^+ and Cl^- channels and the adjacent actin filament network have been described (Cantiello, Stow, Prat & Ausiello, 1991; Suzuki, Miyazaki, Ikeda, Kawaguchi & Sakai, 1993; Schwiebert, Mills & Stanton, 1994; Prat, Xiao, Ausiello & Cantiello, 1995). Moreover, in

neuronal tissues, cytoskeleton breakdown has been shown to affect membrane excitability through the regulation of Ca^{2+} and *N*-methyl-D-aspartic acid (NMDA) channels (Fukuda, Kameyama & Yamaguchi, 1981; Johnson & Byerly, 1993; Rosenmund & Westbrook, 1993). Recently, it has been demonstrated in the myocardium that the opening of K_{ATP} channels is sensitive to mechanical distortions of the membrane (Van Wagoner, 1993; Van Wagoner & Lamorgese, 1994). This could suggest that the integrity of the microenvironment, in particular the actin filament network, surrounding K_{ATP} channel proteins may play a role in modulating this ion conductance.

Therefore, the aim of this study was to define whether disrupters of actin microfilaments affect K_{ATP} channels from guinea-pig ventricular cardiomyocytes. We report that disrupters of actin microfilaments do enhance cardiac K_{ATP} channel activity, apparently by antagonizing the ATP_i-mediated inhibitory gating of these channels. A cytoskeleton-dependent modulation of myocardial K_{ATP} channels would represent a novel component in the regulation of the behaviour of these channels.

Preliminary results of this work have been published in abstract form (Terzic & Kurachi, 1995).

METHODS

Cell preparation

Ventricular myocytes were isolated by an enzymatic dissociation method as described previously (Kurachi, 1985; Terzic, Findlay, Hosoya & Kurachi, 1994*a*). Briefly, the aorta was cannulated in guinea-pigs (200–300 g) anaesthetized with sodium pentobarbitone (40 mg (100 g)$^{-1}$, intraperitoneal injection) and artificially ventilated. Following cardiotomy, the heart was retrogradely perfused (at 37 °C) through the coronary arteries with: first, bathing solution (mM: NaCl, 136·5; KCl, 5·4; $CaCl_2$, 1·8; $MgCl_2$, 0·53; glucose, 5·5; and Hepes–NaOH, 5·5; pH 7·4) for 5–10 min, then nominally Ca^{2+}-free bathing solution, followed by nominally Ca^{2+}-free bathing solution containing collagenase (0·04 g (100 ml)$^{-1}$; Sigma Type I) for 30 min, and finally a high-K^+–low-Cl^- solution for 5 min. The heart was then stored (at 4 °C) in the high-K^+–low-Cl^- solution comprising (mM): taurine, 10; oxalic acid, 10; glutamic acid, 70; KCl, 25; KH_2PO_4, 10; glucose, 11; EGTA, 0·5; and Hepes–KOH, 10 (pH 7·3–7·4). To isolate single cells, a small piece of ventricle was dissected and agitated in the recording chamber filled with the bathing solution. Myocytes were allowed to adhere to the glass floor of the chamber and were then superfused at a constant flow rate (~3–5 ml min^{-1}). Only myocytes with clear striations and a smooth surface were used. Experiments were performed with the approval of the Institutional Animal Care and Use Committee, Mayo Foundation.

Single channel recordings

The gigaohm-seal patch clamp technique was employed in the 'inside-out' configuration. Membrane patches were excised from single ventricular myocytes, and their internal side was perfused with a solution comprising (mM): KCl, 140; EGTA-KOH, 5; and Hepes–KOH buffer, 5 (pH 7·3). Nucleotides, cytoskeletal disrupters or actin were added to the internal side of the patch. The estimated concentration of free Mg^{2+} in the 'internal' solution was kept constant at 0·7 mM by adjusting the concentration of $MgCl_2$ for the Mg^{2+}-binding properties of nucleotides. ATP, uridine diphosphate (UDP), cytochalasin B, nocodazole, as well as actin subunits purified from rabbit heart were purchased from Sigma; deoxyribonuclease Type I (DNase I) was obtained from Boehringer Mannheim (Mannheim, Germany) or Worthington Biochemical Corporation (Freehold, NJ, USA); and taxol was obtained from Calbiochem Corporation (La Jolla, CA, USA). The external side of the patch was exposed to pipette solution which contained (mM): KCl, 140; $CaCl_2$, 1; $MgCl_2$, 1; and Hepes–KOH, 5 (pH 7·4). Patch electrodes, with a fire-polished tip coated with Sylgard, had a resistance of 5–7 MΩ. Single channel currents were recorded, at room temperature (21–23 °C), using a patch clamp amplifier (Axopatch-1C; Axon Instruments, Foster City, CA, USA) and monitored on-line on a high-gain digital storage oscilloscope (VC-6025; Hitachi, Tokyo, Japan). The holding potential was −60 mV. Data were stored on tape using a digital data converter system (VR-10; Instrutech, NY, USA), reproduced, low-pass filtered at 1·5 kHz (−3 dB) by a Bessel filter (Frequency Devices 902, Haverhill, MA, USA), sampled at 4 kHz, and analysed off-line with a computer and software analysis program as described previously (Terzic *et al.* 1994*a*). The threshold for judging the open state was set at one-half the single channel amplitude. The degree of channel activity was assessed, under respective experimental conditions, by digitizing segments of the current records and forming histograms of baseline and open-level data points. Channel activity was expressed as NP_o, where N represents the number of channels in the patch and P_o the probability of each channel being open. In patches in which the fluctuation in NP_o during the experiment made the interpretation of the change in channel activity difficult, NP_o was, in addition, plotted cumulatively, and slopes were fitted to the plots by linear regression and used as an indicator of channel activity. Data are expressed as means ± S.E.M., and statistical significance of differences between two means was determined with Student's t test where appropriate.

RESULTS

DNase I antagonized ATP-induced K_{ATP} channel inhibition

Upon excision of a membrane patch from a ventricular myocyte, multiple openings of K_{ATP} channels (single channel conductance, ~90 pS) appeared (Fig. 1*A a*). ATP (200 μM), added to the internal side of the patch (ATP_i), abolished K_{ATP} channel activity (Fig. 1*A a*). In the continuous presence of ATP_i, addition of DNase I (100 μg ml^{-1}) gradually increased the probability of opening of K_{ATP} channels (Fig. 1*A a*). The mean NP_o ($n = 21$), which was 3·79 ± 0·42 following patch excision, decreased to 0·05 ± 0·02 in the presence of ATP_i (200 μM), but increased to 0·87 ± 0·12 in the combined presence of 200 μM ATP_i plus 100 μg ml^{-1} DNase I (Fig. 1*B*). The effect of DNase I was apparently concentration dependent as the mean NP_o was 0·32 ± 0·16 and 1·14 ± 0·26 in the combined presence of 200 μM ATP_i plus 10 and 200 μg ml^{-1} DNase I, respectively ($n = 6$). Thus, in the presence of ATP_i, DNase I (100 μg ml^{-1}), which is known to form

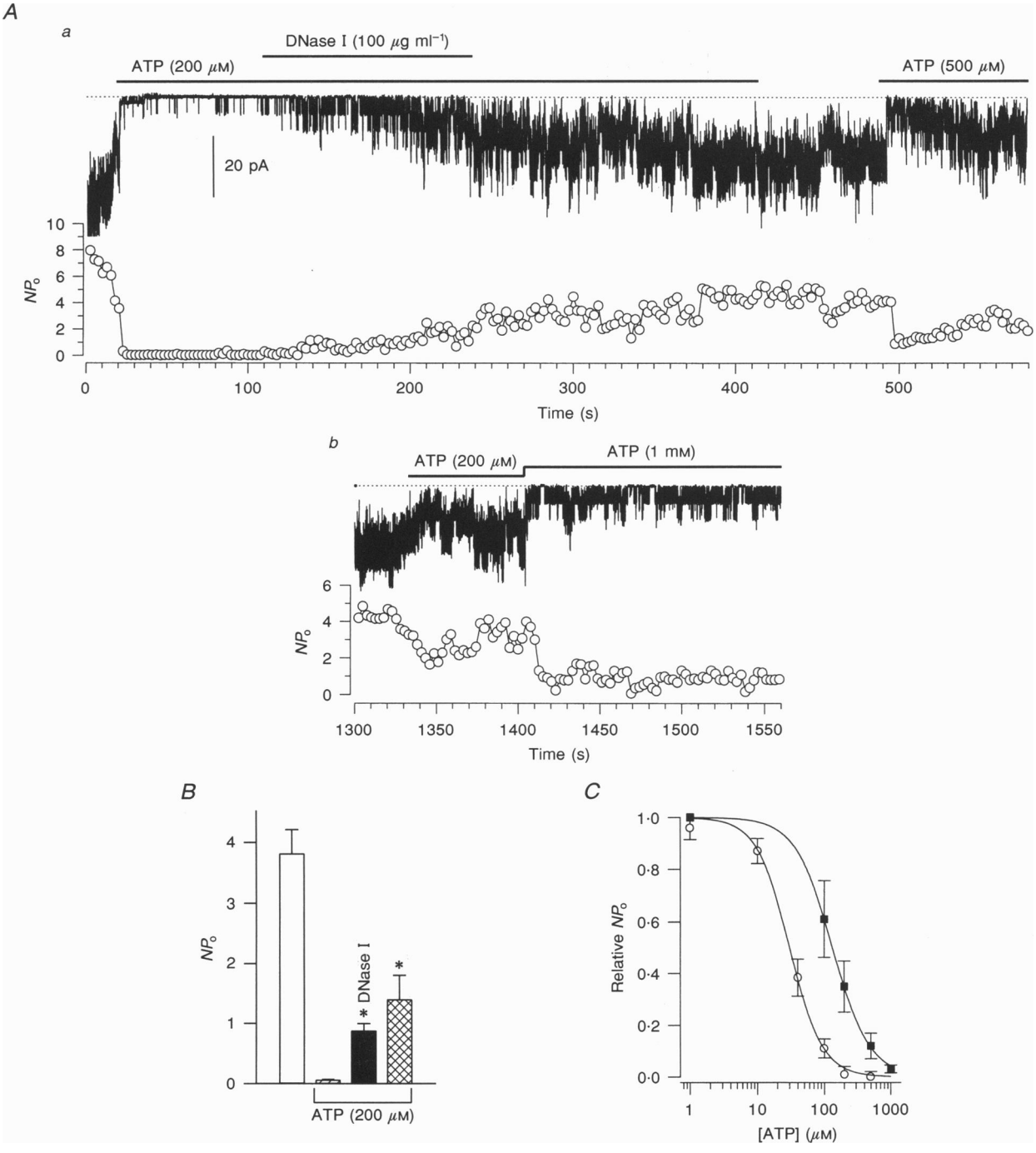

Figure 1. DNase I enhances K_{ATP} channel activity in the presence of ATP_i

A a and *A b*, in an excised membrane patch, K_{ATP} channel activity was inhibited by 200 μM ATP_i. Application of 100 μg ml^{-1} DNase I induced an increase in K_{ATP} channel activity despite the continuous presence of ATP_i. The effect of DNase I was irreversible, and applications of 200 μM, 500 μM or 1 mM ATP_i could not inhibit K_{ATP} channel activity in this DNase-treated patch. *a* and *b*: top traces, channel record from same patch. Dotted lines indicate zero current level. *a* and *b*: bottom traces, channel open probability (NP_o), corresponding to the respective top traces, were calculated over 2·5 s intervals. *N*, number of channels in the patch; P_o, open probability of each channel. The time scale (seconds) indicates the time elapsed after patch excision. *B*, summarized data of control (□), and plus 200 μM ATP_i before (▨), during (■), and after (▩) addition of 100 μg ml^{-1} DNase I. * Significant difference ($P < 0·05$) when compared with the value obtained in 200 μM ATP_i before addition of DNase I. *C*, concentration–response relationships between the relative K_{ATP} channel activity and the concentration of ATP in patches not treated (○), and in those treated (■) with 100 μg ml^{-1} DNase I. Relative NP_o was calculated by normalizing the actual NP_o value obtained at each ATP concentration to the NP_o value recorded in the absence of ATP in each patch. Data are from 3–21 membrane patches for each data point. Continuous lines were fitted by the Hill equation as described in the text.

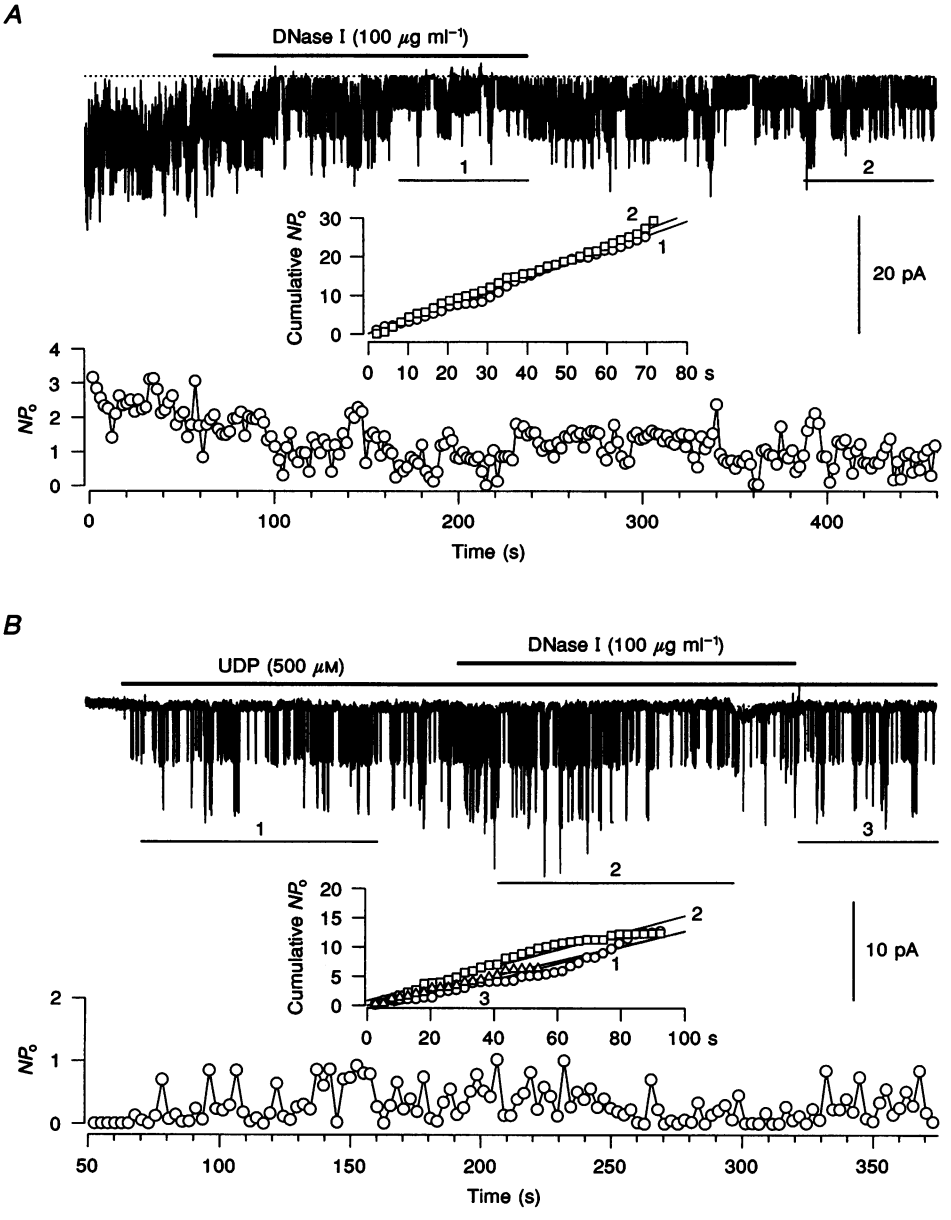

Figure 2. Spontaneous and UDP-induced K_{ATP} channel openings in the presence of DNase I

A: top trace, original trace record depicting spontaneous K_{ATP} channel openings after patch excision in the absence and presence of DNase I (100 μg ml^{-1}); bottom trace, calculated NP_o values, over 2 s intervals, corresponding to channel activity in the top trace; inset, cumulative NP_o values corresponding to segments of original trace (denoted by horizontal bars beneath the original trace), calculated in the presence (○, 1), and after washout (□, 2) of DNase I. Results of linear regression are presented as continuous lines (1 and 2) which correspond to the respective segments. The time scale indicates the time elapsed after patch excision. *B*: top trace, original trace record depicting K_{ATP} channel activity after 'run-down', in the presence of UDP (500 μM), in the presence of UDP plus DNase I (100 μg ml^{-1}), and in the presence of UDP after washout of DNase I. Bottom trace, calculated NP_o values, over 2·5 s intervals, corresponding to channel activity in the top trace. Inset, cumulative NP_o values corresponding to segments of the original trace (denoted by horizontal bars beneath the trace) under the following conditions: in the presence of UDP (○, 1), in the presence of UDP plus DNase I (□, 2), and in the presence of UDP after washout of DNase I (△, 3). Results of linear regression are presented as continuous lines labelled 1, 2 and 3, which correspond to the respective segments.

complexes with G-actin and prevent actin microfilament formation (Kabsch, Mannherz, Suck, Pai & Holmes, 1990), augmented the probability of K_{ATP} channel opening.

Following washout of DNase I (100 μg ml^{-1}), K_{ATP} channel openings remained vigorous despite the continuous presence of 200 μM ATP_i (Fig. 1*A a*). Under these conditions, the mean K_{ATP} channel activity was 1·38 ± 0·42 ($n = 7$) compared with 0·04 ± 0·02 recorded before the addition of DNase I, in the same seven patches, in the presence of 200 μM ATP_i (Fig. 1*B*). Furthermore, in patches previously exposed to DNase I, addition of higher concentrations of ATP_i, i.e. 500 μM (Fig. 1*A a*) or 1 mM (Fig. 1*A b*), did not abolish K_{ATP} channel activity. At different concentrations of ATP_i, the relative NP_o was obtained with reference to values recorded in the absence of ATP_i (Fig. 1*C*). This allowed the data to be fitted by the Hill equation:

$$y = 1/\{1 + ([ATP]/K_i)^{n_H}\},$$

where y is the relative NP_o at each concentration of ATP_i, [ATP] is the concentration of ATP_i, K_i is the ATP_i concentration at half-maximal inhibition of the K_{ATP} channel activity, and n_H is the Hill coefficient. In DNase I-untreated patches, the apparent K_i and Hill coefficient for the inhibitory effect of ATP_i on K_{ATP} channel opening were 30 μM and 1·8, respectively (Fig. 1*C*). Treatment of patches with 100 μg ml^{-1} DNase I (followed by washout of DNase I) provoked an apparent shift to the right of the concentration–response relationship for ATP_i-induced K_{ATP} channel inhibition, as the estimated K_i for the effect of ATP_i increased to 134 μM with the Hill coefficient at 1·6 (Fig. 1*C*). Thus, treatment with DNase I of the internal side of patches excised from ventricular myocytes diminished the apparent sensitivity of K_{ATP} channels towards ATP_i.

DNase I and K_{ATP} channel openings in the absence of ATP_i

Upon excision of a membrane patch in the absence of ATP_i, vigorous openings of K_{ATP} channels were recorded. Under these conditions, application of DNase I (100 μg ml^{-1}) apparently did not increase the probability of opening of K_{ATP} channels ($n = 5$; Fig. 2*A*). After washout of DNase I, K_{ATP} channel activity remained at a similar level (Fig. 2*A*). Channel activities, expressed by the slope of cumulative NP_o (see Methods), were 0·37 s^{-1} (in the presence of DNase I) and 0·38 s^{-1} (following washout of DNase I) in the patch depicted in Fig. 2*A* (see inset). In certain patches, the phenomenon of 'run-down' led to the loss of spontaneous K_{ATP} channel activity (Findlay, 1987; Kozlowski & Ashford, 1990). 'Run-down' channels could be induced to reopen by exposure to a nucleoside diphosphate, such as UDP, as reported previously (Tung & Kurachi, 1991; Terzic *et al.* 1994*a*). In the patch depicted in Fig. 2*B*, spontaneous K_{ATP} channel activity did 'run down', yet application of 500 μM UDP induced K_{ATP} channel openings. Channel activity in the presence of UDP, expressed by the slope of cumulative NP_o, was 0·14 s^{-1} (Fig. 2*B*, inset). Application of DNase I (100 μg ml^{-1}), in the continuous presence of UDP, did not significantly change the UDP-induced K_{ATP} channel activity (Fig. 2*B*) since the slope of cumulative NP_o remained at 0·14 s^{-1} (Fig. 2*B*, inset). Following washout of DNase I, the UDP-induced K_{ATP} channel openings retained their original activity with the slope of cumulative NP_o at 0·13 s^{-1} (Fig. 2*B*, inset). Similar results were obtained in four additional patches. Thus, DNase I apparently does not activate spontaneous channel openings or restore channel activity after 'run-down'.

Boiled DNase I did not enhance K_{ATP} channel opening

When the DNase preparation (140 μg ml^{-1}) was boiled (at 100 °C, 5 min), DNase I could no longer antagonize the inhibitory effect of ATP_i on K_{ATP} channels (Fig. 3). In Fig. 3, the mean values of K_{ATP} channel activity (expressed as NP_o) were 4·52 (control), 0·09 (200 μM ATP_i) and 0·07 (ATP_i plus boiled DNase I). In all five patches tested, boiled DNase I did not affect K_{ATP} channel activity. Yet addition of untreated DNase I (140 μg ml^{-1}) to the internal side of the same patch could induce vigorous opening of K_{ATP} channels inhibited by 200 μM ATP_i (Fig. 3). In the patch depicted in Fig. 3, channel activity increased from 0·07 (ATP_i plus boiled DNase I) to 1·85 (ATP_i plus untreated DNase I). Thus, denaturation by boiling of the DNase I protein led to the loss of its capacity to modulate the activity of K_{ATP} channels.

Actin antagonized the effect of DNase I on K_{ATP} channels

The specificity of the DNase I effect was examined by pre-incubating the enzyme with an excess concentration of purified actin subunits, because it is known that actin forms stable 1 : 1 complexes with DNase I effectively preventing the activity of DNase I (Kabsch *et al.* 1990). Figure 4*A a* shows that DNase I (100 μg ml^{-1}), pretreated and co-applied with actin subunits (200 μg ml^{-1}), did not significantly antagonize ATP_i-induced inhibition of K_{ATP} channel openings. Yet, in the same patch, DNase I (100 μg ml^{-1}) that had not been pre-incubated with actin subunits effectively antagonized ATP_i-induced inhibition of K_{ATP} channel openings (Fig. 4*A*). The mean ($n = 9$) K_{ATP} channel activity (expressed as NP_o) was 3·01 ± 0·72 (control), 0·29 ± 0·12 (in 100 μM ATP_i), and 0·38 ± 0·15 (in ATP_i plus 100 μg ml^{-1} DNase I and 200 μg ml^{-1} actin), but was 1·28 ± 0·23 (in ATP_i plus 100 μg ml^{-1} DNase I alone; Fig. 4*A b*). Thus, co-incubation with exogenous actin subunits apparently protected K_{ATP} channels from the DNase I-evoked antagonism of ATP_i-induced channel inhibition.

In a separate set of experiments, the ability of actin to reverse the effect of DNase I was tested. On its own, DNase I significantly reduced the sensitivity of K_{ATP} channels towards ATP_i-induced inhibition (see Fig. 4*B*). In the patch depicted in Fig. 4*B*, the mean NP_o was 2·3 (control), 0·11 (in 200 μM ATP_i), 1·56 (in ATP_i plus 100 μg ml^{-1} DNase I), and 1·98 (in 200 μM ATP_i following washout of DNase I). However, consecutive addition of purified actin subunits (200 μg ml^{-1}) significantly increased the sensitivity of K_{ATP} channels towards ATP_i-induced channel inhibition (Fig. 4*B*). Consequently, NP_o decreased to 0·83 in 200 μM ATP_i plus 200 μg ml^{-1} actin (Fig. 4*B*). Similar observations were made in three additional patches. Thus, application of actin subunits could partially restore the ability of ATP_i to inhibit K_{ATP} channels in DNase I-treated patches.

Cytochalasin B, but not antimicrotubule agents, enhanced K_{ATP} channel activity

Cytochalasin B is another well-known disrupter of actin filaments (Fukuda *et al.* 1981). As shown in Fig. 5*A*, addition of cytochalasin B (10 μM) to the internal side of membrane patches augmented the probability of opening of K_{ATP} channels pre-inhibited by 200 μM ATP_i. Channel activities, expressed by the slope of cumulative NP_o (see Fig. 5*A b*), were 1·57 s^{-1} (control), decreased to 0·02 s^{-1} (in 200 μM ATP_i) and increased to 0·40 s^{-1} (in ATP_i plus 10 μM cytochalasin B) in the patch depicted in Fig. 5*A*. Mean K_{ATP} channel activity (expressed as NP_o; $n = 5$; Fig. 5*B*) was 4·87 ± 0·54 (control), 0·03 ± 0·01 (in 200 μM ATP_i) and 0·68 ± 0·25 (in ATP_i plus 10 μM cytochalasin B). In contrast, addition to the intracellular side of a patch of agents that act on microtubules (Rowinsky, Cazenave & Donehower, 1990; Kuznetsov, Langford & Weiss, 1992), such as taxol (30–100 μM; $n = 3$; Fig. 6) or nocodazole (100 μM; $n = 2$; not illustrated), did not significantly affect K_{ATP} channel activity in the presence of ATP_i. In corresponding segments of the patch depicted in Fig. 6 (see inset), channel activities expressed by the slope of cumulative NP_o, were 1·32 s^{-1} (control), 0·10 s^{-1} (in 200 μM ATP_i), 0·11 s^{-1} (in ATP_i plus 30 μM taxol), 0·13 s^{-1} (in ATP_i after washout of 30 μM taxol) and 0·14 s^{-1} (in ATP_i plus 100 μM taxol). Thus, an actin microfilament disrupter, but not antimicrotubule agents, enhanced the probability of opening of K_{ATP} channels pre-inhibited by ATP_i.

DISCUSSION

This study demonstrates that DNase I, as well as cytochalasin B, when applied to the internal surface of excised membrane patches modulate the ATP_i-dependent gating of myocardial K_{ATP} channels. Specifically, these agents, known to prevent or disrupt actin filament formation, antagonized the ATP_i-induced inhibition of K_{ATP} channels. This suggests that actin microfilament networks may play a significant role in regulating the activity of cardiac K_{ATP} channels.

It could be argued that the effect of DNase I on K_{ATP} channels was not the consequence of an action on actin microfilaments. However, there are several arguments against the interpretation that the action of DNase I was due to a non-specific interaction with membrane proteins.

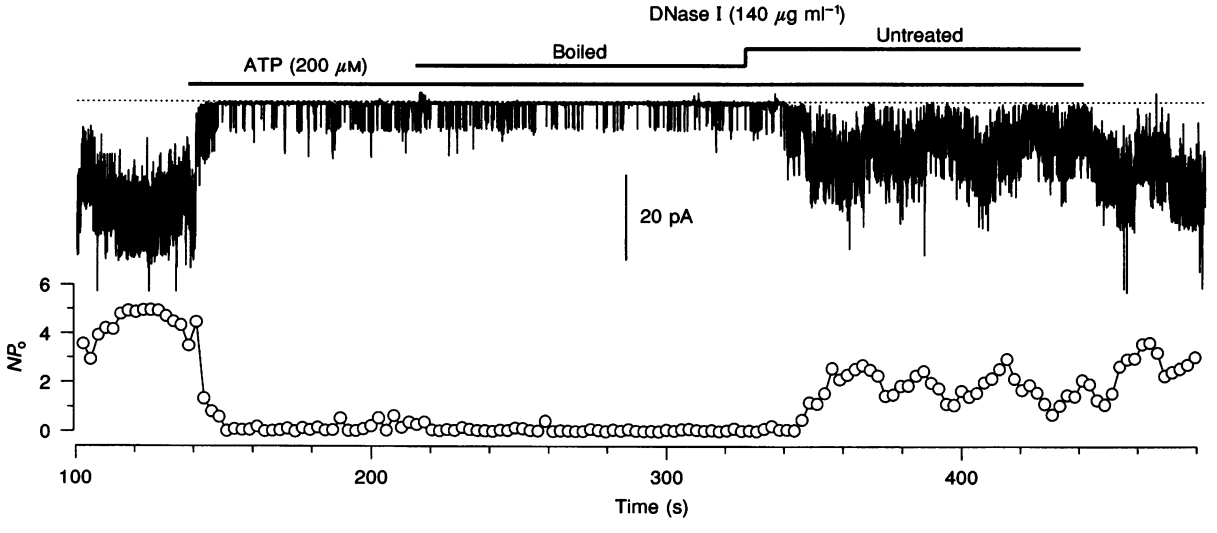

Figure 3. Boiled DNase I does not enhance K_{ATP} channel opening

DNase I preparation (140 μg ml^{-1}) boiled at 100 °C for 5 min did not antagonize ATP_i-induced inhibition of K_{ATP} channel openings. By contrast, in the same patch, untreated DNase I antagonized ATP_i-induced channel inhibition. Dotted line represents zero current level (top trace). Channel open probability (NP_o) was calculated over 2·5 s intervals (bottom trace). Time scale indicates time elapsed following patch excision.

First, denatured DNase I could not antagonize ATP_i-induced K_{ATP} channel inhibition, which is consistent with the notion that it is the native structure of the protein that is essential for DNase I to form 1 : 1 molar complexes with G-actin and prevent actin filament formation (Kabsch *et al.* 1990). Second, it was a structurally distinct disrupter of actin microfilaments, i.e. cytochalasin B (Fukuda *et al.* 1981), but not antimicrotubule drugs such as taxol or nocodazole (Rowinsky *et al.* 1990; Kuznetsov *et al.* 1992), that could mimic the effect of DNase I on K_{ATP} channel activation. This suggests that the ability of DNase I to modulate K_{ATP} channel openings is shared by other agents known to act on actin microfilaments, such as cytochalasins. Third, incubation with exogenous actin subunits prevented the action of DNase I, suggesting that actin binding sites on the DNase I molecule (Kabsch *et al.* 1990) are required for this protein to modulate K_{ATP} channel activity. Also, actin subunits partially restored the DNase I-induced loss of K_{ATP} channel sensitivity towards ATP_i-mediated inhibition, suggesting that DNase I targeted an actin-dependent process. Taken together, these observations may fulfil the established criteria for a disrupter of actin microfilaments to regulate a specific ion channel (Cantiello *et al.* 1991), and support the possibility that DNase I acted on actin filaments to modulate K_{ATP} channel activity.

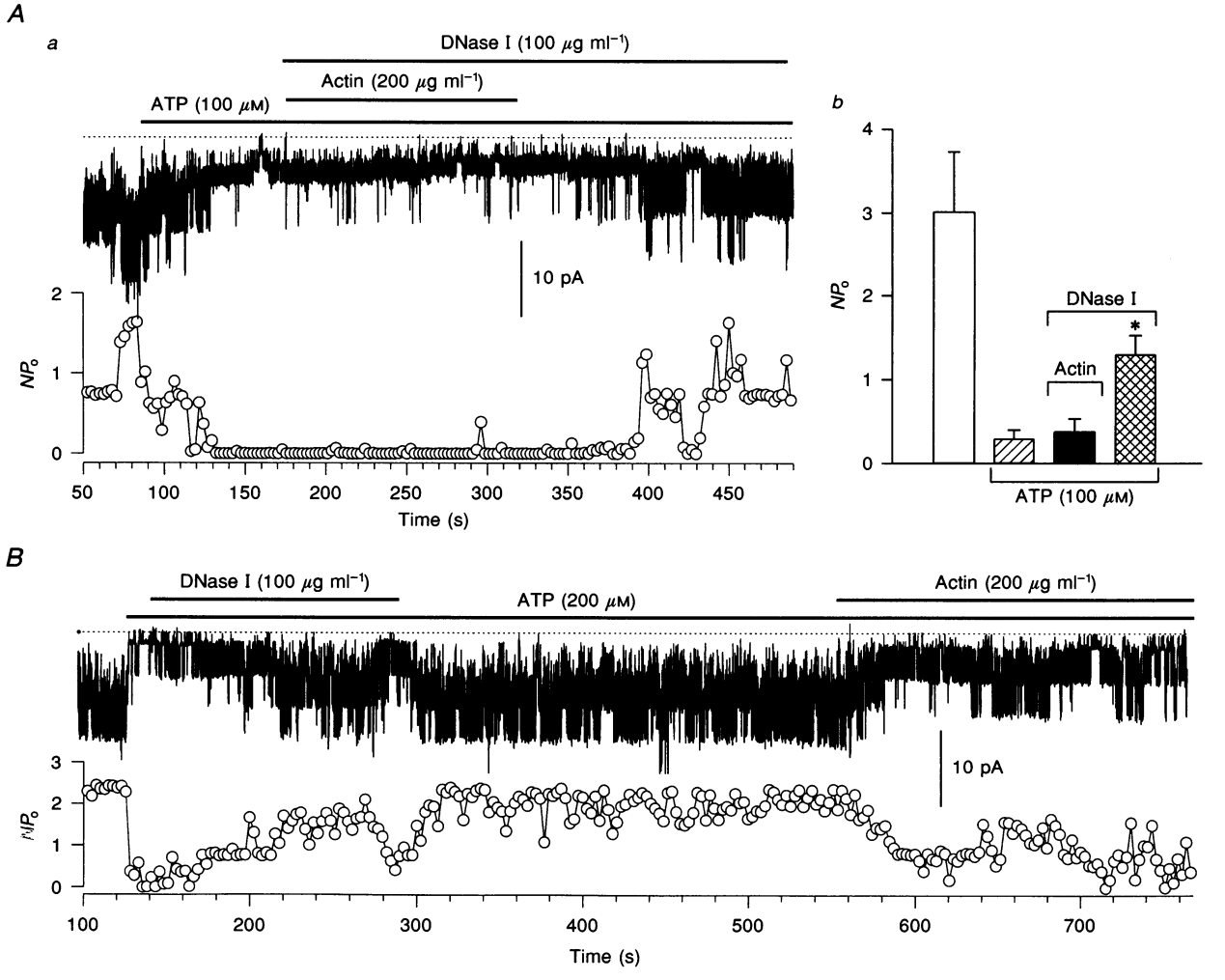

Figure 4. Actin apparently antagonizes the DNase I effect on K_{ATP} channel activity

A a, co-incubation and co-application of purified actin subunits (200 μg ml^{-1}) with DNase I (100 μg ml^{-1}) prevented the effect of DNase I on K_{ATP} channel activity. *b*, summarized data of control (□), plus 100 μM ATP_i (▨), ATP_i plus DNase I with exogenous actin subunits (200 μg ml; ■), and ATP_i plus DNase I without exogenous actin subunits (▩). * Significant difference ($P < 0{\cdot}05$) when compared with the value obtained in 100 μM ATP_i alone. *B*, application of purified actin subunits partially restored the sensitivity of K_{ATP} channels towards ATP_i-induced inhibition in a DNase-pretreated patch. In *A a* and *B*, the corresponding channel open probabilities (NP_o) were calculated over 2·5 s intervals, and are given underneath the original trace records. Dotted lines represent the zero current level, and the time scales indicate the time elapsed following patch excision.

We observed the modulatory effect of actin microfilament disrupters in excised membrane patches. In this regard, the regulation of myocardial K_{ATP} channels resembles the modulation of Na^+ and Cl^- channels by actin filament disrupters, which can also proceed in excised membrane patches (Cantiello *et al.* 1991; Prat *et al.* 1995; Udrovinas, Shander & Makielski, 1995). This, however, does not necessarily mean that the action of actin filament disrupters could occur in a 'cytosol-free' environment since membrane patches do not consist of just the plasmalemma, but are composed of membrane-covered cytoplasm with cytoskeletal structures (Ruknudin *et al.* 1991; Horber *et al.* 1995). It has been proposed previously that actin, and actin binding proteins, are linked to various transport proteins including the α-subunit of the Na^+,K^+-ATPase (Nelson & Veshmock, 1987), Na^+ and Ca^{2+} channels in the brain (Srimivasan, Elmer, Davis, Bennett & Angelides, 1988; Johnson & Byerly, 1993), NMDA channels in the hippocampal neurons (Rosenmund & Westbrook, 1993), Na^+ and Cl^- channels in kidney (Cantiello *et al.* 1991; Suzuki *et al.* 1993) and the cystic fibrosis transmembrane conductance regulator (Prat *et al.* 1995). The present study is in accord with the notion that actin networks could interact and regulate ion channel activity and extends such a concept to the modulation of myocardial K_{ATP} channels.

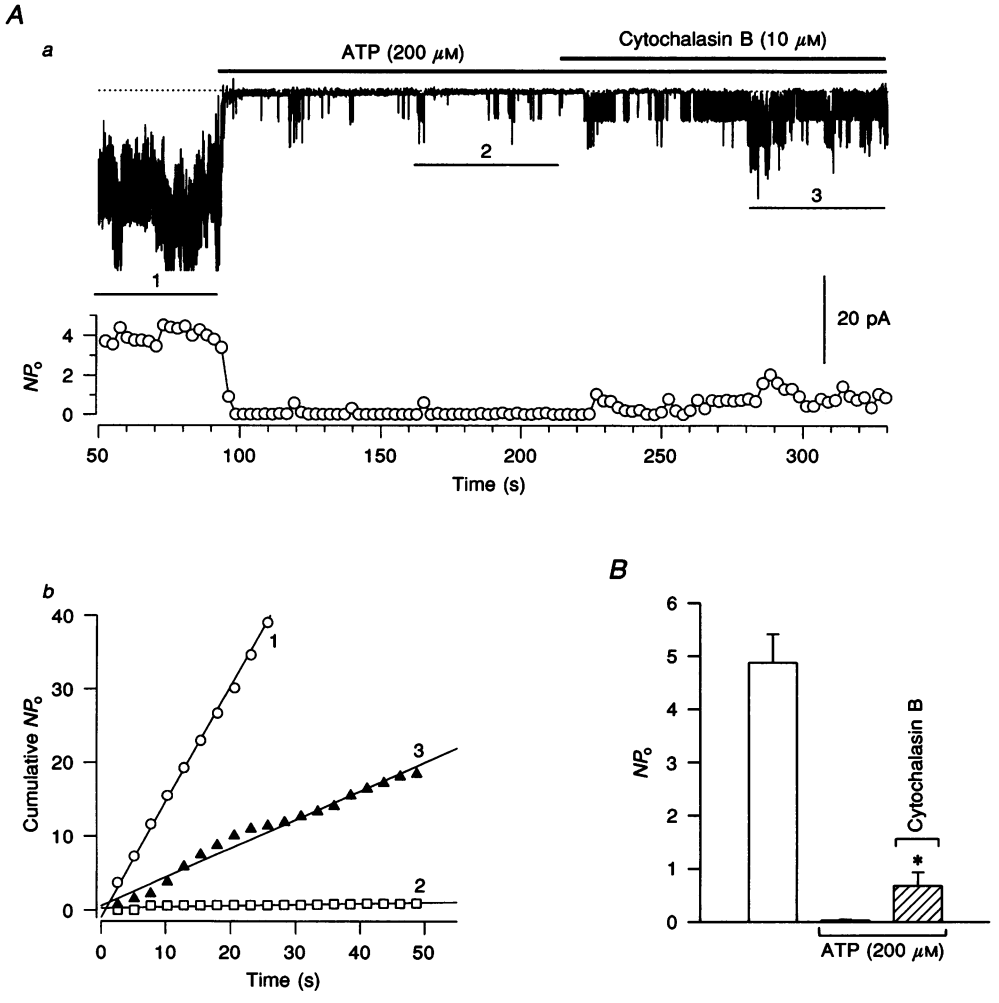

Figure 5. Cytochalasin B enhances K_{ATP} channel activity inhibited by ATP_i

A a: top trace, original trace record depicting spontaneous K_{ATP} channel openings in control, in ATP_i (200 μM), and in ATP_i (200 μM) plus cytochalasin B (10 μM); bottom trace, calculated NP_o values, over 2·5 s intervals, corresponding to channel activity in top trace. The dotted line in the top trace represents the zero current level, and the time scale indicates time elapsed following patch excision. *b*, cumulative NP_o values, corresponding to segments of original trace (denoted by horizontal bars beneath the original trace), under the following conditions: control (○, 1), in ATP_i (□, 2), and in ATP_i plus cytochalasin B (▲, 3). Results of linear regression are presented as continuous lines labelled 1, 2 and 3, which correspond to the respective segments. *B*, summarized data of control (□), plus 200 μM ATP_i (■), and ATP_i plus cytochalasin B (10 μM; ▨). * Significant difference ($P < 0·05$) when compared with the value obtained in 200 μM ATP_i alone.

A notable feature of the putative actin-dependent regulation of K_{ATP} channels is that actin filament disrupters appear to modulate the ATP_i-dependent gating of K_{ATP} channels. In this regard, the K_{ATP} channel activation induced by actin filament disrupters is similar to that of G-proteins (Terzic, Tung, Inanobe, Katada & Kurachi, 1994*b*), and of a particular class of K^+ channel openers which activate K_{ATP} channels by decreasing the sensitivity of K_{ATP} channels towards the ATP_i-induced channel inhibition, but do not activate K_{ATP} channels in the absence of ATP_i (Terzic *et al.* 1994*c*, 1995). Partly due to the experimental variations observed from patch to patch, the present study does not distinguish whether actin microfilament disrupters decreased the sensitivity of K_{ATP} channels towards ATP_i by antagonizing ATP_i at the ATP_i binding site or whether they countered the effect of ATP_i by acting at another site which affects the sensitivity of the channel towards ATP_i. Further studies are required to define the precise nature of the interaction of actin filaments and K_{ATP} channels or associated regulatory proteins. It has been established previously that DNase I acts on short actin filaments, whereas cytochalasin B disrupts long actin filaments more effectively (Cantiello *et al.* 1991). This may suggest that the integrity of both short and intermediate actin filaments is essential for the regulation of the ATP_i sensitivity of myocardial K_{ATP} channels.

The role of the plasma membrane environment in the regulation of cardiac ion channel function is poorly understood. Yet mechanical pressure applied to a cardiac cell membrane leads to an increase in K_{ATP} channel activity, suggesting that the probability of K_{ATP} channels opening in the presence of millimolar concentrations of ATP_i is regulated, in part, through a mechanosensitive mechanism (Van Wagoner, 1993). It is known that mechanical disturbances of the cytoskeleton can occur under various conditions, including ischaemia and hypoxia (Ganote & Armstrong, 1993). Therefore, it is conceivable that under such conditions the behaviour of ion conductances that depend on the integrity of the subsarcolemmal actin filament network could be dramatically altered. Indeed, the mechanosensitive modulation of K_{ATP} channels is potentiated under ischaemic conditions (Van Wagoner & Lamorgese, 1994), while the open channel probability of myocardial K_{ATP} channels increases severalfold under ischaemia and hypoxia despite the millimolar concentration of ATP_i (Nichols & Lederer, 1991; Weiss & Venkatesh, 1993; Findlay, 1994). Further investigation is required to define the precise role of actin filament-dependent regulation of myocardial K_{ATP} channels under physiological and pathophysiological challenges.

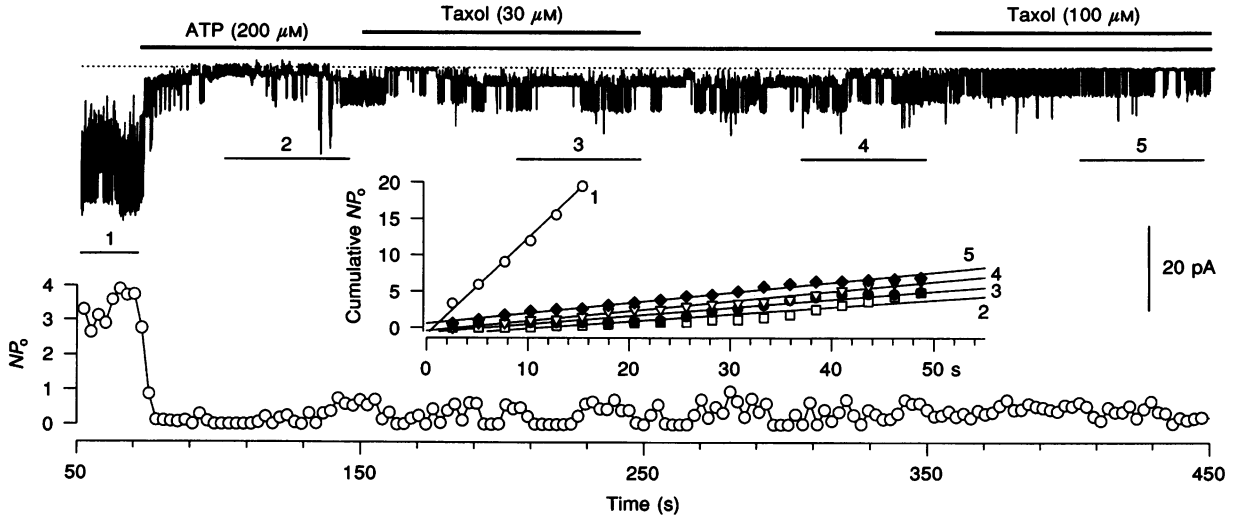

Figure 6. Lack of significant action of taxol on K_{ATP} channel activity

Top trace, original trace record depicting spontaneous K_{ATP} channel openings in control, in ATP_i (200 μM), in ATP_i (200 μM) plus taxol (30 μM), in ATP_i (200 μM) after washout of taxol (30 μM), and in ATP_i (200 μM) plus taxol (100 μM). Bottom trace, calculated NP_o values, over 2·5 s intervals, corresponding to channel activity in the top trace. The dotted line represents the zero current level (top trace), and the time scale indicates time elapsed following patch excision. Inset, cumulative NP_o values, corresponding to segments of original trace (denoted by horizontal bars beneath the original trace), obtained in control (○, 1), ATP_i (□, 2), ATP_i plus 30 μM taxol (●, 3), ATP_i after washout of 30 μM taxol (▽, 4), and ATP_i plus 100 μM taxol (◆, 5). Results of linear regression are presented as continuous lines labelled 1, 2, 3, 4 and 5, which correspond to the respective segments.

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Acknowledgements

This work was supported by the American Heart Association, the Pharmaceutical Research and Manufacturers of America Foundation, the National Heart Foundation (A.T.), and the Ministry of Education, Science, Sports and Culture of Japan (Y.K.).

Received 5 September 1995; accepted 16 November 1995.