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**Article:**

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Non-genomic regulation of intermediate conductance potassium channels by aldosterone in human colonic crypt cells

K A Bowley, M J Morton, M Hunter, G I Sandle

Background: Aldosterone has a rapid, non-genomic, inhibitory effect on macroscopic basolateral K+ conductance in the human colon, reducing its capacity for Cl- secretion. The molecular identity of the K+ channels constituting this aldosterone inhibitable K+ conductance is unclear.

Aim: To characterise the K+ channel inhibited by aldosterone present in the basolateral membrane of human colonic crypt cells.

Methods: Crypts were isolated from biopsies of healthy sigmoid colon obtained during colonoscopy. The effect of aldosterone on basolateral K+ channels, and the possible involvement of Na+\textendash:H+ exchange, were studied by patch clamp techniques. Total RNA from isolated crypts was subjected to reverse transcriptase-polymerase chain reaction (RT-PCR) using primers specific to intermediate conductance K+ channels (KCNN4) previously identified in other human tissues.

Results: In cell attached patches, 1 mmol/l aldosterone significantly decreased the activity of intermediate conductance (27 pS) K+ channels by 31%, 53%, and 54% after 1, 5 and 10 minutes, respectively. Increasing aldosterone concentration to 10 mmol/l produced a further 56% decrease in channel activity after five minutes. Aldosterone 1–10 mmol/l had no effect on channel activity in the presence of 20 µmol/l ethylisopropylamiloride, an inhibitor of Na+\textendash:H+ exchange. RT-PCR identified KCNN4 mRNA, which is likely to encode the 27 pS K+ channel inhibited by aldosterone.

Conclusion: Intermediate conductance K+ channels (KCNN4) present in the basolateral membranes of human colonic crypt cells are a target for the non-genomic inhibitory effect of aldosterone, which involves stimulation of Na+\textendash:H+ exchange, thereby reducing the capacity of the colon for Cl- secretion.
patients undergoing colonoscopy or flexible sigmoidoscopy for investigation of altered bowel habit and/or abdominal pain.

None of the patients was receiving medication at the time of the procedure. Patients undergoing colonoscopy had received Klean-Prep (Norgine, Harefield, UK) during the preceding 24 hours, and those undergoing flexible sigmoidoscopy received Fleet ready to use enema (De Witt, Runcorn, UK) before the procedure. Routine histology was normal in all cases. The study was approved by Leeds Health Authority Ethics Committee. Intact crypts were isolated by a modification of a procedure. Patients undergoing colonoscopy had received Klean-Prep (Norgine, Harefield, UK) during the preceding 24 hours, and those undergoing flexible sigmoidoscopy received Fleet ready to use enema (De Witt, Runcorn, UK) before the procedure. Routine histology was normal in all cases. The study was approved by Leeds Health Authority Ethics Committee. Intact crypts were isolated by a modification of a

Figure 1 Photomicrograph of intact colonic crypts isolated by Ca²⁺ chelation and visualised using Hoffman modulated optics (×400).

Note that the crypts were isolated devoid of non-epithelial cells originating from the lamina propria. The luminal opening and base of each crypt can be clearly distinguished. Arrows indicate the continuous layer of crypt epithelial cells (A) and the crypt lumen (B).

Reverse transcriptase-polymerase chain reaction (RT-PCR) studies

Colonic crypts were pelletted by centrifugation and total RNA was extracted using the guanidium thiocyanate-acid phenol-chloroform method of Chomczynsky and Sacchi,12 and resuspended in RNase free water. Total RNA was reverse transcribed at 37°C using Superscript I reverse transcriptase with oligo (dT)₁₅ primers according to the manufacturer’s instructions (Invitrogen, Paisley, UK). To control for possible genomic DNA contamination, reverse transcription was also performed in the absence of Superscript. Oligonucleotide primers were also designed to span intron/exon boundaries so as to generate a differently sized PCR product when amplified from genomic or complementary DNA (cDNA). cDNA was subsequently used as a template for two rounds of PCR with primers specific for human KCNN4 (GenBank accession No AF000972). Each PCR was performed with 2.5 U of Taq DNA polymerase (prepared inhouse) with the following cycling parameters: 30 seconds at 94°C, 45 seconds at 58°C, and 30 seconds at 72°C. The first 35 cycles generated PCR products of 830 base pairs (sense 5'-CAG AGA TGC TGT GGT TCG GGG-3' and antisense 5'-CTT GTA GCA CTC GGG CAG CGG-3'). To increase the amplification, a second round of 35 cycles was performed which generated PCR products of 761 base pairs (sense 5'-TTT CCA CCT TCT TAC TTC TCC GCC-3' and the above antisense primer). A human placental cDNA library amplified with sense and antisense primers to KCNN4 served as a positive control for PCR. PCR products were separated by agarose gel electrophoresis, visualised by UV illumination, and photographed using the Kodak digital science documentation and analysis system. Wide range DNA marker (Sigma, Poole, UK) was run with each gel for molecular weight comparison. PCR products were isolated from the gel (Gene Clean protocol) and identified by automated fluorescence sequencing (Lark Technologies, Saffron Walden, UK). The resulting sequence was aligned with that in GenBank (AF000972) using the CLUSTAL X algorithm.

Statistical analysis

Results are expressed as mean (SEM). Statistical analyses were performed using the Student’s t test where p<0.05 was considered significant.
RESULTS
Properties of basolateral K⁺ channels in human colonic crypts

We have previously described Ba²⁺ blockable Ca²⁺ sensitive intermediate conductance K⁺ channels in the basolateral membrane of human colonic crypts which were present in approximately 75% of patches. In this study, similar channels were observed in 58/113 (51%) cell attached basolateral membrane patches in the mid third of isolated human colonic crypts. With NaCl solution in the bath and KCl solution in the pipette, inward currents were seen at the resting membrane potential (zero holding voltage) in all cell attached basolateral membrane patches, and linear current-voltage relationships indicated a mean slope conductance and reversal potential of 27 (1) pS and 28 (6) mV, respectively (n=15), and channel activity was voltage independent between −80 mV and −20 mV. Curvilinear current-voltage relationships were seen after excision of the patches into the inside out configuration (n=11) from which the K⁺:Na⁺ permeability ratio (Pₒ/Pᵢ) and reversal potential were calculated (41 (3):1 and 74 (1) mV, respectively), using the Goldman-Hodgkin-Katz current and voltage equations. When inside out patches were exposed to KCl solution in the bath and pipette, the current-voltage relationships indicated inward rectification of the K⁺ channel, single channel conductance being greater at −60 mV than at +60 mV (29 (2) pS vs 12 (2) pS; p<0.0008, n=7).

Non-genomic inhibition of basolateral K⁺ channels by aldosterone

In cell attached basolateral membrane patches (holding voltage −40 mV), the addition of 1 nmol/l aldosterone to the bath solution rapidly decreased spontaneous 27 pS K⁺ channel activity, and this decreased further when the concentration of aldosterone was increased to 10 nmol/l (fig 2A). Data from 11 patches are summarised in fig 2B which shows that adding 1 nmol/l aldosterone decreased Pₒ from 0.59 (0.06) to 0.41 (0.07) (by 31%) after one minute (p<0.004), to 0.28 (0.07) (by 53%) after five minutes (p<0.0005 compared with basal values), and to 0.27 (0.04) (by 54%) after 10 minutes (p<0.0005 compared with basal values). In seven patches, increasing aldosterone concentration to 10 nmol/l decreased Pₒ further from 0.32 (0.02) to 0.14 (0.05) (by a further 56%) after five minutes (p<0.01), a low level of activity which was maintained for an additional 10 minutes. In six cell attached patches not exposed to aldosterone, which served as time controls, channel activity did not change significantly during the 25 minute recording period (Pₒ 0.58 (0.12) at time zero, Pₒ 0.57 (0.16) after 25 minutes; NS).

Single channel current amplitude decreased significantly from 1.86 (0.30) pA to 1.46 (0.29) pA (p<0.015) 10 minutes after addition of 1 nmol/l aldosterone, suggesting depolarisation of the cell membrane. In contrast, in the time control studies, there was no change in current amplitude after 10 minutes (1.77 (0.27) pA v 1.71 (0.32) pA; NS). Cell membrane depolarisation induced by aldosterone was consistent with inhibition of basolateral K⁺ channels.

Close inspection of fig 2A reveals that aldosterone decreased the apparent number of K⁺ channels in the patch. Thus the mineralocorticoid may stimulate recycling of channels into a pool of cytoplasmic vesicles rather than have a direct inhibitory effect on channels located in the basolateral membrane. However, as shown in fig 3, in five patches containing a single K⁺ channel, 10 nmol/l aldosterone decreased channel activity rather than abolishing it entirely, which is consistent with inhibition of a single K⁺ channel by aldosterone.
with direct inhibition of the channels rather than stimulation of channel recycling.

**Effect of EIPA on K⁺ channel inhibition by aldosterone**

In a separate series of experiments, crypts were pretreated with 20 µmol/l EIPA, a specific Na⁺:H⁺ exchange inhibitor, which had no effect on channel activity in cell attached basolateral membrane patches after five minutes at a holding voltage of −40 mV (P̄o 0.53 (0.12) vs P̄o 0.58 (0.10); n=6, NS). As shown in fig 4A, the subsequent addition of 1–10 nmol/l aldosterone had no effect on 27 pS K⁺ channel activity in the continuing presence of EIPA. Data from six experiments, summarised in fig 4B, indicate that channel activity was unchanged by 1 nmol/l aldosterone (P̄o 0.59 (0.08) after one minute, 0.63 (0.07) after five minutes, and 0.54 (0.08) after 10 minutes). In four patches, the inhibitory effect of 10 nmol/l aldosterone was also prevented by EIPA (P̄o 0.55 (0.14) after one minute, 0.54 (0.16) after five minutes, and 0.54 (0.20) after 10 minutes). Single channel current amplitude in the presence of EIPA was similar before and 10 minutes after addition of 1 nmol/l aldosterone (1.80 (0.38) pA and 2.04 (0.36) pA, respectively; NS), indicating that EIPA prevented the cell membrane depolarisation and decrease in current observed when aldosterone was added in the absence of EIPA (see above).

An additional set of experiments was performed to determine whether prolonged exposure to EIPA alone affected channel activity. In five cell attached patches, channel activity was similar before and 25 minutes after exposure to 20 µmol/l EIPA (P̄o 0.54 (0.07) and 0.55 (0.01), respectively; NS). Thus EIPA per se had no effect on the activity of the 27 pS K⁺ channel during the time course of these experiments.

**pH sensitivity of basolateral K⁺ channels**

As shown above, EIPA prevented the non-genomic inhibitory effect of aldosterone on the 27 pS K⁺ channels, which suggests
that aldosterone stimulates Na⁺:H⁺ exchange. This would have the effect of increasing intracellular pH, raising the possibility that cellular alkalinisation causes K⁺ channel inhibition. To investigate this further, recordings were obtained from excised inside out patches bathed in an “intracellular” high K⁺ solution at different values of pH between pH 6.8 to 7.6. Figure 5 shows that the 27 pS K⁺ channel was moderately pH sensitive, Pᵢ in eight patches increasing from 0.15 (0.06) at pH 6.8 to 0.37 (0.07) at pH 7.6 (p<0.04). These data indicate that alkalisation alone causes stimulation rather than inhibition of the 27 pS K⁺ channel.

Detection of KCNN4 mRNA in human colonic crypt cells
To investigate whether KCNN4 is expressed in colonic epithelial cells, total RNA was extracted from a human colonic crypt isolate which was devoid of all non-epithelial cell types such as fibroblasts, endothelial cells, and lamina propria cells (fig 1). Using primers specific to human KCNN4, RT-PCR (performed in triplicate) produced a single 761 base pair product (fig 6) which when sequenced showed 100% homology with human placental KCNN4 (GenBank accession No AF000972). These data confirm that KCNN4 mRNA is expressed in human colonic epithelial cells.

DISCUSSION
Mammalian distal colon is a major target epithelium for the genomic effects of aldosterone, which include stimulation of electrogenic Na⁺ absorption and an electrogenic K⁺ secretory process. These changes appear within hours of type 1 mineralocorticoid receptor activation although the full transport effects of sustained aldosterone stimulation occur after 2–3 days. Aldosterone also has non-genomic effects on colonic ion transport, increasing intracellular Ca²⁺ and stimulating protein kinase C activity in rat and human colonic epithelia. These effects are associated with an increase in Na⁺:H⁺ exchange that leads to intracellular alkalinisation. It has been suggested that the non-genomic effects of aldosterone inhibit the macroscopic basolateral, Ca²⁺ sensitive, basolateral K⁺ conductance involved in Cl⁻ secretion, although this K⁺ conductance was not characterised at the single channel level. The present study provides the first indication that 27 pS basolateral K⁺ channels in human colonic crypt cells constitute the aldosterone inhibitable macroscopic basolateral K⁺ conductance in this epithelium. Aldosterone at low concentration (1 mmol/l) produced significant and sustained channel inhibition after one minute, which was even more marked at higher concentrations (10 mmol/l) (fig 2). This effect was too rapid to be accounted for by changes in gene transcription or translation of mRNA. It is also unlikely to have reflected stimulation of K⁺ channel recycling because limited channel activity persisted (rather than disappearing entirely) in patches containing a single K⁺ channel after addition of aldosterone (fig 3). The non-genomic inhibitory effect of aldosterone on colonic Cl⁻ secretion seems to be a direct consequence of downregulation of Ca²⁺ sensitive basolateral K⁺ channels, which are likely to be the inwardly rectifying 27 pS K⁺ channels we have identified in human colonic crypt cells. Similar basolateral K⁺ channels exist in Tₘ cells and rat colonic crypts, where they play a critical role in the Cl⁻ secretory process. Their inhibition leads to a reduction in K⁺ recycling across the basolateral membrane (thus decreasing cellular Cl⁻ uptake by Na⁺:K⁺:2Cl⁻ cotransport), and failure to maintain the cell hyperpolarisation necessary for sustained Cl⁻ exit via apical Cl⁻ channels. The rapid inhibitory effect of aldosterone on Ca²⁺ dependent macroscopic basolateral K⁺ conductance in the rat and human colon has been linked to stimulation of protein kinase C sensitive Na⁺:H⁺ exchange and a rise in cytosolic pH. Aldosterone has a variety of non-genomic effects on signalling pathways in non-epithelial cells, changing intracellular ion concentrations, cell volume, and Na⁺:H⁺ exchange in human mononuclear leucocytes, Na⁺:H⁺ exchange in vascular smooth muscle cells, increasing intracellular Ca²⁺ in vascular smooth muscle cells and porcine aortic endothelial cells, and enhancing phosphoinositide hydrolysis in human mononuclear leucocytes and vascular smooth muscle cells. In the present study, the presence of EIPA prevented the inhibitory effect of aldosterone on the 27 pS K⁺ channels, which suggests that aldosterone stimulated Na⁺:H⁺ exchange and caused intracellular pH to rise. Aldosterone has been shown to produce a rapid increase in intracellular pH from 7.11 to 7.32 in isolated human colonic crypts, the implication being that cellular alkalinisation is linked in some way to inhibition of the Ca²⁺ dependent macroscopic basolateral K⁺ conductance, which we believe is equivalent to 27 pS K⁺.
channels. However, our studies with excised inside-out patches indicate that alkalinisation alone stimulates these K⁺ channels (Fig 5). Thus it seems that intracellular alkalinisation per se does not mediate the non-genomic inhibition of aldosterone of 27 pS basolateral K⁺ channels although it is possible that another pH sensitive signalling pathway may be involved.

The second aspect of this study was the molecular characterisation of 27 pS basolateral K⁺ channels. The biophysical and pharmacological properties of these K⁺ channels in human and rat colonic crypts, and in Tc₆₅ cells, are similar to the IKca channel cloned from cDNA libraries of a variety of tissues. The IKca channel shows inward rectification, is extremely sensitive to Ca²⁺, binds calmodulin to the C terminus of each of its four subunits, and has minimal voltage sensitivity. In Cl⁻ secreting epithelia such as the lung and colon, the role of IKca channels is to hyperpolarise the cells and maintain the drive for electrogenic Cl⁻ secretion. Using specific primers for human KCNN4, RT-PCR confirmed the presence of KCNN4 mRNA in human colonic crypts (Fig 6). KCNN4 mRNA has previously been identified by RT-PCR in mucosal scrapings of rat and human colon and in malignantly transformed Tc₆₅ cells. The crypt isolation method we used has the advantage of providing non-malignant human colonic epithelial cells devoid of red blood cells, lymphoid cells, fibroblasts, endothelial cells, and smooth muscle cells (Fig 1), all of which express IKca (KCNN4) channels. It therefore seems reasonable to assume that the KCNN4 mRNA originated from colonic crypt cells. We cannot exclude the possibility that apical IKca channels may also be present in human colonic crypt cells (adherent mucus prevented single channel recording from the apical membrane in crypts opened longitudinally) but we have only ever seen high conductance (220 pS) K⁺ channels in the apical membrane of surface cells around the luminal openings of human colonic crypts (and unpublished data). Thus we take the view that KCNN4 mRNA encodes 27 pS basolateral K⁺ channels in human colonic crypt cells, which are identical to the IKca channels found in many other epithelial and non-epithelial cell types.

In summary, nanomolar concentrations of aldosterone exert a non-genomic inhibitory effect on 27 pS K⁺ channels in the basolateral membranes of human colonic crypt cells, thereby reducing the capacity of the colon for Cl⁻ secretion. Although aldosterone has an associated non-genomic stimulatory effect on Na⁺-H⁺ exchange (which increases intracellular pH), our data suggest that alkalinisation in isolation enhances rather than inhibits the activity of these K⁺ channels, and the exact role of Na⁺-H⁺ exchange remains unclear. Nevertheless, these channels have similar biophysical properties to KCNN4 channels present in a variety of other human tissues. Having identified KCNN4 mRNA transcripts in these colonic crypt cells, we conclude that KCNN4 encodes 27 pS K⁺ channels residing in the basolateral (but not the apical) membrane domain.

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Authors' affiliations

K A Bowley, G I Sandle, Molecular Medicine Unit, St James’s University Hospital, Leeds, UK
M J Morton, M Hunter, Department of Biomedical Sciences, University of Leeds, Leeds, UK

REFERENCES


35 Dunn PM. The action of blocking agents applied to the inner face of Ca2+-activated K+ channels from human erythrocytes. J Membrane Biol 1998; 165:133–43.

