Effect of High Hydrostatic Pressure on the BK Channel in Bovine Chromaffin Cells

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ABSTRACT The activity of the BK channel of bovine chromaffin cells was studied at high hydrostatic pressure, using inside-out patches in symmetrical KCl solution, Ca^{2+}-free and at $V_H = -60$ to -40 mV. Pressure increased the probability of channels being open (900 atm increasing the probability 30-fold), and it increased the minimum number of channels apparent in the patches. The pressure activation of the channel was reversed on decompression. Channel conductance was unaffected. It was shown that pressure did not act by raising the temperature, or by affecting [Ca] or pH, or the order of the membrane bilayer, and it was concluded that pressure most likely acted directly on the channel proteins and/or their modulating reactions.

INTRODUCTION

The BK (or Maxi K) channel in the plasmamembrane of bovine chromaffin cells is typical of a large group of high-conductance channels, selective for K ions, which are gated by voltage, [Ca]i, and in some cases by fatty acids and stretch (Marty, 1981, 1983; McManus, 1991; Kirber et al., 1992; Latorre, 1994). In this paper high hydrostatic pressure is also shown to activate the channel.

High pressure is a thermodynamic intensity parameter which has been used to good effect in the study of lipid bilayers and proteins. Pressure increases the order of lipid bilayers (Macdonald, 1992), exerts a reduction in bilayer area and in certain cases a slight thickening (Braganza and Worcester, 1986), and induces isothermal phase transitions (Wong et al., 1988). Pressures of several thousand atmospheres denature proteins, whilst lesser pressures, relevant here, dissociate multimeric proteins (Jonas and Jonas, 1994; Gross and Jaenicke, 1994; Weber, 1993; Silva and Weber, 1993; Balny et al., 1989; Weber and Drickamer, 1983). (An atmosphere closely approximates one bar and 100 kPa.) Quantitatively, an equilibrium (e.g., monomer-multimer) responds to high pressure according to $d \ln K/dp = -\Delta V/RT$. Reaction rate constants (k) are related to p (pressure) and $\Delta V\#$, the activation volume in the rate-determining step, in an analogous equation ($d \ln k/dp = -\Delta V\#/RT$). Typically molar volume changes (ΔV) of 10 or more ml mol^{-1} are sufficient to cause significant changes in K or k in the 1000-atm pressure range commonly used in biological studies (Edsall and Gutfreund, 1983).

The widespread pressure-dissociation of soluble multimeric proteins has stimulated interest in the stability of membrane-bound proteins under pressure. In the case of the Ca^{2+} ATPase from sarcoplasmic reticulum and erythrocytes, high pressure has been shown to dissociate the enzyme to subunits (Verjovski-Almedia et al., 1986; Buchet et al., 1990; Coelho-Sampaio et al., 1991). The Ca^{2+}-dependent ATPase activity is reduced by high pressure, consistent with a shift in its conformational equilibrium (Jona and Martonosi, 1991) and with the ordering of the annular lipids (Heremans and Wuytack, 1980). Intermediate reactions are also pressure sensitive; for example, calcium binding to the high-affinity site of the enzyme entails a ΔV of +30–50 ml mol^{-1}, similar to that of calcium binding to calmodulin (Stephan and Hasselbach, 1991). Knowledge of how high pressure affects channel structures is limited to a study of the peptide melittin. This exists in aqueous solution, in a monomer-tetramer equilibrium in which the dominant intersubunit interactions are hydrophobic. High pressure dissociates these, favoring the monomeric state (Thompson and Lakowicz, 1984). In a lipid bilayer, melittin exists in aggregates in addition to tetramers and responds to an appropriate transmembrane voltage by forming channels. In such an environment melittin's solution by lipid and water is stable at high pressure (Khalique Ahmed et al., 1992); aggregation is little affected and the peptide is not extruded from the bilayer, unlike some membrane proteins (Tong and Scarlata, 1993; Plager and Nelsestruan, 1992). Its rotational motion in well-ordered bilayers is, however, reduced at high pressure.

Ion channels studied at high pressure using electrophysiological methods, either classical voltage clamp or patch clamp, manifest a variety of kinetic effects but no change in conductance. No other generalization seems justifiable at present, although the number of published experiments has grown very significantly since the pioneer days (Spyropoulos, 1957; Henderson and Gilbert, 1975), through a period of development and refinement (Harper et al., 1981; Conti, 1986), resulting in the first high-pressure patch-clamp paper (Heinemann et al., 1987). A good introduction to the subject is a paper by Kendig et al. (1993).

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The present experiments were undertaken to explore the effects of pressure on a major class of ion channel which has distinctive properties and certain technical advantages. BK channels behave as ligand-gated channels with a high conductance, and some have been reconstituted in defined bilayers, while the subunits of others are accessible for study through expression techniques (McManus et al., 1995; Meera et al., 1996).

MATERIALS AND METHODS

Bovine chromaffin cells were prepared by standard methods (Peters et al., 1989) and grown on poly-L-lysine-coated coverslips in HAMS F10 solution (Gibco BRL, from Life Technologies, Paisley, Scotland), which also contained, per liter, 120 ml fetal calf serum, 50 mg gentamycin, 60 mg streptomycin, 60,000 i.u. penicillin (all from above), and 1.2 g albumin (Sigma A-3350). The cells were used within 6 days. For most experiments the coverslips were transferred to a Ca^{2+}-free intracellular solution (Kirber et al., 1992) containing (in mM) 140 KCl, 2 $MgCl_2$, 5 EGTA, and Tris buffer (Sigma). The latter comprised the Tris base Tris(hydroxymethyl-)aminomethane and Tris (hydroxymethyl)aminomethane hydrochloride in the ratio of 1:11.4 by weight, contributing 10 mM. The pH of the Tris buffer is unaffected by high pressure but varies with temperature by $-.028$ pH unit $°C^{-1}$ (Disteche, 1972). The final pH was adjusted to 7.2 at 23°C, and the osmolarity of the complete solution was 290–300 mOsm. A few experiments were also carried out using a solution containing (in mM) 140 KCl, 2 $MgCl_2$, 1.1 EGTA, 0.1 $CaCl_2$, and 10 HEPES (Sigma) (pH 7.2), which buffered Ca^{2+} at 10^{-8} M (Marty, 1981). In both cases the same solution filled the bath and patch pipette; hence inside-out patches were used with symmetrical [KCl], with the membrane potential clamped between +40 and +60 mV, to give an initially low level of channel activity. Channel currents were recorded on videotape, using an Axon Instruments Axopatch 200A amplifier and a modified Sony PCM 701-ES. Off-line analysis was carried out with a computer program kindly supplied by Dr. Dempster (Dempster, 1993) on data that were low-pass filtered at 3.3 kHz (-dB, four-pole Bessel filter) and digitized at 30 kHz. Usually recordings of 2 min duration were made at appropriate intervals throughout the experiment, and 40–60-s segments of the recording were subsequently analyzed by a half-amplitude threshold crossing program using a CED 1401 interface and an Acer 1100 computer. Incremental threshold settings were used to detect the duration of simultaneous channel openings (Barrett et al., 1982). The probability with which patches manifested channel openings (P_{po}) was calculated from the aggregate open time/total recording time. The maximum number of simultaneously open channels was noted from inspection of analog recordings and amplitude histograms and provided a lower limit to the number of channels in a patch (N_c). At low values of P_{po} there was insufficient recording time in the experimental protocol (see below) to determine thoroughly whether one or more channels were, in fact, present (Colquhoun and Hawkes, 1995), but N_c is nevertheless interesting and probably reasonably accurate at higher P_{po} values. Dividing P_{po} by N_c provides an index of the probability of a channel being open (P_o), subject to the above. The identity of the channel studied was confirmed as the BK channel described by Marty (1983) by its sensitivity to [Ca]; its conductance (240 pS in symmetrical solution); and its susceptibility to block by tetraethylammonium chloride, trihexylammonium bromide (Yellen, 1984), and Penitrem A (Knaus et al., 1994).

Single-channel recording was carried out at high pressure by a method similar to that introduced by Heinemann et al. (1987).

After a satisfactory seal was made (Fig. 1), an inside-out patch was formed, but sometimes the cell remained attached, either loosely tethered or more intimately, the latter doubtless forming cell-attached patches. No difference was noted in the subsequent results. The petri dish was flooded to a depth of 15 mm, enabling the patch pipette to be elevated, and the transfer bath first swung beneath the pipette tip, and then was raised by sliding the connecting rod up the supporting shaft. With the patch safely maneuvered into the transfer bath, the apparatus could now "fly" (Heinemann et al., 1987) and be inserted into the pressure vessel.

The design of the experiments was influenced by the heat generated by the pressurized liquid paraffin and by the variability of the channel's activity. Dummy experiments using a thermistor (YSI type 427) mounted in the center of the pressure vessel showed that compression to 300 atm in 20 s, at 23°C, caused a 3°C rise in the temperature of the oil. This was followed by an exponential decrease to the initial temperature over ~20 min (Fig. 2). Similarly, compression to 100 atm caused a rise of 1°C. The patch, at the tip of the pipette immersed in 0.3 ml of solution in the transfer bath (Fig. 1), would experience the same temperature change as the oil. The heat of compression of aqueous solutions is an order of magnitude smaller than that of oil; thus the inclusion of a polymethacrylate beaker (Fig. 1) containing 7 ml of aqueous solution reduced the heat generated close to the pipette tip and insulated the pipette from the heat generated in the surrounding liquid paraffin. The "heat shield" beaker enabled compression to 900 atm to be carried out in 60 s with a rise in the water temperature (and hence the pipette temperature) similar to that seen in the oil at 300 atm. A pressure of 600 atm caused a 2°C increase, and in both cases cooling was slightly slower than seen in the absence of the beaker (Fig. 2).

Control experiments were conducted to measure channel activity, over 60 min, in Tris-buffered "Ca-free" solution (above) at 23°C and at room pressure, with the membrane potential clamped between +40 and +60 mV. P_{po} proved variable, but mode switching was largely absent. In three experiments P_{po} showed a significant trend over time (two decreasing, the correlation coefficient $r = -0.68, -0.92$; one increasing, $r = 0.84$), and in four experiments P_{po} varied up to fivefold and showed no particular trend (Silberberg et al., 1996). Experiments comparing P_{po} before compression, at high pressure and then after decompression, would have to take 60 min to ensure temperature equilibration, and over such a time scale the variability of the channel, as we have noted, would be likely to introduce serious error and the chances of patches surviving that long would be low. Accordingly, short experiments were adopted, in which channel activity was recorded at high pressure and a slightly increased temperature, beginning within 5 min of compression and within 7 min of a short period of precompression recording. Thus recordings at 300 atm, without the heat shield beaker, were at a temperature 0.75–2.5°C above the initial 23°C at normal pressure, and 100 atm experiments incurred a rise of one-third of that amount. Inclusion of the heat-shield beaker reduced the heat rise to one-third. Experiments at 600 and 900 atm, using the heat-shield beaker in all cases, involved a rise of nearly 2°C and 3°C, respectively, at the start of the recording period. The significance of these temperature changes was assessed at normal pressure by heat transient control experiments. These were carried out by placing a patch, submerged in its transfer bath, in a beaker and immersing it in warm liquid paraffin to simulate the heat of compression. P_{po} was determined at 23°C and then 4–7 min after the temperature increase caused by the warm oil. In eight experiments a mean rise of 2.7°C caused P_{po} to decrease by a mean of 25%. The results of the pressure experiments given in the next section are not corrected for this effect, for reasons given in the Discussion.

RESULTS

Patches typically contained a minimum of one to three channels at normal atmospheric pressure during the precompression, control period of recording, with P_o values around 0.005–0.02. In those cases in which a minimum of one channel appeared to be present in the precompression control period, the treatment of Colquhoun and Hawkes (1995) showed that it was unlikely that only one channel was, in fact, present. Generally high pressure increased the minimum number of active channels, P_{po} and P_o (Table 1; Fig. 3), but had no effect on channel conductance (Fig. 4). A few experiments using the pH buffer HEPES and Ca^{2+} at 10^{-8} M (Materials and Methods) produced results similar to

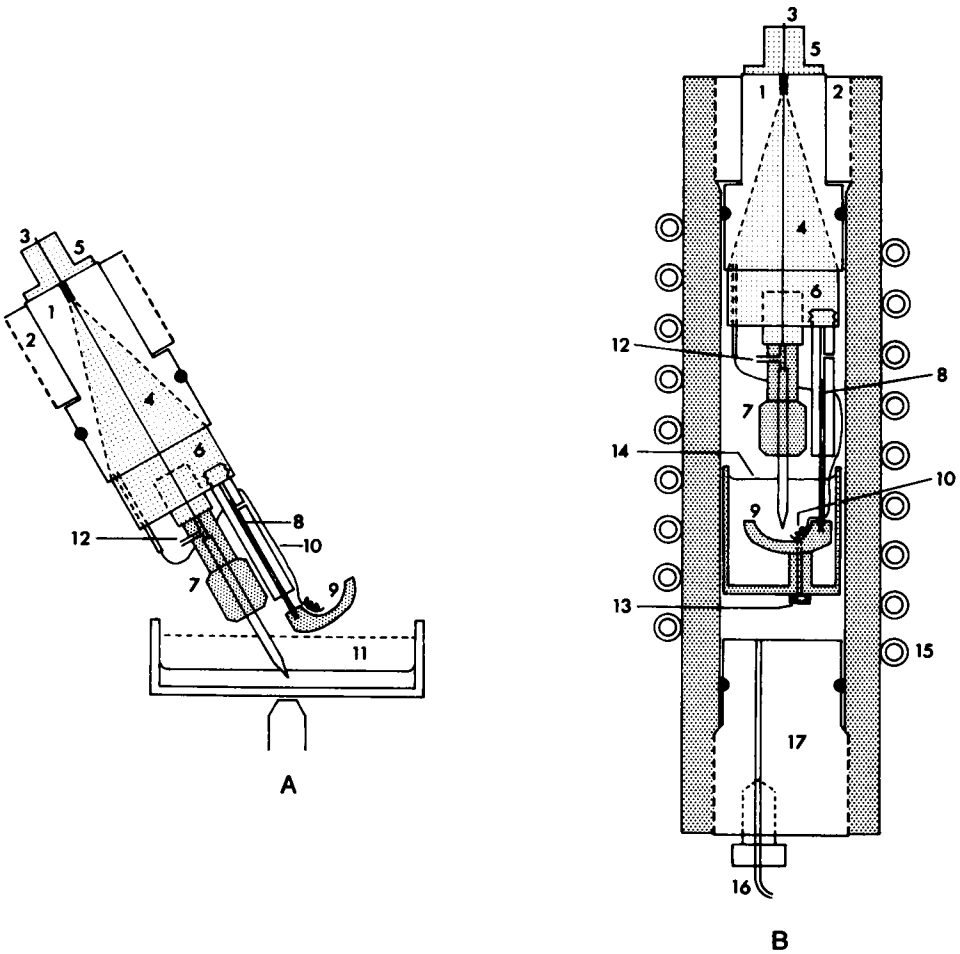

FIGURE 1 High-pressure patch-clamp apparatus. (*A*) Pressure vessel plug (*1*) and its retaining ring (*2*), which are held in a modified Leitz micromanipulator (not shown). The electrical connection (*3*) through the plug is insulated by a teflon cone (*4*), which terminates in a socket (*5*) on the outer face of the plug, and on the inner face, it terminates as a cylindrical block (*6*), which supports the electrode holder (*7*) and the sliding support (*8*) for the transfer bath (*9*). The patch-clamp amplifier headstage (not shown) connects to the socket (*5*). The transfer bath electrode (*10*) connection passes through the teflon block (*6*) to the vessel plug. The apparatus is shown with the tip of the patch pipette in a giant petri dish (*11*) (63-mm internal diameter × 17 mm deep) containing a reference electrode (not shown) and the solution bathing the cells, with the latter supported by a coverslip. After an excised patch is formed in the usual way, the level of the bathing solution is raised, the pipette is also raised, and the transfer bath (*9*) is slid down and swung under and then up to provide a 0.3-ml volume of solution surrounding the pipette tip. The transfer bath reference electrode then takes over from the main bath electrode, the suction line (*12*) is disconnected from the electrode holder, and the whole assembly is moved from the micromanipulator to a stand where the heat shield beaker (*13*) is bolted to the underside of the transfer bath (*9*). The apparatus is then inserted into the pressure vessel (internal diameter 29 mm). (*B*) Liquid paraffin fills all air spaces and floats at its interface (*14*) with the solution bathing and filling the pipette. Temperature control is achieved by circulating water through an external copper coil (*15*). Pressure was increased by a Haskel pump (not shown), which injected liquid paraffin through a capillary (*16*) connecting to the bottom plug (*17*) of the pressure vessel, and was measured by a calibrated Bourdon tube pressure gauge (not shown). Temperature measurements at high pressure were made separately, using a special top plug fitted with high-pressure electrical connections for the thermistor placed inside the vessel. Materials: vessel body, high-strength stainless steel; retaining ring and vessel plugs, high-strength bronze alloy; insulating cone, electrode holder socket, headstage socket, Teflon; sliding support for transfer bath, brass and stainless steel; dish, transfer bath, heat shield beaker, and electrode holder, polymethacrylate; o-ring, neoprene.

those obtained under standard conditions (Table 1A). The effect of pressure on channel activity was obvious during the first few seconds of compression, but it was not practical to quantify at that stage. Decompression reverses the effect (Table 1C), but not always completely. In all experiments above ~300 atm and in more than half of those at 100 and 300 atm, pressure increases the minimum number of channels, sometimes spectacularly (Fig. 4 *A*). The "additional" channel openings showed the same unit current as at normal pressure (Fig. 4 *B*). In the presence of TEA in contact with the inner face of the channel, the characteristic "fast blocking" artefactual reduction in channel conductance at normal pressure (Yellen, 1984) is also seen in openings at high pressure (Fig. 4 *C*). Presumably pressure has no significant effect on the residence time of TEA (Yellen, 1984).

A few experiments were carried out in which two transmembrane voltages were successively applied. These required the time between the precompression recording and the high-pressure recording to be increased to 13 min, and they revealed no particular effect of voltage within the

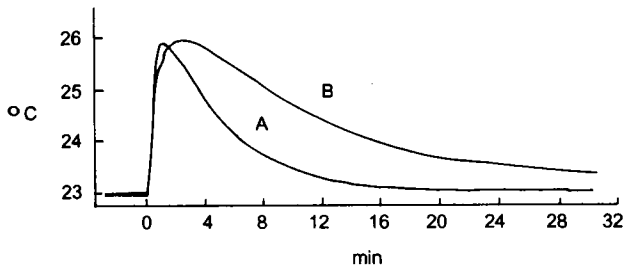

FIGURE 2 Heat of compression. *Curve A*: The rise in the temperature of liquid paraffin in the center of the pressure vessel (Fig. 1) on compression to 300 atm in 20 s, and its subsequent decline at constant pressure. *Curve B*: The rise in the temperature of the aqueous solution in the heat-shield beaker (Fig. 1) on compression to 900 atm in 60 s, and its subsequent decline at constant pressure.

limited range involved (Table 1B). One result, at 435 atm, comparing −45 mV and −70 mV, appears to be anomalous with high ratios, but when plotted in Fig. 3, *A* and *B*, the results may be seen to lie within the normal variability of the data.

Finally, a series of experiments were carried out in which, at normal pressure, patches were subjected to a decrease of 10°C, by a procedure similar to that of the heat transient control experiments (Materials and Methods). The purpose of these experiments was to use low temperature to mimic the bilayer-ordering effect of high pressure. The ratio of P_{po} at low temperature to P_{po} at the initial room temperature (22–23°C) was determined in the same way as for the high pressure experiments. The results of six experiments, with a mean temperature decrease of 10.04°C, were consistent, yielding a mean P_{po} ratio of 1.86. Cooling increased channel activity slightly, but it did not increase the minimum number of active channels.

DISCUSSION

The results show that high hydrostatic pressure activates the BK channel in bovine chromaffin cells at membrane potentials in the +40 to +80 mV range. The channels activated at high pressure have the same conductance and susceptibility to TEA as channels at normal pressure. Is this a direct

TABLE 1 Effect of high hydrostatic pressure on the activity of the bovine chromaffin cell BK channel

A. Compression

100 atm			300 atm			600 atm			900 atm		
1.88	(2/2)	1.88	8.65	(2/2)	8.65	14.11	(2/3)	9.45	201.81	(2/11)	36.69
2.35	(1/2)	1.17	3.54	(1/2)	1.76	12.37	(1/2)	6.26	20.18	(4/7)	11.55
6.39	(1/3)	2.14	20.50	(3/9)	6.85	17.46	(2/6)	5.83	40.65	(2/9)	9.04
1.69	(2/2)	1.69	2.70	(3/5)	1.62	69.40	(2/5)	27.73	54.73	(2/8)	13.68
1.94	(2/2)	1.94	16.09	(1/2)	8.15	19.13	(2/4)	9.59	190.50	(1/6)	31.74
2.23	(5/4)	2.78	5.79	(2/2)	5.79	12.17	(2/5)	4.87	146.50	(1/4)	36.63
1.96	(2/2)	1.96	5.27	(1/2)	2.64*	10.99	(1/2)	5.50	259.50	(1/3)	86.32
2.76	(1/2)	1.37	4.65	(3/3)	4.65						
3.00	(8/11)	2.18	10.55	(2/3)	7.00						
7.95	(1/2)	3.52#	6.66	(2/2)	6.66						
4.9	(1/2)	2.45#	16.54	(3/7)	7.08						
			3.70	(1/1)	3.70						
435 atm			6.27	(1/3)	2.09*						
			12.70	(1/2)	6.34						
40.32	(2/5)	16.25	3.84	(3/3)	3.84#						
			13.51	(2/4)	6.78#						

B. Comparing voltages, V_H

300 atm				435 atm				600 atm			
V_H −40 mV	9.46	(1/1)	9.46	V_H −45 mV	40.32	(2/5)	16.25	V_H −40 mV	10.99	(1/2)	5.5
V_H −55 mV	10.55	(2/3)	7.00	V_H −70 mV	7.84	(3/5)	4.71	V_H −80 mV	11.43	(2/3)	7.62
V_H −50 mV	3.70	(1/1)	3.70								
V_H −80 mV	4.47	(1/1)	4.47								

C. After decompression to atmospheric pressure from

300 atm			600 atm			900 atm		
12.13	(3/11)	3.31	38.28	(2/4)	19.3	298.15	(1/9)	33.12
1.07	(2/3)	0.71	35.80	(2/6)	12.0			
			9.54	(1/2)	4.76			

Data are for inside-out patches in symmetrical [KCl], calcium-free, V_H −40/−60 mV unless stated otherwise. The ratio P_{po} high pressure: P_{po} atmospheric pressure is followed, in brackets, by the minimum number of channels observed at atmospheric pressure/high pressure, followed by P_o high pressure:P_o atmospheric pressure (see text). Each set of data relates to a single experiment, except in B, where pairs of data relate to an experiment.

*Heat-shield beaker used, as at all higher pressures (see Materials and Methods).

#10^{-8} M [Ca^{2+}], buffered with HEPES.

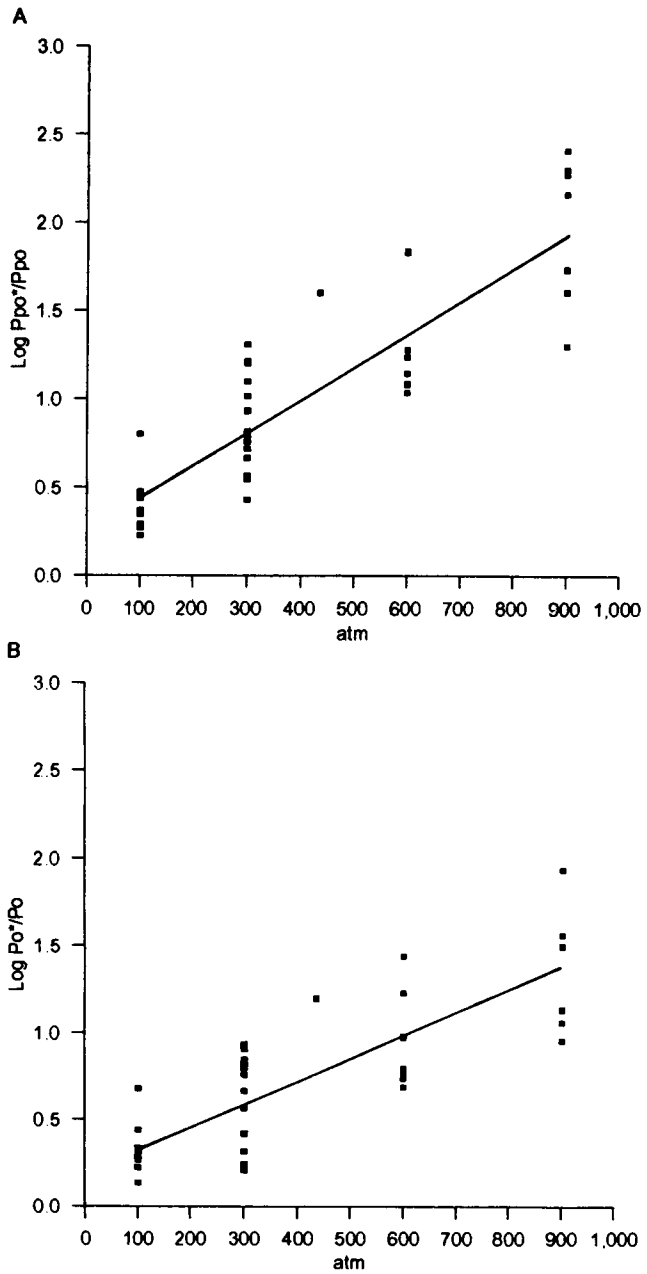

FIGURE 3 Effect of pressure on the channel open probability. (*A*) Log ratio P_{po}* (pressure):P_{po} (normal atmospheric pressure) against pressure. The linear regression has a slope corresponding to $\Delta V = -105$ ml/mol^{-1} at 24°C from dln $K/dp = -\Delta V/RT$. See text. (*B*) Log ratio P_o* (pressure):P_o (atmospheric pressure) against pressure. The linear regression has a slope corresponding to $\Delta V = -74$ ml mol^{-1}.

effect on the channel protein, or is it an indirect effect, arising from pressure affecting the channel's environment?

The highest pressure used here has a negligible effect on the pH of Tris buffer (Disteche, 1972). The Ca + EGTA $\rightleftharpoons$ CaEGTA association reaction involves a ΔV of +20.4 ml mol^{-1} (Hasselbach and Stephan, 1987), and accordingly its equilibrium constant is halved by 900 atm, doubling the free calcium concentration. At the negligible level used here [Ca^{2+}] would nevertheless remain negligible. At normal pressure [Ca^{2+}] increases channel P_o only above a concentration of 10^{-8}M (unpublished observations, this study). No obvious difference was seen at pressure between the P_{po} in calcium-free solution and 10^{-8}M Ca^{2+}. The latter was buffered by HEPES, the pH of which would decrease slightly at high pressure, and the decreased pH would favor an increase in [Ca^{2+}], reinforcing the pressure effect (Table 1).

High pressure might be imagined to affect channel kinetics by increasing the viscous resistance of the lipid bilayer surrounding the channel. In so far as equilibrium states were measured, viscous resistance may be discounted because it cannot affect equilibrium properties (Lee, 1991). However, bilayer order, lateral spacing, and other structural details may affect channel kinetics. Cooling by 10°C increases the order of lipid bilayers by an amount similar to a hydrostatic pressure of 500 atm (Macdonald, 1992). The former increased P_{po} by a factor of 1.86, whereas the latter increased it 16-fold (Fig. 3), so clearly order per se is not a major factor in the pressure activation of the channel. It is interesting that Bolotina et al. (1989) reported that cholesterol enrichment of rabbit aorta smooth muscle membranes reduced the activity of the BK channel there, presumably by some mechanism other than viscous resistance to molecular motion.

The effect of the transient heating caused by compression, which was shown to be very small in Materials and Methods, has been ignored in the results. This is justified by the data at 300 atm. Experiments with and without the heat-shield beaker produced similar results (Table 1A), yet the temperature transients differed by ~2°C. Furthermore, the temperature experienced by patches compressed to 900 atm with the heat-shield beaker and its 7 ml of aqueous solution in place, is very similar to that experienced on compression to 300 atm without the beaker. Therefore the difference between the temperature transients experienced at various pressures is very small (Fig. 2) and may be ignored. The general conclusion is that pressure is not acting indirectly by raising the temperature, or by affecting [Ca^{2+}], pH, or the order of the membrane bilayer.

In the absence of other plausible indirect means of influencing the channel, it seems reasonable to interpret the results as a thermodynamic effect of pressure on the channel proteins and or their modulating reactions. The increase in the minimum number of channels that pressure brings about is best interpreted as arising from an increase in P_o. It was not possible to demonstrate conclusively the presence of only one channel during the precompression period of recording, and so there is no firm evidence that pressure activates hitherto inactive channels. Furthermore, the "additional" channel openings apparent at high pressure had a conductance and sensitivity to TEA similar to those active at normal atmospheric pressure (Fig. 4), consistent with the presence of a homogeneous population of BK channels in the patches. However, modification to the high-pressure apparatus which is in hand will reduce the time taken to position the transfer bath and mount the assembly in the pressure vessel, thereby increasing the recording life of the

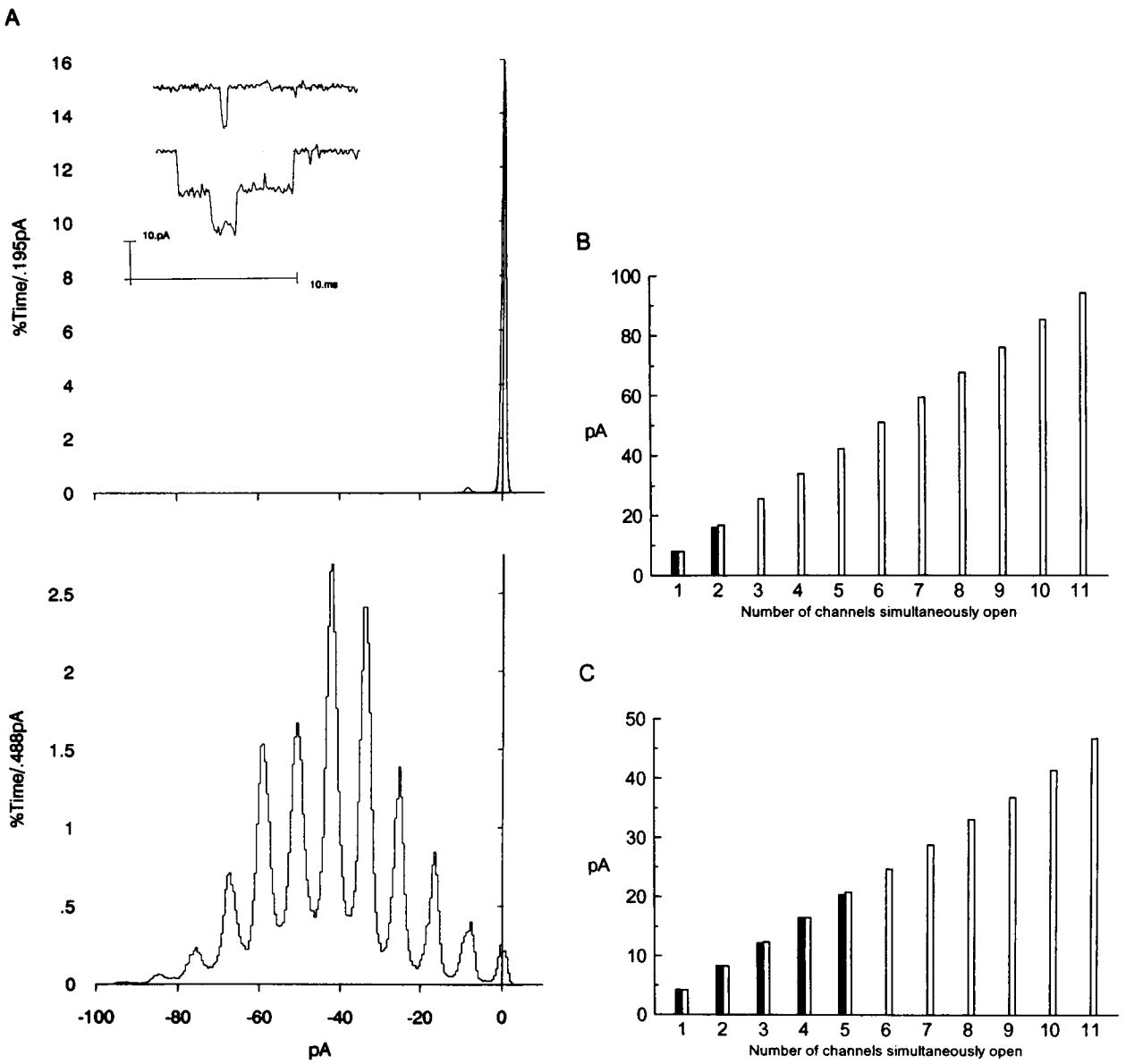

FIGURE 4 High pressure activates channels and has no effect on conductance. (*A*) *Inset*: Channel currents, upper at normal pressure, and lower at 600 atm pressure. Upper amplitude histogram (V_H = −45 mV) at atmospheric pressure; lower histogram same patch and voltage at 900 atm. (*B*) Channel currents at V_H = −45 mV taken from amplitude histograms similar to those in *A*. Filled columns represent currents when one and two channels are simultaneously open at normal atmospheric pressure. Open columns show the current when up to 11 channels open simultaneously in the same patch at the same voltage at 900 atm. (*C*) Channel currents at V_H = −40 mV taken from amplitude histograms and in the presence of 30 mM TEA in the bath. Filled columns represent currents when five channels simultaneously open at normal pressure. Open columns show the currents when 11 channels open in the same patch at the same voltage at 100 atm.

patch within the vessel. This will make it possible to study any patches, shown by prolonged precompression recordings to contain a single channel, at high pressure, and it will make other desirable experiments practical.

The experiments reported here did not investigate the reactions that are thought to modulate BK channels: ligand binding, phosphorylation-dephosphorylation, and G-protein binding (Toro and Stefani, 1993; Levitan, 1994), and which, in the case of the bovine chromaffin cell BK channel, are not known. In inside-out patches the channel is probably maximally dephosphorylated, and although pressure might activate the channel by favouring phosphorylation, this would be the reverse of the dephosphorylation proposed by Godart and Ellory (1996) to account for the pressure stimulation of silent KCl transporters in human red cells. The linear relationship of the data (Fig. 3) is consistent with one reaction determining the channel open-closed equilibrium. A pressure of 900 atm increases P_o by 30-fold, with no sign of saturation. The ΔV of −105 ml mol^{-1}, the pressure coefficient of the channel open-closed equilibrium, is probably more realistic than the lesser figure calculated from P_o, which contains some uncertainty in the minimum number of

channels. The $-\Delta V$ in the dissociation of multimeric enzymes brackets the ΔV measured here (Silva and Weber, 1993). For example, the dissociation of enolase proceeds with a ΔV of -65 ml mol^{-1}, whereas that of glyceraldehyde dehydrogenase entails up to -230 ml mol^{-1} (Paladini and Weber, 1981; Ruan and Weber, 1989). It is not obvious, however, how such dissociations might be relevant to the functioning of the channel in question. Tubulin and certain types of microtubule are highly pressure labile, with ΔV values in the region of 100–400 ml mol^{-1}, depending on reaction details (Inoue et al., 1975; Gross and Jaenicke, 1994). As cytoskeletal microtubules appear to influence BK channel gating (Kitzmiller and Rosenberg, 1997), high pressure might conceivably act by the same route. A $-\Delta V$ of 105 ml mol^{-1} is also consistent with the dissociation of a ligand, perhaps an endogenous blocker (Methfessel and Boheim, 1982), or with conformational changes in proteins (Jonas and Jonas, 1994; Gross and Jaenicke, 1994; Weber, 1993; Van Eldik et al., 1989; Hasselbach and Stephan, 1987). Finally, as the mobility of ions in aqueous solution is not affected by the pressure used here (Brummer and Hills, 1961), and as the BK channel conductance is not affected by 900 atm (like alamethicin channels at 1000 atm; Brunner and Hall, 1983), there is no reason to suppose that the geometry of the BK channel pore is affected by pressure. This would be consistent with the most appealing and simplest view, that pressure acts directly on the gating reaction.

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