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POTASSIUM CHANNELS OPENED BY NORADRENALINE AND OTHER TRANSMITTERS IN EXCISED MEMBRANE PATCHES OF GUINEA-PIG SUBMUCOSAL NEURONES

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SUMMARY

1. Unitary potassium currents were recorded in outside-out patches of membrane from guinea-pig submucosal neurones. The actions of α_2-adrenoceptor agonists, somatostatin and [Met^5]enkephalin were studied.

2. Three main groups of background potassium channels were active. At -70 mV with 160 mM-potassium on both sides of the membrane, they had conductances of 30–65 (small), 120–160 (intermediate) and 220–260 pS (large).

3. The open channel current–voltage relation showed only constant-field rectification. Extracellular barium (2 mM) and caesium (2 mM) decreased inward but not outward currents. Tetraethylammonium (10 mM) had no effect.

4. Noradrenaline, somatostatin and [Met^5]enkephalin each increased the open probability of all three classes of channel when two or more unitary amplitude channels were active in the membrane patch. Agonists were ineffective when no channel, or a single channel, was discernible in the patch. Agonists did not cause the appearance of unitary currents distinct from those seen prior to their application.

5. The effect of the agonists required intracellular guanosine 5′-triphosphate.

6. The results show that the hyperpolarization of submucosal plexus neurones by noradrenaline, somatostatin and [Met^5]enkephalin results from the increased opening of at least three types of background potassium channel, and that the coupling from the receptors to the channels is maintained in excised membrane patches.

INTRODUCTION

Guinea-pig submucosal neurones are hyperpolarized by noradrenaline, somatostatin and enkephalin, acting through α_2-adrenoceptors, somatostatin receptors and δ-opioid receptors (Surprenant & North; 1988; Tatsumi, Costa, Schimerlik & North, 1990). Noradrenaline released from sympathetic nerves causes a large inhibitory postsynaptic potential, as apparently does somatostatin released from intrinsic enteric nerves (Mihara, Nishi, North & Surprenant, 1987; Surprenant & North, 1988; Bornstein, Costa & Furness, 1988); the main functional significance would be to reduce the activity of secretomotor neurones and promote water and electrolyte reabsorption. The hyperpolarization results from an increase in potassium

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conductance, and a common potassium conductance is likely because the potassium conductance increase evoked by an agonist at any one of the three receptors occludes the action of agonists at either of the other two (see North, 1989). This suggests the possibility that a common cytoplasmic second messenger underlies the convergence, although tests of the involvement of protein kinase A or protein kinase C have been negative (Surprenant & North, 1988). The general purpose of the present experiments was to investigate the mechanism underlying the coupling from these receptors to the potassium channels.

An increase in potassium conductance by agonists acting at guanosine 5′-triphosphate-binding proteins (G proteins) has been extensively studied in cardiac tissue. In this case, three lines of evidence suggest that the coupling between receptor and channel does not involve the cell cytoplasm. First, agonists applied to the intact cell can increase potassium currents under conditions in which known intracellular messengers are inoperative (Pfaffinger, Martin, Hunter, Nathanson & Hille, 1985). Second, potassium channel activity can be increased by applying activated guanosine 5′-triphosphate (GTP)-binding proteins directly to the cytoplasmic surface of excised membrane patches (Brown & Birnbaumer, 1990). Third, the activity of single potassium channels recorded in the cell-attached configuration is altered when agonists are in the pipette but not when they are added to the solution which bathes the remainder of the cell surface (Sakmann, Noma & Trautwein, 1983; Soejima & Noma, 1984; Kurachi, Nakajima & Sugimoto, 1986; Brown & Birnbaumer, 1990). However, there may be differences between the coupling mechanism that operates in the cardiac tissue with that in neurones. For example, application of G proteins to the cytoplasmic surface of membrane patches excised from hippocampal neurones suggested that G proteins can activate not one but four different types of potassium channel (Van Dongen, Condina, Olate, Mattera, Joho, Birnbaumer & Brown, 1988). Moreover, cell-attached recordings from hippocampal neurones showed that $GABA_B$ agonists activated potassium channels in the membrane patch when they were applied to the outside of the cell, suggesting the involvement of a diffusible second messenger system (Premkumar, Chung & Gage, 1990).

The specific purpose of the present work was to study the action of noradrenaline and the other agonists on potassium channels in excised membrane patches, to define the general properties of the channels, and to test directly the hypothesis that a single channel can be opened by agonists at different receptors without the need for cytoplasmic second messengers. A preliminary report has been communicated (Shen, North & Surprenant, 1990).

METHODS

Tissue preparation and solutions

The submucosal plexus was dissected from the ileum of adult guinea-pigs that had been killed by exsanguination from carotid arteries after halothane anaesthesia. Acute dissociations of submucosal neurones were as previously described in detail (Surprenant, Shen, North & Tatsumi, 1990); the tissues were incubated in the collagenase-zero calcium–magnesium solution at 37 °C for variable durations (10–45 min) as determined by microscopic observation of the preparation. Tissues were triturated and plated onto glass cover-slips in Dulbecco's modified Eagle's medium. Recordings were made up to 12 h after dissociation, at room temperature. The following solutions were used (mM): external solution KCl, 160; $MgCl_2$, 1; glucose, 10; HEPES, 10; and tetrodotoxin, 0·001. Osmolarity was maintained at 320 mosM by adding sucrose in those experiments in which lower concentrations (105, 55, 25 and 10 mM) of potassium were used. Internal solution contained:

potassium gluconate, 160; $MgCl_2$ 1; EGTA, 11; HEPES, 10; ATP, 1·5; and GTP, 0·25–0·5. Calcium chloride (1 mM) replaced $MgCl_2$ in those experiments in which magnesium-free internal solution was used. In some cases (see Results) GTP-γ-S or GDP-β-S was used in place of GTP. All solutions were applied to the outside surface of the membrane patch by means of a 'fast-flow' delivery pipette. In most cases, six such pipettes (each with a diameter of 400 μm) were glued together so that several different solutions could be applied rapidly to an excised patch (Surprenant *et al.* 1990). Agonists used were noradrenaline, UK 14304 (5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline, a gift from Pfizer) and clonidine (α_2-adrenoceptors), somatostatin and [Met^5]enkephalin. Antagonists used were idazoxan, yohimbine (α_2-receptors) and naloxone (opioid receptors).

Electrophysiological procedures

Tight-seal recordings were made in the outside-out configuration using fire-polished glass electrodes (8–30 MΩ) coated with Sylgard. All membrane potentials were corrected for liquid-junction potentials as described in detail by Hagiwara & Ohmori (1982). An Axopatch 1B amplifier was used to record patch currents, which were stored on videocassette tape for off-line analysis. Currents were digitized at 5–10 kHz, filtered at 1–2 kHz (−3 dB) using an 8-pole Bessel filter (Frequency devices) and analysed using modified pClamp v 5·5 software (Axon Instruments) and Kaleidagraph (Albelbeck Software).

Potassium channel data analysis

Patches in which only a single unitary amplitude potassium channel was present, or in which total open probability (Np_o) was $< 0{\cdot}05$ and overlap of channel activity was $< 5\%$ of total openings, were analysed by creating events lists using 50% threshold criteria, ignoring openings $\leqslant 0{\cdot}2$ ms (Colquhoun & Sigworth, 1983). Events containing multiple level openings were manually eliminated from the lists and then open and closed time histograms were fitted with the sum of two exponentials. Open probability (p_o) for single channels was calculated from $p_o = (\tau_{o1}+\tau_{o2})/(\tau_{o1}+\tau_{o2}+\tau_{c1}+\tau_{c2})$. Amplitude histograms for small and large conductance potassium channels were obtained separately by first setting threshold at a low level so that all channels > 12 pS were included and the histogram was obtained manually. Threshold was then set so that only the large conductance channels were included and a new histogram obtained automatically. Amplitude histograms were fitted by the sum of one or more Gaussian functions using the least-squares method.

Because patches in which a single-amplitude channel was present usually did not respond to agonist (see Results), patches containing multiple, overlapping channels were analysed by creating probability density histograms (0·1–0·5 pA bins) as described by Sakmann & Trube (1984). Total open probability (Np_o) was obtained by subtracting the area under the Gaussian fit of the closed state distribution from one, the total area under the curve. In each of three patches containing two well-resolved channels, Np_o calculated by this method was within 8% of the Np_o value obtained by the former method. Gaussian functions were also fitted to the open state distributions and the area under these curves were used to estimate single-channel p_o in the absence and presence of agonist. Tests of significance were by Student's t test.

RESULTS

Properties of background potassium channels

Outside-out patches pulled from submucosal neurones typically showed unitary potassium currents of several different amplitudes. In 160 mM-external potassium they fell into three groups: small (30–65 pS), intermediate (120–160 pS) and large (220–260 pS) (Fig. 1). Small amplitude channels occurred with either intermediate or large amplitude channels (fifty-one patches had small with intermediate channels and twenty-three patches had small with large channels) but intermediate and large channels without small channels were never observed in the same patch (six patches had all three amplitude channels). A single small amplitude channel without intermediate or large channel was discerned in ninety-four patches, a single intermediate channel without small or large channel was observed in seven patches

and a single large amplitude channel without small or intermediate channel was observed in only one of over 200 recordings. In each case examined, the unitary currents reversed polarity at about 0 mV, and this reversal potential changed approximately according to the Nernst equation when the external potassium

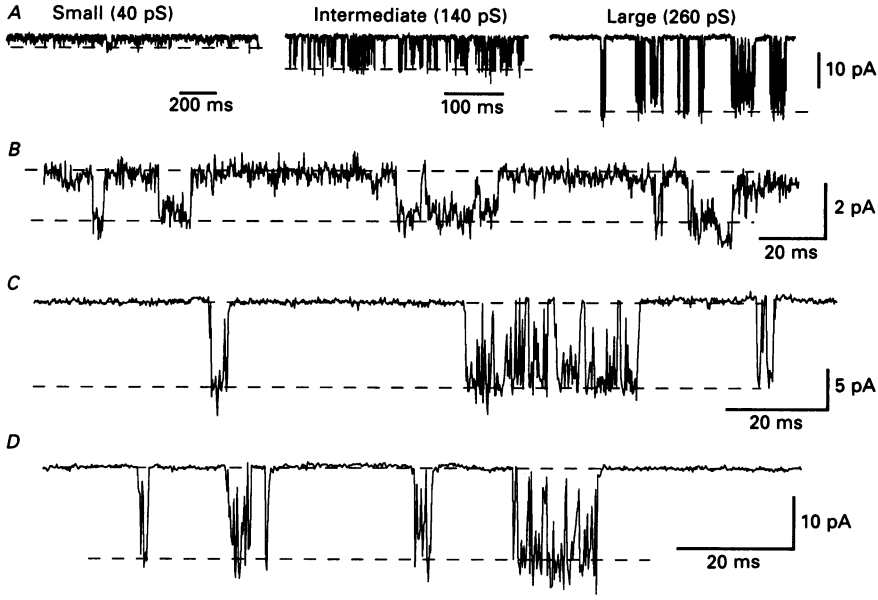

Fig. 1. Several distinct potassium channels are active in excised neuronal membranes. *A*, current activity recorded at a holding potential of −50 mV from three different outside-out membrane patches of submucosal neurones with internal and external potassium concentration of 160 mM. A single unitary amplitude current was present in each patch; unitary conductances were 40, 140 and 260 pS respectively. These examples are not representative of the majority of patches, which contained a combination of these sized channels. *B*, *C* and *D*, segments of recordings from patches shown in *A* at higher sweep speed and gain; all channels show flickery burst behaviour with many of the fast closures within bursts not resolved.

concentration was altered (Fig. 2), except at low external potassium concentrations (Fig. 2*C*). In the majority of patches, the open channel *I–V* relation was well fitted by the Goldman–Hodgkin–Katz equation (Fig. 2*B*). There was no significant change in the single-channel *I–V* relation when internal magnesium was raised to 2 mM and external magnesium was removed, nor when internal magnesium was absent and external magnesium was 2 mM. Single-channel *I–V* relations measured from two patches (total of twenty-four patches examined) did show an increased conductance for inward unitary currents; one example of the inward rectification recorded from one of these patches is shown in Fig. 4*A* and *B*. No other obvious differences in channel activity were noted in these two patches.

All channels showed clear burst of openings separated by periods of relative inactivity. Open and closed time distributions were measured in thirteen patches in which only a single small conductance channel was present and in six patches in which only the intermediate channel was present. The large conductance channel

occurred alone in only one patch but these large amplitude currents were sufficiently well resolved from the small amplitude currents in three patches to allow distribution analysis (i.e. overlap occurred in $< 5\%$ of total openings and total open probability, Np_o, was $< 0{\cdot}05$; see Methods).

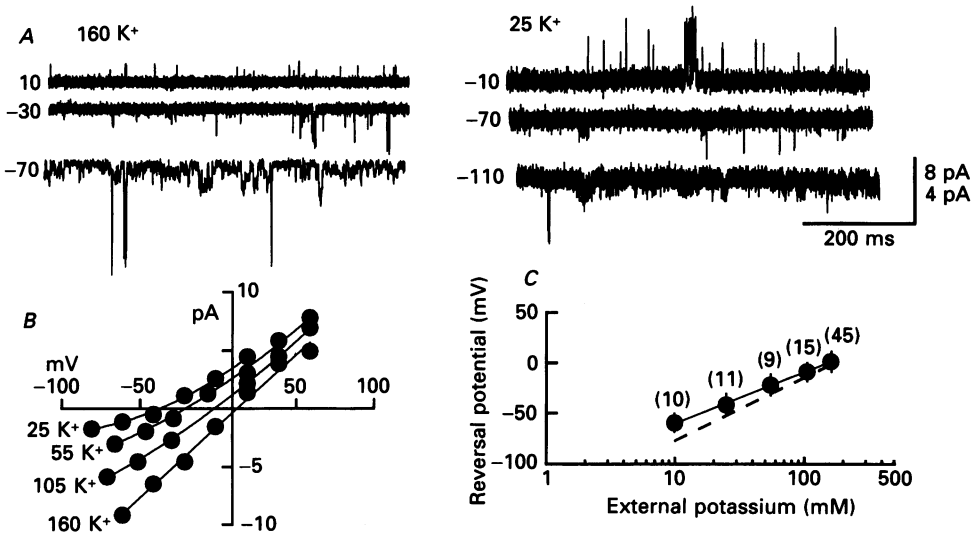

Fig. 2. *A*, channel activity recorded at different membrane potentials (as indicated in mV) from one patch in equal potassium (160 mM, left-hand traces) and when external potassium was reduced to 25 mM (right-hand traces); gain for traces in 25 mM-K^+ is twice that for currents in 160 mM-K^+. At least two distinct unitary amplitude currents were present. *B*, current–voltage relation obtained for a single large amplitude channel in 25, 55, 105 and 160 mM-K^+ as indicated. Lines are best fits to constant-field equation over the potentials shown. *C*, summary of all experiments in which reversal potentials as a function of external potassium were determined; results from all unitary amplitude channels are pooled; numbers in parentheses are numbers of channels measured. Dashed line is predicted from the Nernst equation.

The distribution of open times could always be fitted by the sum of two exponential functions (Fig. 3); time constants (τ_{o1}, τ_{o2}) were similar for channels of all three conductances. For the small, intermediate and large conductance channels, the values for τ_{o1} were $0{\cdot}24 \pm 0{\cdot}06$, $0{\cdot}32 \pm 0{\cdot}05$ and $0{\cdot}30 \pm 0{\cdot}02$ ms and of τ_{o2} were $1{\cdot}8 \pm 0{\cdot}8$, $1{\cdot}7 \pm 0{\cdot}2$ and $3 \pm 0{\cdot}3$ ms respectively. Closed times were also well fitted by two exponential functions, with time constants τ_{c1} and τ_{c2}. For the small, intermediate and large conductance channels the values of τ_{c1} were 11 ± 4, 12 ± 4 and 20 ± 9 ms; values for τ_{c2} varied enormously among patches with average values of 235 ± 95, 290 ± 87 and 290 ± 100 ms respectively. The above values were measured with equal potassium concentrations at a potential of -60 mV.

The overall probability (p_o) of these channels being open was quite variable for different patches; p_o for individually resolved channels ranged from 0·006 to 0·2 at -40 mV. The relationship between membrane potential and probability of channel opening was examined in two ways: by calculating dwell times from events lists obtained at -60 and 20 mV and by constructing probability density distributions

over a wider range of potentials. Dwell times for small ($n = 3$) and intermediate ($n = 2$) channels were measured both at -60 and 20 mV. The main effect of depolarization was to reduce both closed times with no significant alteration in open times; at -60 mV τ_{c1} and τ_{c2} were 11 ± 3 and 400 ± 60 ms for the small channel and

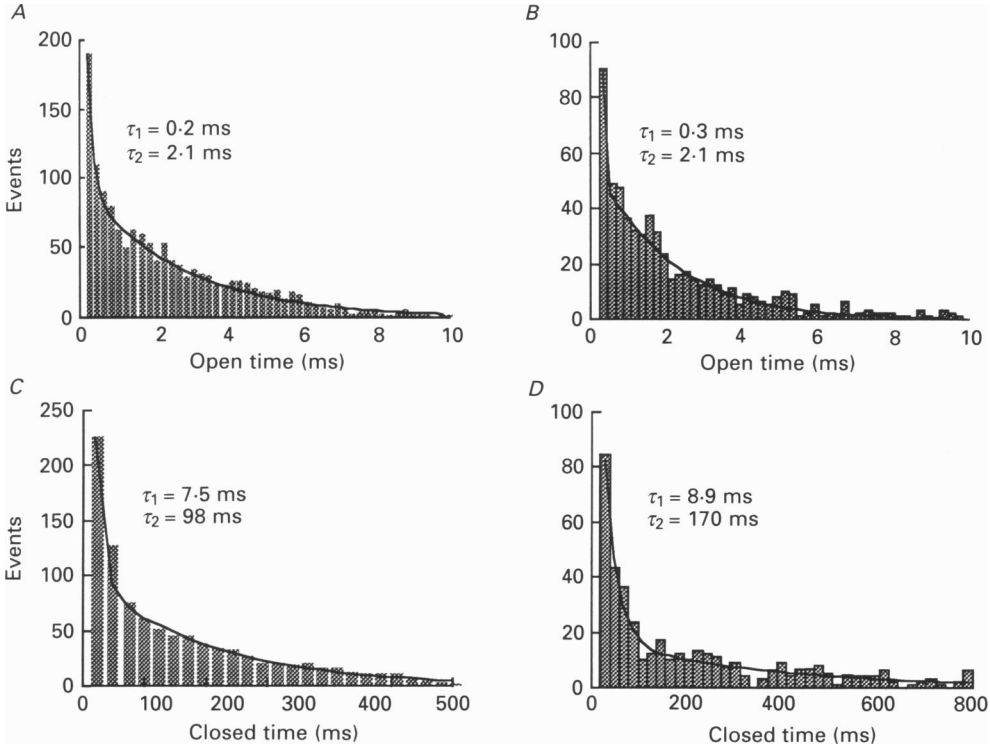

Fig. 3. Examples of open (upper graphs) and closed (lower graphs) time distributions obtained from one patch containing a small amplitude channel (*A* and *C*, 50 pS in equal potassium) and from another patch containing a large amplitude channel (*B* and *D*, 250 pS). Lines show best fit to the sum of two exponential functions, time constants of which are as indicated in each graph. Data were obtained at a holding potential of -70 mV. No significant differences among mean open times of small, intermediate or large amplitude channels were observed.

22 ± 7 and 290 ± 40 ms for the intermediate channels; at 20 mV these values were $6{\cdot}5 \pm 0{\cdot}8$, 208 ± 78 ms (small channel) and $4{\cdot}6 \pm 0{\cdot}5$, 134 ± 46 ms (intermediate channel). The Np_o values calculated from probability density distributions of multichannel patches also increased with depolarizations ($n = 8$; e.g. Fig. 5*C–E*). An increase in p_o at depolarized potentials has also been seen for the inward rectifier potassium channel in cardiac muscle (Sakmann & Trube, 1984).

Voltage-activated delayed rectifier potassium channels did not contribute significantly to channel openings at depolarized levels because (1) the amplitude of the delayed rectifier channels (γ approximately 12–14 pS, unpublished observations; see Rudy, 1988; Stuhmer, Ruppersberg, Schroter, Sakmann, Stocker, Giese, Perschke, Baumann & Pongs, 1989) was below our usual resolution of measurement

for potassium channel recordings and (2) the activity of the delayed rectifier channels, as evidenced by increased baseline noise, generally disappeared 30–45 s after initiation of the depolarizing command and all our measurements were taken more than 60 s after the depolarization.

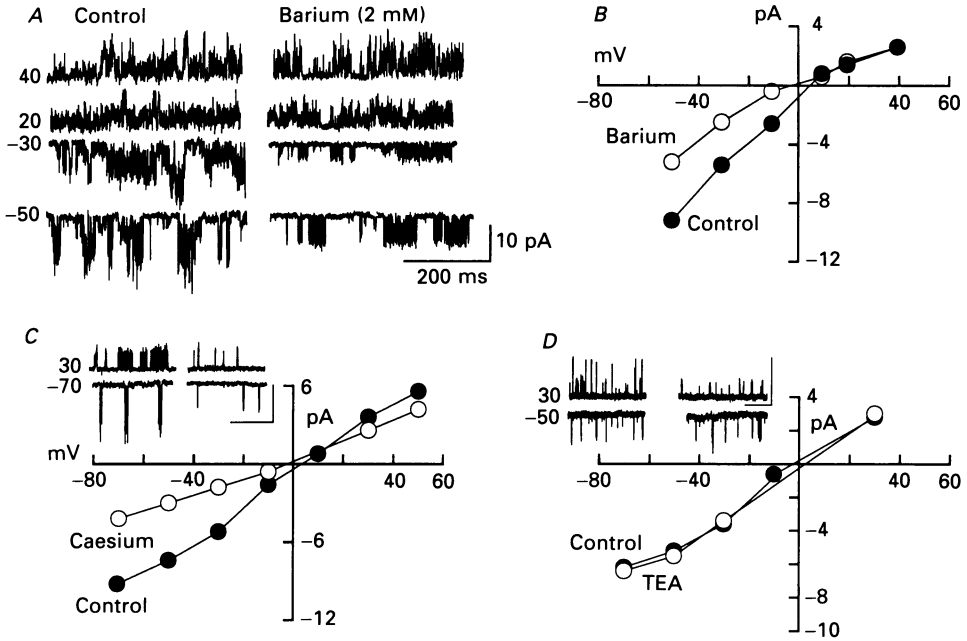

Fig. 4. *A*, single-channel activity recorded at −50, −30, 20 and 40 mV as indicated in the absence and presence of 2 mM-external barium. *B*, *I–V* relation obtained from experiment shown in *A*; barium reduced inward but not outward unitary amplitudes. *C* and *D*, *I–V* relations obtained from experiments similar to that of *A*, in which external caesium (2 mM, *C*) or TEA (10 mM, *D*) was applied. Caesium reduced inward current only; TEA was without effect on leakage K^+ channel. Note the occurrence of a large amplitude outward current when the holding potential was 30 mV (*D*, inset); this was abolished by TEA (10 mM), see Fig. 5*E*. Calibrations for traces shown in insets (*C* and *D*) are as in *A*.

Effects of potassium channel blockers on single-channel activity

Current–voltage relations recorded from intact submucosal neurones show an inwardly rectifying resting conductance which is characterized by its dependence on the external potassium concentration; the inward potassium current (flowing negative to E_K) is inhibited by low concentrations of barium (10–100 μM) and by caesium (2 mM) but not by tetraethylammonium (TEA, 10 mM) or 4-aminopyridine (4-AP, 1 mM; Surprenant & North, 1988). Therefore, we examined the effects of these potassium channel blockers on single-channel activity in outside-out membranes.

Barium and caesium (2 mM) both reduced unitary potassium currents recorded in symmetrical potassium concentrations by about 50% at potentials negative to E_K but had little effect on amplitudes of outward currents recorded at positive potentials (Fig. 4*A–C*). Barium decreased unitary amplitude of the large conductance channel by 41·5±6 and 11±4% at −60 and 40 mV respectively ($n = 5$). We have not

examined this reduction in unitary amplitude in further detail; it presumably results from rapid 'flickery' channel closures not resolved at our sampling frequency (see Fukushima, 1982).

Barium (2 mM) reduced Np_o measured at −60 mV in all membrane patches examined ($n = 6$); total open probability for inward currents at this potential was

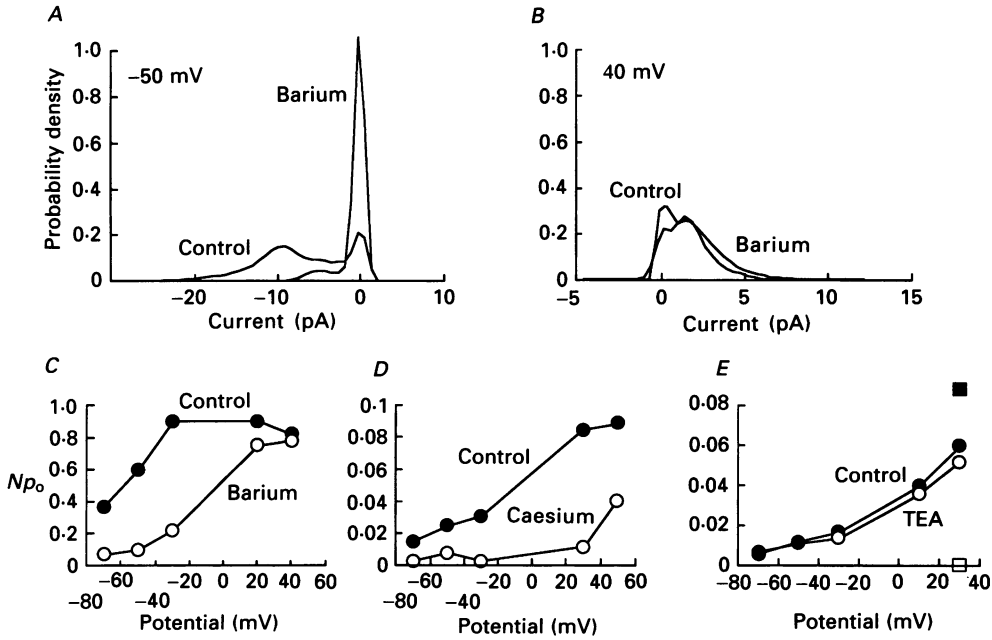

Fig. 5. *A* and *B*, probability density functions obtained from 30 s recording periods in control solution and in 2 mM-barium at −50 mV (*A*) and at 40 mV (*B*); 0·4 pA bins were used to construct the histogram; data are from the experiment shown in Fig. 4*A* and *B*. Np_o values were estimated by fitting a single Gaussian to the rising phase of the zero-current peak (i.e. closed state) and subtracting the area under this Guassian from one. *C*, *D* and *E*, probability of channel opening (Np_o) as a function of membrane potential in control solution and in barium (*C*), caesium (*D*) and TEA (*E*). Values are calculated from probability density distributions of experiments shown in Fig. 4. In *E*, the squares refer to the appearance (■) of a distinct channel at the membrane potential of 30 mV and to its blockade (□) by TEA.

decreased by 57·8±9% ($n = 6$) while outward current Np_o measured at 40 mV was reduced by only 12±3% (Fig. 5*A–C*). Actions of barium on channel activity recorded in lower external potassium and over a wider potential range were not examined in the present study; thus, our data do not allow us to distinguish between voltage-dependent and/or current-dependent inhibition of channel opening by barium. In contrast, caesium significantly reduced Np_o at all potentials ($n = 4$; Fig. 5*D*). TEA (10 mM) did not alter unitary amplitude or Np_o of currents active at potentials between −70 mV and about 20 mV; however, a large amplitude outward current which occasionally was observed at potentials positive to 20 mV was abolished (Figs 4*D* and 5*E*). The large unitary amplitude of this voltage-activated potassium channel and its high sensitivity to blockade by TEA most likely mean it

is the 'big K' calcium-activated potassium channel (Marty, 1983; Petersen & Maruyama, 1984). 4-Aminopyridine (0·1–1 mM) had no obvious effect on unitary potassium currents or Np_o ($n = 9$; data not shown).

Effects of α_2-adrenoceptor agonists on potassium channels

Noradrenaline (0·1–10 μM) and the α_2-adrenoceptor agonist, UK 14304 (40–600 nM), caused an obvious increase in potassium channel activity without any change in the amplitude of the unitary currents (Figs 6 and 7). Noradrenaline or UK 14304 increased channel activity in 75% (24/32) of patches that showed two or more distinct currents; noradrenaline (3 μM) or UK 14304 (200 nM) increased Np_o by 3- to 43-fold in these patches. Patches in which only a single unitary event was present, such as those illustrated in Fig. 1, rarely responded to agonist (0/1 large, 1/6 intermediate and 4/94 small channel patches); therefore, the majority of data reported herein are from membrane patches containing channels of two or three unitary amplitudes.

Noradrenaline and UK 14304 had no effect when applied to patches in which no channels were active ($n = 10$). Moreover, these agonists did not cause the appearance of currents through new channels having distinct unitary conductances. Figure 7 shows recordings from one patch in which channel openings of three distinct unitary amplitudes occurred at low frequency in control solution ($Np_o = 0{\cdot}036$); UK 14304 increased channel activity about fourfold ($Np_o = 0{\cdot}127$) by increasing p_o of each channel without altering mean current amplitude. No clear, maintained transitions from one current amplitude to another were detected when agonist was applied (e.g. Fig. 7*B* and *C*), providing further support for the belief that channel activity represented openings of distinct potassium channels rather than subconductance states of a single channel type. However, we cannot rule out the possibility that very fast transitions may occur which are beyond the resolution of our recordings, especially in view of the bursting nature of open channel activity (Fig. 7).

The action of agonist was readily reversible when its application was discontinued (Fig. 6*A*), and noradrenaline increased Np_o to a similar degree during repeated applications for 25–45 min following patch excision. The α_2-adrenoceptor antagonists idazoxan (0·2–0·5 μM, $n = 28$; Fig. 6*B*) and yohimbine (200 nM, $n = 3$) did not themselves alter channel activity, but in their presence both noradrenaline and UK 14304 were ineffective.

In six patches (at -40 mV) in which a single small amplitude K^+ channel was present and noradrenaline application was effective, the increase in p_o resulted primarily from a reduction in the longer of the two closed times (i.e. the interval between bursts, τ_{c2}). Control values (ms) were τ_{o1} 0·22 ± 0·02, τ_{o2} 1·54 ± 0·25, τ_{c1} 5·05 ± 1·5 and τ_{c2} 148 ± 37 (p_o 0·019 ± 0·007) and the values in 3 μM-noradrenaline were τ_{o1} 0·21 ± 0·02, τ_{o2} 1·9 ± 0·4, τ_{c1} 3·9 ± 1·9 and τ_{c2} 28 ± 6 (p_o 0·076 ± 0·02). Probability density distributions constructed from these same patches gave control and noradrenaline values for open channel density of 0·022 ± 0·009 and 0·086 ± 0·01 respectively. In another patch in which a large amplitude channel was well resolved from a second one of small amplitude, large channel p_o calculated from dwell times increased from 0·06 to 0·23 in the presence of 3 μM-noradrenaline, again because of a reduction in τ_{c2}. The corresponding channel density probabilities calculated from

all-points probability density histograms for this channel were 0·056 (control) and 0·28 (noradrenaline). Therefore, we used probability density distributions to examine whether noradrenaline or the α_2-adrenoceptor agonist, UK 14304, differentially altered probability of opening of the three different sized potassium channels (see Methods).

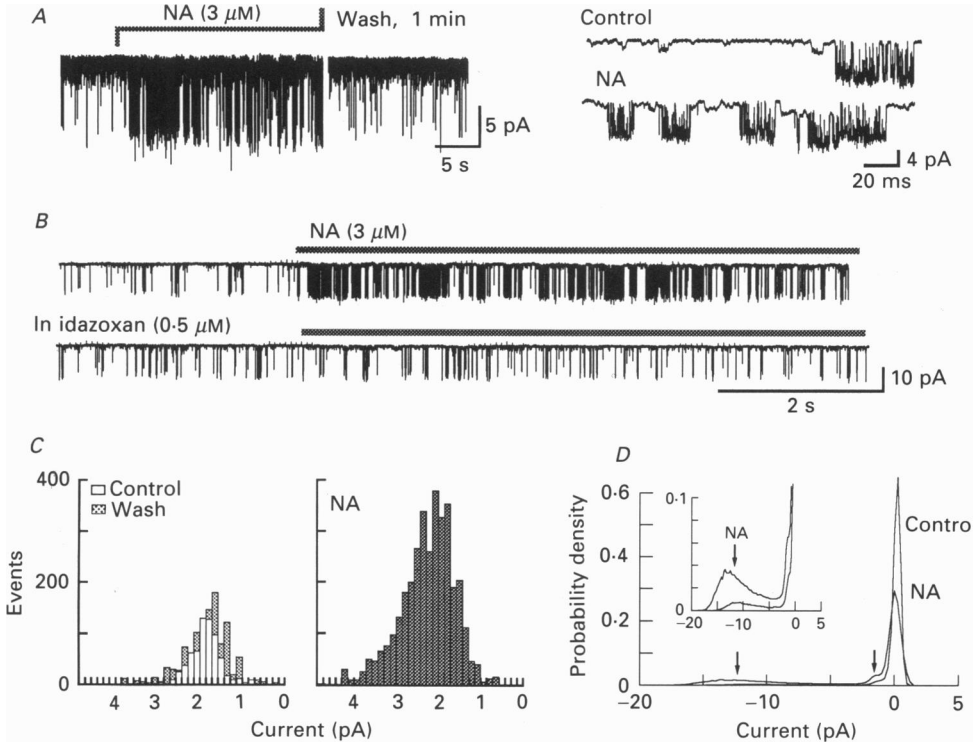

Fig. 6. Noradrenaline (NA) increases probability of opening of resting K^+ channels. *A*, channel activity recorded in an outside-out membrane patch at −70 mV; noradrenaline was applied for duration indicated by bar above trace. Right-hand traces show segments from control and noradrenaline at faster sweep; unitary amplitudes are unaffected by noradrenaline and no new unitary amplitude channels are apparent. Unitary conductances of the two distinct channels present in this patch were 36 and 125 pS. *B*, single-channel recording obtained from another patch in which two channels (35 and 260 pS) were active in control solution; noradrenaline increased frequency of channel opening (upper trace). Repeated application of noradrenaline in the presence of idazoxan was without effect (lower trace). Noradrenaline was again effective in increasing channel activity when idazoxan was removed (data not shown). *C* and *D*, two examples of the increase in p_o by noradrenaline. In the experiment shown in *C*, only a single small amplitude channel was present in the patch and amplitude histograms were obtained from events lists collected over 30 s periods in control, noradrenaline and wash. Mean peak amplitudes obtained from Gaussian fits to the data were −1·7, −1·8 and −1·7 pA respectively; holding potential −40 mV. *D* shows probability density distribution obtained from the experiment shown in *B*; 30 s recording periods were measured. Arrows mark peaks (from Gaussian functions) of open states for the small amplitude (−1·6 pA) and large amplitude (−12 pA, holding potential −45 mV) channel present in this patch. The shift in distribution profile of the large amplitude channel in noradrenaline (obvious in inset) is due to contribution from overlapping small amplitude channel.

Both noradrenaline and UK 14304 increased open channel probability for each potassium channel active in normal solution (Fig. 10*A*). Noradrenaline and UK 14304 did increase p_o of the small amplitude channel significantly more than the intermediate or large amplitude channel, producing an average 7-fold increase in

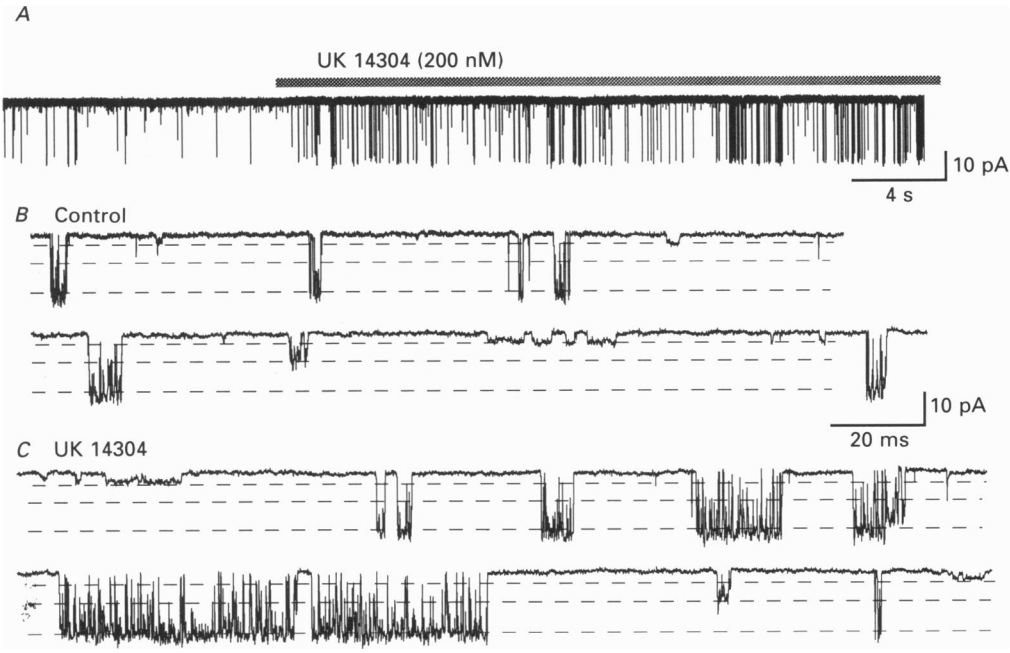

Fig. 7. Example of actions of the α_2-adrenoceptor agonist, UK 14304, on single-channel activity recorded from one membrane patch in which three distinct unitary amplitude currents were active prior to application of agonist (applied for time indicated by bar). *B* and *C* are expanded segments from recording shown in *A*; dashed lines indicate average unitary amplitude for small (3 pA), intermediate (7·9 pA) and large (17 pA) amplitude channel present prior to agonist application. Clear transitions from one conductance state to another were not apparent before or after agonist application in this, or other, membrane patches. p_o values calculated from probability density plots for small, intermediate and large channels were 0·014, 0·002 and 0·02 in control solution and 0·054, 0·008 and 0·066 in UK 14304.

small channel activity and an approximately 5-fold increase in intermediate and large channel probability of opening (Fig. 10*B*). The actions of α_2-receptor agonists showed no consistent dependence on membrane potential; that is, there were no significant differences in the agonist-induced increases in Np_o recorded at membrane potentials between -70 and 20 mV ($n = 4$).

Actions of somatostatin and [Met⁵]enkephalin on potassium channels

Somatostatin (40–200 nM, $n = 21$) and [Met⁵]enkephalin (2–20 μM, $n = 6$) had the same actions as noradrenaline (Fig. 8). As for noradrenaline, these agonists had no action when applied to patches which showed no activity ($n = 3$ for each agonist). In

three patches, UK 14304, somatostatin and [Met^5]enkephalin were applied sequentially (Fig. 8); all agonists reversibly increased activity of the same channels. Similar results were obtained with two of the three agonists in a further seven patches. The action of [Met^5]enkephalin, but not that of somatostatin or

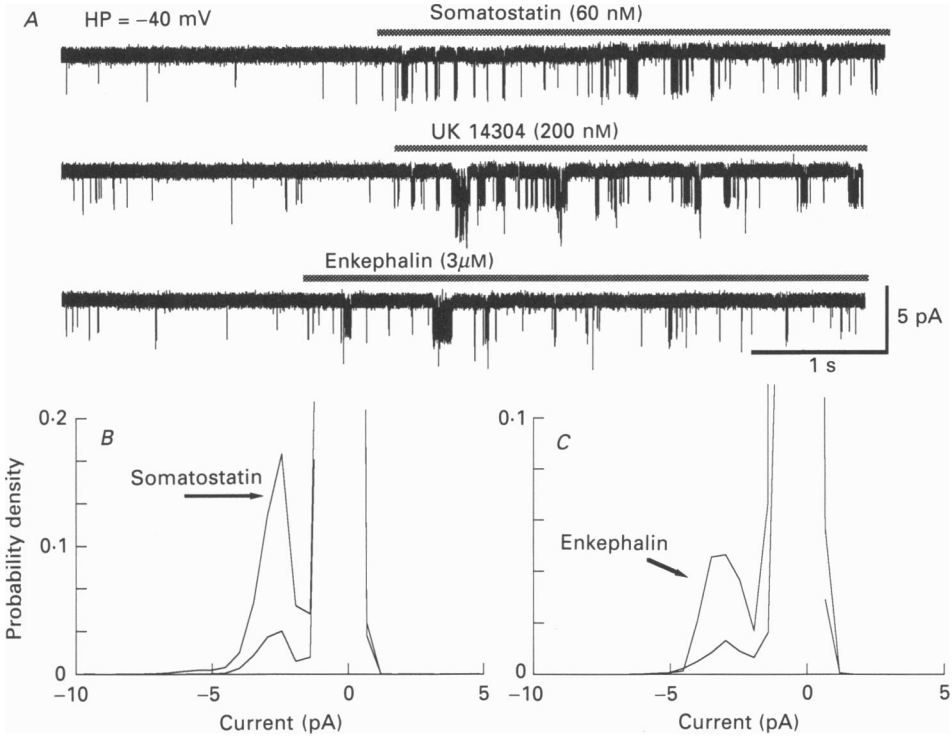

Fig. 8. Multiple receptors couple to the same K^+ channels in an isolated patch. *A*, recordings obtained from one outside-out membrane patch in response to superfusion with somatostatin, the α_2-adrenoceptor agonist UK 14304, and [Met^5]enkephalin; each 1 min application of agonist was followed by 5 min superfusion in control solution. *B* and *C* show probability distributions for experiment in *A*; closed state profile is off-scale.

noradrenaline, was prevented by prior exposure to naloxone (0·2 μM, $n = 2$). Figure 9 shows open and closed time distributions measured in one patch in which only a single small amplitude channel was active; application of enkephalin to this membrane reversibly increased p_o by 6-fold (from 0·007 to 0·04) by decreasing the intervals between bursts (τ_{c2}). This was the only membrane patch containing a single potassium channel which responded to application of [Met^5]enkephalin (total of seven patches examined). Somatostatin increased Np_o in 21/28 patches recorded from showing two or more unitary amplitude channels but had no effect on 4/4 patches exhibiting a single channel.

Figure 10 summarizes the actions of α_2-adrenoceptor agonists, somatostatin and [Met^5]enkephalin, to increase activity of potassium channels present in outside-out membrane patches. Open channel probability density of intermediate and large amplitude channels was increased to a similar degree (approximately 5-fold increase

over control values) by all agonists; noradrenaline and somatostatin, but not [Met^5]enkephalin, increased the probability density of the small amplitude channel to a significantly greater degree (Fig. 10*B–D*).

Involvement of G proteins in receptor–channel coupling in outside-out membranes

When GTP-γ-S (500 μM) was included in the pipette solution, more channel activity was observed than in its absence. In some cases this was apparent from the

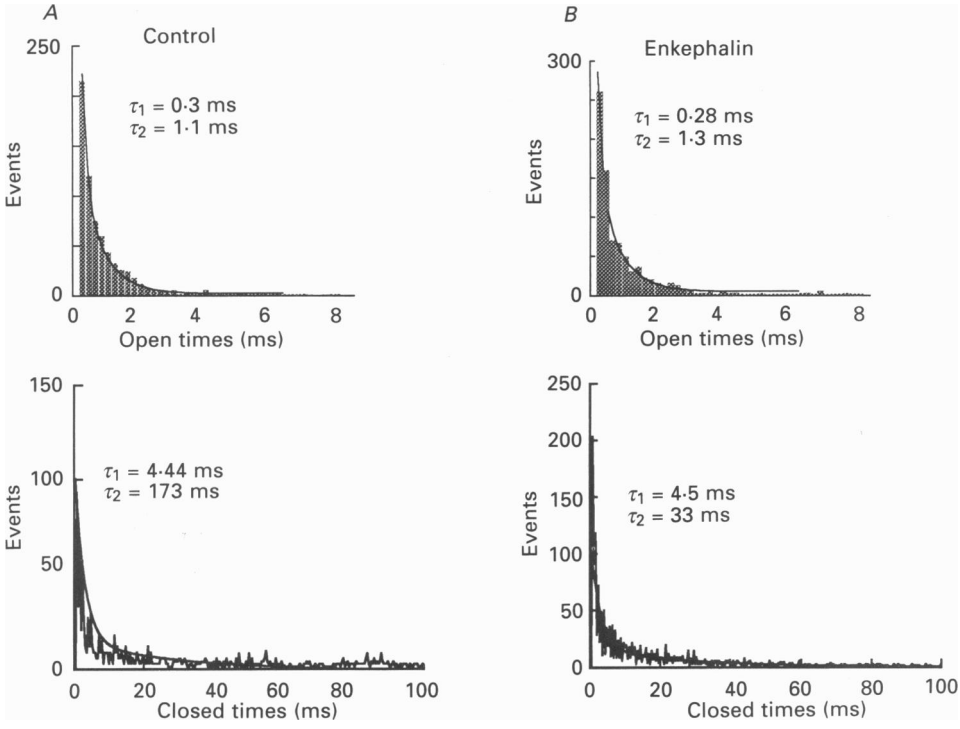

Fig. 9. Open (upper graphs) and closed (lower graphs) time distributions measured in control (*A*) and in the presence of 3 μM-[Met^5]enkephalin (*B*) from one patch in which a single 36 pS small amplitude channel was present. Data obtained at −60 mV. [Met^5]enkephalin markedly shortened τ_{c2} (from 173 to 33 ms) without altering other time constants.

moment of patch excision, whereas in others there was a continuous increase in channel activity during several minutes after excision. In eleven experiments in which the pipettes contained the usual GTP concentration (500 μM), Np_o values recorded 5–10 min after patch excision averaged 0·06 ± 0·03, whereas in twelve patches in which the pipette contained GTP-γ-S Np_o was 0·28 ± 0·1. Application of noradrenaline further increased Np_o in these patches but this increase did not show a significant reversal within 10 min after wash-out of agonist. When the pipette contained neither GTP nor GTP-γ-S, noradrenaline stimulated channel activity in only one of ten experiments; in that case, the action of noradrenaline disappeared 8 min after patch excision. The exclusion of GTP from the patch pipette did not have obvious effects on resting potassium channel activity itself; three unitary amplitude

channels were still present in different patches and Np_o values recorded 5–20 min after patch excision were within the range observed under our normal recording conditions (i.e. GTP in the patch pipette).

When the pipette solution contained GDP-β-S (500 μM) channel activity (Np_o) appeared to be less than in control conditions; in six patches Np_o values (range

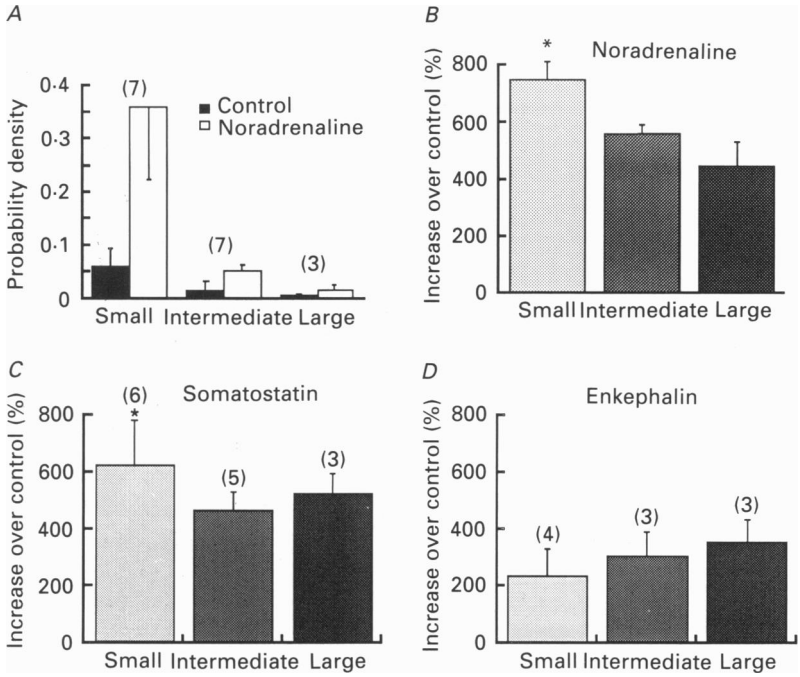

Fig. 10. Agonists promiscuously increase activity of each resting K^+ channel. Summary of actions of noradrenaline (*A* and *B*), somatostatin (*C*) and [Met^5]enkephalin (*D*) applied to outside-out membrane patches containing one or more leak K^+ channel. *A* shows average probability density distributions for each channel size in control and in the presence of maximum concentrations of noradrenaline (3 or 10 μM) or UK 14304 (600 nM). *B* shows data from *A* replotted as percentage increase over control value; asterisk indicates significantly different from intermediate and large amplitude channel values. *C* and *D*, data expressed as in *B* for experiments using somatostatin (200 nM) or [Met^5]enkephalin (3 or 10 μM). Numbers in parentheses are number of patches examined.

0·003–0·008) recorded 5–10 min after patch excision were all in the lowest range recorded from control patches. Neither noradrenaline nor somatostatin had any effect when GDP-β-S was in the pipette.

DISCUSSION

Background potassium channel activity

The potassium channels distinguished by their unitary conductances fell into three groups: small (30–65 pS), intermediate (120–160 pS) and large (220–280 pS) in symmetrical potassium concentrations. The small conductance channel overwhelmingly predominated, occurring in more than 90% of recordings. The wide range in

unitary current amplitude within this 'small amplitude' group can presumably be divided into several further subclasses that have not been distinguished. Such large variability in current amplitudes from patch to patch is probably not an artifact of patch excision because (a) a similar range of unitary potassium current amplitudes was seen in cell-attached recordings (unpublished observations) and (b) no similar variability was observed in the amplitudes of unitary voltage-dependent calcium currents (Shen & Surprenant, 1991) or 5-HT-activated cation currents (Derkach, Surprenant & North, 1989) recorded under similar conditions. On the other hand, in respects such as block by barium and caesium, weak voltage dependence, open and closed time distributions, insensitivity to tetraethylammonium, and activation by agonists (see below), the three classes were not distinguishable, and this raises the possibility that they represent subconductance levels of a single molecular species of potassium channel. The main evidence against this, though formally inconclusive (see Colquhoun & Hawkes, 1990), was the observation of patches which showed during long recordings (30–60 min) only a single unitary current amplitude (Fig. 1).

Are the properties of the unitary potassium currents observed consistent with the notion that they contribute to the resting potassium currents of submucous plexus neurones recorded with microelectrodes under more physiological circumstances (Surprenant & North, 1988)? In the single-channel recordings, calcium-dependent potassium currents should be blocked by the absence of external calcium and the buffered intracellular solution used, and TEA-sensitive currents were seen only at positive membrane potentials (Fig. 4). The block of inward current by barium and caesium is similar to the action on the inwardly rectifying potassium conductance that operates in the intact cell in the potential range -60 to -120 mV (extracellular potassium 5 mM). Furthermore, it is this inwardly rectifying potassium conductance that is increased by the agonists in the whole-cell recordings (Surprenant & North, 1988).

On the other hand, the single-channel properties do not obviously account for inward rectification in the whole-cell studies. The probability of opening increased with positive potentials, a change opposite to that required to account for whole-cell rectification. When the extracellular potassium concentration was changed, unitary currents showed no rectification other than that predicted from the constant-field assumptions. The inwardly rectifying potassium conductance of heart muscle continues to show single-channel rectification in cell-attached membrane patches (Sakmann & Trube, 1984) and later recordings showed that this results from block of outward current by cytoplasmic magnesium ions (Matsuda, Saigusa & Irisawa, 1987; Vandenberg, 1987). In the present experiments, there was no obvious effect of different magnesium concentrations within the pipette, at the intracellular aspect of the patch. It is possible that inward rectification in the whole cell results from current through another set of channels, perhaps undetected due to their small amplitude, or washing out after patch excision (see McCloskey & Cahalan, 1990). In this respect, of interest are recent reports demonstrating significant changes in the properties of ion channels as a result of patch excision (Milton & Caldwell, 1990; Morris & Horn, 1991).

Although effects of agonists on potassium channels absolutely required GTP (see Results and below), basal activity of resting potassium channels showed no obvious

dependence on the presence of GTP in the pipette solution. That is, there were no consistent differences (for periods up to 1 h) in unitary amplitudes, numbers of potassium channels active per patch or probability of opening of individual channels whether GTP was included or omitted from the recording pipette. The S channel in *Aplysia*, which comprises greater than 70% of the resting membrane conductance of the cell, is also active in cell-free (inside-out) membrane patches without any requirement for GTP (Siegelbaum, 1987). Our results differ from those reported by VanDongen and colleagues (VanDongen *et al.* 1988) for cultured hippocampal neurones where no channel activity was observed in excised, inside-out membrane patches until GTP, GTP-γ-S or purified G proteins were applied to the inner surface of the membrane, at which time one of four distinct potassium channels with unitary conductances between 12 and 55 pS appeared. Similarly, the mammalian cardiac I_{K1} channel quickly becomes inactive when an inside-out patch is obtained unless GTP is present at the internal surface (Trube & Hescheler, 1984) although amphibian I_{K1} channels show no such GTP dependence (Clark, Nakajima, Giles, Kanai, Momose & Szabo, 1990). It will be important to extend these types of studies in submucosal as well as other neurones known to possess one of the family of pertussis-sensitive G protein-coupled receptors.

Agonists increase on-going activity of resting K^+ channels

A striking finding was that noradrenaline and the other agonists increased channel activity of potassium channels already active prior to their application; the agonist never recruited a channel of novel conductance, nor was it particularly selective in increasing the probability of opening of any active potassium channel. This is in distinct contrast to muscarinic actions studied in detail with cell-attached and inside-out recordings in heart: there, acetylcholine (ACh) increases activity of the 40–55 pS K_{ACh} channel without affecting single I_{K1} channels even when both are active in the same patch in the absence of agonist. Further, when no channel activity is present or I_{K1} channels alone are tonically active, ACh induces the appearance of the K_{ACh} channel (Sakmann *et al.* 1983; Soejima & Noma, 1984). Similarly, a single 55 pS potassium channel has been observed in locus coeruleus neurones (Miyake, Christie & North, 1989) and in GH3 pituitary tumour cells (Yatani, Codina, Sekura, Birnbaumer & Brown, 1987) only when agonist is present in the pipette during on-cell recordings; in both cases a smaller amplitude potassium channel ($\sim$ 30 pS) was unaltered by the presence or absence of agonist. In all of these studies, as well as in our study, the presence of GTP at the inside surface of the membrane was essential for agonist effect; these observations are in keeping with the grander body of evidence linking G proteins to the coupling of inwardly rectifying potassium channels by this family of inhibitory receptors (North, 1989; Brown & Birnbaumer, 1990).

In every membrane patch in which activation of α_2-adrenoceptors was effective in increasing single-channel activity, so too was activation of somatostatin receptors, echoing a similar 1:1 correspondence observed during intracellular microelectrode and whole-cell studies on submucosal neurones (Surprenant & North, 1988; Tatsumi *et al.* 1990). Conversely, when noradrenaline did not increase channel activity in excised patches, then neither did somatostatin or enkephalin. Agonists were usually effective in membrane patches containing more than one tonically active potassium channel and almost always ineffective when only a single potassium channel was

active prior to agonist application. Patches in which only a single channel was active were generally obtained with higher resistance electrodes and may have been smaller. It is conceivable that such patches lacked receptors if significant clustering occurs. Alternatively, the concentration of G protein at the cytoplasmic surface of the membrane might become critically low in small excised patches, a hypothesis that could be tested by adding exogenous G protein to the pipette solution.

Conclusions

Brown and his colleagues (see Brown & Birnbaumer, 1990) have marshalled extensive evidence that G proteins can activate a variety of ion channels when applied to the inner surface of excised membrane patches; some channels are voltage dependent and others are not. It therefore becomes important to understand which receptors access *which* channels under physiological circumstances, and how this can be controlled. In a general sense, the present findings argue against specificity in receptor–channel coupling at the level of a membrane patch: we did not find, for example, that somatostatin opened only large conductance channels and noradrenaline opened only smaller conductance channels. The corollary of this convergence of several receptors onto a common channel is that specificity is conferred by controlling the level of receptors and channels present in the membrane rather than by 'hard-wired' molecular pathways from given receptor to given channel.

The divergence of signal from a receptor to not one but several, presumably distinct molecular species of potassium channels could reflect the importance of this signalling pathway for normal cell function. Noradrenaline released from sympathetic nerves exerts a tonic inhibitory influence on the activity of submucosal plexus neurones, without which there would be fluid and electrolyte loss from the intestinal mucosa. It does this by increasing the probability of opening of several types of potassium channels that are in any case contributing to the polarization of the neurone.

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