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Enhancement of conductive anion permeability in cultured cells by cetiedil

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Cetiedil, a drug that is reported to block K^+-channels, substantially increases the conductive Cl^- permeability of Chinese hamster ovary (CHO) cells. The permeability was monitored by volume changes in cells treated with gramicidin to increase the cation permeability. Under this circumstance, increases in Cl^- conductances result in volume changes detectable by electronic sizing, with the direction determined by the gradients of the permeating ions. In NaCl or KCl media, swelling occurs, but in *N*-methylglucamine chloride, shrinking. The increases in Cl^- conductance could also be measured as an increased $^{36}Cl^-$ flux or by changes in membrane potential (measured by fluorescence of a potential-sensitive dye) toward the Cl^- equilibrium potential. The effect of cetiedil was concentration dependent, with maximal effect at 50 μM. The anion specificity for the conductance was $NO_3^- > Cl^- = Br^- \gg SO_4^{-2}$ or isethionate. A number of other drugs that influence transport activities had no effect on Cl^- conductance. The cetiedil effect on Cl^- conductance was observed in one other cell line, but was absent in several other cell types. The cetiedil-induced Cl^- conductance in CHO cells appears to involve a different pathway than that induced by exposure to hypotonic medium.

Introduction

During studies of the action of the drug cetiedil on volume changes of Chinese hamster ovary (CHO) cells, we were surprised to note the induction of a substantial increase in conductive chloride permeability. The drug is known to act as a blocker of K^+-channels [1,2], in keeping with its chemical structure as a tertiary amine. At the clinical level it is a peripheral vasodilator [3] and a potential anti-sickling agent [4].

In salt-secreting and -reabsorbing epithelia, Cl^- channels are activated by various secretagogues via the cyclic AMP system [5,6], a system that appears to be deficient in cystic fibrosis [7,8]; in several suspended cell types, such as lymphocytes, Ehrlich ascites, and CHO, Cl^- conductances can be activated by hypotonic shock [9–11], or in some cases by Ca^{2+} loading; and in certain nerve pathways, synaptic conductances appear to involve activation of Cl^- channels [12]. The relationship, if any, between these various Cl^- conductance pathways is not clear. Activation by drugs has not been widely reported.

In the present study we have characterized the cetiedil-induced Cl^- pathways in CHO cells by measuring volume changes, $^{36}Cl^-$ fluxes and membrane potentials. We have also examined the effects of cetiedil in several other types.

Abbreviations: cetiedil, 2-(hexahydro-1*H*-azepin-1-yl)ethyl α-cyclohexyl-3-thiophenacetate; CHO, Chinese hamster ovary; 2-[^{3}H]dGlc, 2-[^{3}H]deoxyglucose; di-b-cAMP, N^6-2′-*O*-dibutyryladenosine 3′:5′-(cyclic)monophosphate sodium salt; DMSO, dimethylsulfoxide; E_m, membrane potential; $t_{1/2}$, half-time.

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Materials and Methods

Cetiedil ethyl ester was a kind gift from Dr. Gerrard Pilley (Innothera Pharmaceuticals, Arcueil, France). Bisoxonol was from Molecular Probes. Verapamil was from Knoll Pharmaceuticals, Canada. The following compounds were from Sigma Chemical Co: quinine sulfate, trifluoperazine dihydrochloride, procaine hydrochloride, A23187 ionophore, di-b-cAMP, gramicidin D. Chlorpromazine sulfoxide · HCl was from Smith Kline & French Labs, Philadelphia, PA. All of the compounds with the exception of verapamil and di-b-cAMP were dissolved as 100 mM stock solutions in DMSO. $Na^{36}Cl$ and tritiated 2-deoxyglucose were from ICN Radiochemicals.

Solutions. The various media used contained as major salts NaCl, KCl, *N*-methylglucamine chloride, NaBr, $NaNO_3$, and sodium isethionate at 140 mM or Na_2SO_4 at 100 mM concentration. All media also contained 1 mM KCl 0.5 mM $CaCl_2$, 0.5 mM $MgSO_4$ 10 mM D-glucose and 20 mM Hepes. The pH was adjusted to pH 7.4 and osmolarity to 280–290 mosM.

Cells. CHO cells (wild-type, Toronto, strain) were cultured in suspension and harvested by centrifugation [11]. Viability of the cells was assessed by Trypan blue dye exclusion and was invariably higher than 90%. Rat thymic lymphocytes were obtained from Dr. J. Dixon (The Hospital for Sick Children, Toronto). L6H9 rat skeletal muscle cells, and C6 rat glioma cells were from Dr. A. Klip (The Hospital for Sick Children, Toronto), and LLC-PK_1, pig kidney cells from Dr. K. Skorecki (Banting Institute, University of Toronto). All cells except lymphocytes were released into suspension by trypsinization, and allowed to recover for 1 h in RPMI-1640 medium at 37°C prior to use. Viability exceeded 85% in all cases.

Cell volume measurements. Changes in cell volumes were measured by electronic sizing using a Coulter counter equipped with a channelyzer, as described previously for CHO cells and lymphocytes [9,11]. The average cell volume varied between 967 and 1033 μm^3 depending on the cell batch and the suspending medium, and was taken as the initial volume. Experiments were initiated by suspending approx. 10^5 cells in 20 ml of medium at 37°C, followed by the sequential addition of the required reagents (completed within 30 s).

Conductive chloride flux measurement. The procedure is based on measurements of the rate of change in cell volume, using the Coulter counter system (as described above), under conditions in which the conductive chloride flux is the limiting factor in salt movement across the membrane. The method has previously been applied to lymphocytes [9] and to CHO cells [11]. Briefly, the antibiotic gramicidin is added to induce a high membrane permeability to Na^+ and K^+. Cells will tend to shrink or swell, depending on the direction of the ion gradients. Because the conductive anion permeability is rate-limiting under these circumstances, this parameter can be calculated from the rate of volume change. The direction of the volume change can be manipulated by ion substitution. In the presence of a gramicidin-impermeant cation such as the *N*-methylglucamine cation, the net salt gradient is outward, so the cells tend to shrink, whereas in the presence of gramicidin-permeant cations such as Na^+ or K^+, the cells tend to swell. In shrinking experiments the permeating anion is primarily cellular Cl^-. In swelling experiments, the permeabilities of other anions can be tested by substituting them in the medium.

^{36}Cl / 2-[^{3}H]dGlc efflux measurements. CHO cells were loaded with ^{36}Cl and 2-[^{3}H]dGlc by incubation for 1 h at room temp. in bicarbonate-free RPMI-1640 medium buffered with 20 mM Hepes to pH 7.4, at $6 \cdot 10^6$ cells/ml, containing 13.3 μCi/ml ^{36}Cl (sodium salt) and 3.3 μCi/ml 2-[^{3}H]dGlc. The cells were then rapidly washed once in Na_2SO_4 medium in an Eppendorf microcentrifuge. The flux was initiated by dilution of the cells (final density approx. $2 \cdot 10^6$ cells/ml) into Na_2SO_4 medium at room temperature, with or without gramicidin, cetiedil, or the vehicle DMSO. At the indicated times 0.5 ml aliquots of the cell suspension were removed and centrifuged for 5 s in a microcentrifuge through 0.6 ml of dibutylphthalate/vegetable oil (10:2, v/v). The cell pellets were dispersed in 0.1 ml NaCl medium and counted with a double-label $^3H/^{14}C$ program in an LKB Wallac scintillation counter.

Determination of the transmembrane potential. The fluorometric method for the determination of

transmembrane potential (E_m) described by Rink et al. [13] was employed, using a Perkin-Elmer 650-40 spectrofluorometer. CHO cells were suspended at $7 \cdot 10^5$ cells/ml in appropriate media, bisoxonol was added to a final concentration of 0.2 μM and fluorescence was recorded at an excitation of 540 nm and emission of 580 mm until stable. Calibration was done with cells suspended in solutions of decreasing Na^+ content (isosmotically substituted by *N*-methylglucamine) in the presence of the Na^+/K^+ ionophore, gramicidin. The membrane potential of cells suspended in NaCl solution containing 40 nM gramicidin was taken as zero. The resting membrane potential was estimated at -52 mV, based on intracellular concentrations of Na^+ and K^+ of 13 and 136 mM, respectively, as previously determined by flame photometry [11]. To determine the Cl^- content, CHO cells were equilibrated for 1.5 h with ^{36}Cl. Then the cells were separated from the medium by sedimentation through oil as described for the efflux experiments, and the trapped extracellular space was estimated with [^{14}C]poly(ethylene glycol), M_r 4000 (New England Nuclear). The Cl^- concentration by this method was 29 ± 3 mmol per liter of cell water.

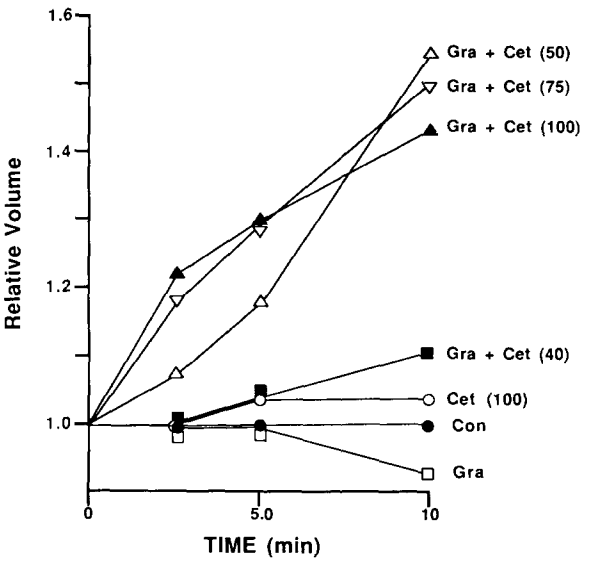

Fig. 1. Changes in CHO cell volume induced by cetiedil and gramicidin in NaCl medium. CHO cells were suspended in NaCl medium containing 140 mM NaCl, 1 mM KCl, 0.5 mM $CaCl_2$, 0.5 mM $MgSO_4$, 10 mM glucose, 20 mM Hepes (pH 7.4) at 37°C. Gramicidin (0.5 μM) was added to the cell suspension, followed by cetiedil at different concentrations. Controls received DMSO only (DMSO is the solvent for gramicidin). Control (●); 0.5 μM gramicidin (□); gramicidin + 40 μM cetiedil (■); gramicidin + 50 μM cetiedil (△); gramicidin + 75 μM cetiedil (▽); gramicidin + 100 μM cetiedil (▲); cetiedil alone (○). Data are representative of three similar experiments.

Results

Anion-dependent cell volume responses

When CHO cells are suspended in isotonic saline containing the Na^+/K^+ ionophore gramicidin, they undergo little change in cell volume (Fig. 1, [11]). The cell volume after a 10 min exposure to 0.5 μM gramicidin alone was 0.99 ± 0.07 ($n = 8$) relative to control. Since the intracellular Cl^- concentration is only about 29 mM, the absence of swelling in 140 mM NaCl under these conditions is indicative of a relatively low Cl^- conductance. On the other hand, addition of cetiedil in conjunction with gramicidin resulted in rapid swelling (Fig. 1). The effect was dose- and time-dependent. At 40 μM cetiedil a slight swelling was usually observed after a short lag period, while at 75 or 100 μM, no delay was observed and the rate of swelling was about 5% per min relative to the initial volume. With 75 μM cetiedil and 0.5 μM gramicidin the cell volume after 10 min was 1.49 ± 0.09 ($n = 7$). Cetiedil alone at concentrations up to 100 μM had little effect, the cell volume after a 10 min incubation was 1.03 ± 0.06 ($n = 8$) relative to control.

The responses of cells suspended in KCl media were essentially similar. Gramicidin alone had no effect on volume, but when added together with cetiedil (75 μM) swelling resulted that was comparable in rate to that found with NaCl (not shown). As expected, valinomycin (1 μM), a specific K^+-ionophore, produced effects similar to those found with gramicidin: no effect when added alone, but swelling when added with cetiedil. The rate of swelling was, however, about half that with gramicidin. One minor difference was found in KCl medium. The effect of cetiedil alone was somewhat greater. In NaCl medium, a small swelling was noted of about 4% after 5 min (Fig. 1), whereas in KCl medium the swelling was some-

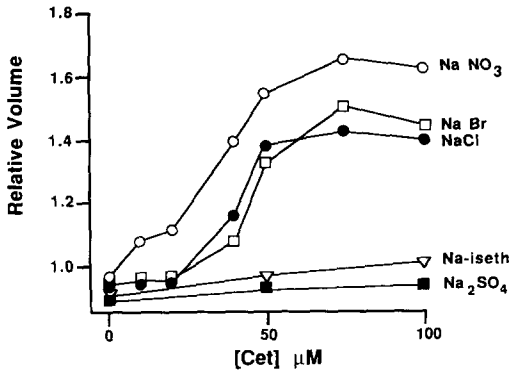

Fig. 2. Effect of anion substitution on the CHO cell volume response to cetiedil. CHO cells were suspended in various media containing Cl^-, Br^-, NO_3^-, SO_4^{-2} or isethionate as the major anion. Gramicidin (0.5 μM) was included in each case, and cetiedil was added to obtain the desired concentration. Cell volume was determined after incubation for 10 min at 37°C. The major salt in various media was: NaCl (●); NaBr (□); $NaNO_3$ (○); Na_2SO_4 (■); sodium isethionate (▽). Results are average of two separate experiments.

what greater, 7.5% after 5 min (data not shown).

The concentration dependence of the cetiedil-gramicidin-induced swelling is illustrated in Fig. 2 for a series of anions. Little or no swelling occurred in Na_2SO_4 or sodium isethionate; the swelling was most rapid in $NaNO_3$; a threshold concentration of over 20 μM was required in the case of NaCl and NaBr; and maximal effects were observed at about 70 μM concentration.

In a medium in which Na^+ was replaced by the impermeant cation *N*-methylglucamine, normal cells shrink slowly (Fig. 3). Cetiedil alone had no effect; gramicidin alone increased the rate of shrinkage somewhat; however, cetiedil plus gramicidin substantially increased the rate and magnitude of the response.

The effect of cetiedil plus gramicidin on Cl^- conductivity was also assessed by measurements of ^{36}Cl efflux from cells preloaded with ^{36}Cl (Fig. 4). The efflux was quite rapid in NaCl, with a half-time ($t_{1/2}$) of 9.8 min (not shown). In order to minimize the contribution of Cl^-/Cl^- exchange, the experimental medium was isotonic Na_2SO_4. The $t_{1/2}$ for ^{36}Cl egress was increased to 12.4 min in this medium. Gramicidin (0.25 μM) alone did not have any effect on the $t_{1/2}$, indicat-

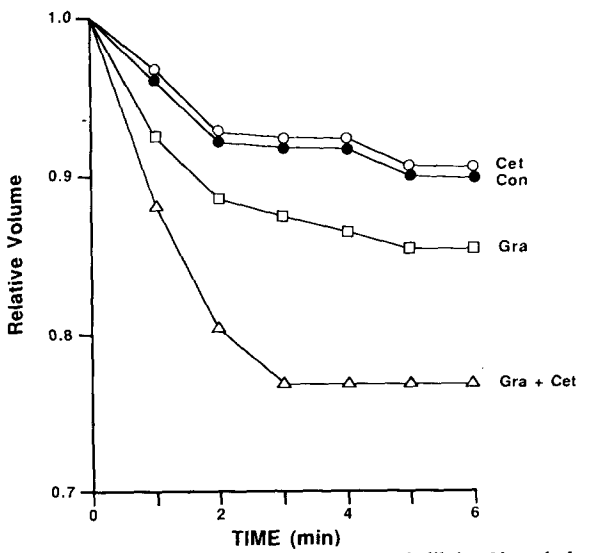

Fig. 3. CHO cell volume response to cetiedil in *N*-methylglucamine chloride medium. CHO cells were suspended in *N*-methylglucamine chloride medium containing 140 mM *N*-methylglucamine chloride, 1 mM KCl, 0.5 mM $CaCl_2$, 0.5 mM $MgSO_4$, 10 mM glucose, 20 mM Hepes (pH 7.4) at 37°C. The initial cell volume was determined prior to the addition of reagents and recorded every 1 min for the next 6 min. Control (●); 0.5 μM gramicidin (□); 75 μM cetiedil (○); gramicidin+cetiedil (△). Data are representative of eight separate experiments.

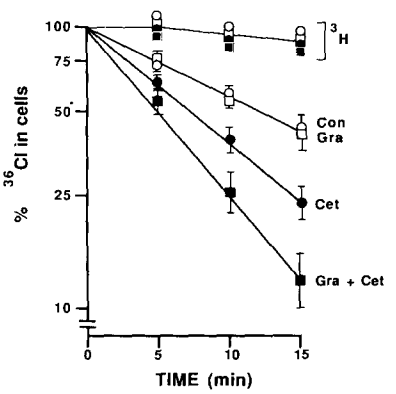

Fig. 4. Effect of cetiedil on chloride efflux. CHO cells were loaded with ^{36}Cl and 2-[^{3}H]dGlc for 1.5 h at room temperature. The cells were then sedimented and washed once in a medium composed of 100 mM Na_2SO_4, 1 mM KCl, 0.5 mM $CaCl_2$, 0.5 mM $MgSO_4$, 10 mM glucose, 20 mM Hepes (pH 7.4). At zero time, equal aliquots of the cell suspension were diluted into the same medium at room temperature, with or without 0.25 μM gramicidin. Cetiedil or DMSO were added last. Efflux was stopped at the intervals indicated by sedimentation through an oil layer. Control (○); 100 μM cetiedil (●); 0.25 μM gramicidin (□); gramicidin+cetiedil (■). Ordinate: percent of initial ^{36}Cl remaining in the cells (log scale), the same scale applies for 2-[^{3}H]dGlc efflux, which is indicated as ^{3}H. The results are the mean ± S.E. of six determinations.

ing that cation conductivity is not limiting for ^{36}Cl efflux. The $t_{1/2}$ was shortened to 7.2 min in the presence of cetiedil (100 μM) alone and was further decreased to 5.0 min with 0.25 μM gramicidin. The efflux of 2-[^{3}H]dGlc was not affected by cetiedil or gramicidin or the two agents together.

Several other compounds which produce a wide range of responses at the cell membrane level, were tested for their effects on the Cl^- conductivity of CHO cells as measured by their swelling after 10 min in NaCl medium containing 0.5 μM gramicidin and the test compound. Chlorpromazine (100 μM) induced a 14% swelling and a slight swelling was consistently produced by the Ca^{+2} ionophore A23187 (2 μM), about 4%. No changes were observed upon exposure of the cells to 100 μM verapamil, trifluoperazine, procaine, quinine, caffeine or 1 mM dibutyryl cAMP (data not shown).

The cell volume response of several cell types to 0.5 μM gramicidin and 50 μM cetiedil was compared (Table I). All of the cell types tested showed relatively low Cl^- conductance as indicated by the small or negligible volume changes elicited by gramicidin alone. Cetiedil alone (50 μM) produced little volume change in any of the cells tested. Aside from CHO cells, only L6H9 myoblasts were found to swell with 50 μM cetiedil and gramicidin (31% in 10 min); rat thymocyts, C6 rat glioma cells and LLC-PK_1, pig kidney cells remained essentially unresponsive.

Higher concentrations of cetiedil produced nonspecific effects in some cells. At 100 μM and higher, lysis was observed in thymocytes and volume changes were noted without addition of gramicidin in L6H9 cells.

Substantial alterations in conductive chloride permeability would be expected to result in changes in membrane potential (E_m). Using the fluorescent dye, bisoxonol, the resting E_m of CHO cells was estimated to be -52 mV (see Materials and Methods). A series of measurements were made to evaluate the effects of cetiedil and gramicidin, on the E_m of cells suspended in different media, NaCl, Na_2SO_4, and KCl (Fig. 5). The baseline potential was the same in NaCl, Na_2SO_4, and *N*-methylglucamine chloride, but as expected, depolarization occurred in KCl (higher fluorescence). These observations suggest that as is the case for most cells, the K^+ gradient is the predominant determinant of E_m, so that substitution of impermeant cations or anions for Na^+ and Cl^- do not substantively change the potential. Depolarization was observed, as expected, in Na^+ media on addition of gramicidin, which induces high permeability to Na^+ as well as K^+. In *N*-methylglucamine chloride (*N*-methylglucamine is an impermeant cation), gramicidin causes hyperpolarization, probably by enhancing K^+ permeability, and K^+ efflux.

Several qualitative predictions can be made regarding the direction of change of the E_m resulting from a cetiedil-induced increase in Cl^- conductance. The equilibrium potential for Cl^- (E_{Cl}) was estimated at about -41 mV, based on an intracellular Cl^- concentration of 29 mM. Thus an increase in Cl^- conductance would be expected to depolarize the cells in NaCl and *N*-methyl-

TABLE I

COMPARISON OF THE VOLUME RESPONSES OF DIFFERENT CELL TYPES TO GRAMICIDIN AND CETIEDIL

All determinations were performed in NaCl medium, after a 1 h recovery from trypsinization in the case of L6H9, C6 and L6H9 cells. Percent volume change refers to the percent change in cell volume relative to the initial value, obtained after 10 min of exposure to drugs at 37°C. Negative values indicate a volume decrease. Results are the average of two separate determinations done in duplicate.

Cell type	Gramicidin (μM)	Cetiedil (μM)	% Volume change
CHO	0.5	0	−7
	0	50	0
	0.5	50	45
L6H9	0.5	0	0
	0	50	6
	0.5	50	31
C6	0.5	0	0
	0	50	0
	0.5	50	0
Rat thymocytes	0.5	0	10
	0	50	5
	0.5	50	10
LLC-PK_1	0.5	0	−8
	0	50	0
	0.5	50	0

glucamine chloride medium, and this depolarization should be more pronounced in low Cl^- medium. Two effects were observed on addition of cetiedil (10 μM), an initial small transient change, followed by a slower response that results in an altered steady-state, E_m. The transient response involved a hyperpolarization in NaCl and *N*-methylglucamine chloride media, and a depolarization in KCl medium. The underlying cause is not known, but the changes are consistent with a small transient change in K^+ permeability. The slow change to a new steady state appears to reflect changes in Cl^- permeability. A partial depolarization occurs, to the same extent in NaCl and *N*-methylglucamine chloride and to a somewhat greater extent in Na_2SO_4. In KCl medium, cetiedil causes a small decrease in potential. These results are consistent with an increased Cl^- conductance with the E_m moving in each case toward the Cl^- equilibrium potential.

Discussion

Our primary method for measuring Cl^- conductance is based on the principle that conductive movements of cations and anions in the direction of their net electrochemical gradients leads to gains or losses of salts that are expressed as measurable changes in cell volume associated with water equilibration. Water permeability of CHO cells is relatively high so they equilibrate rapidly [11]. In the presence of Na^+/K^+ specific ionophore, gramicidin, to enhance the Na^+ and K^+ permeabilities, the limiting factor in volume changes will be the conductive Cl^- permeability. For cells suspended in NaCl, the net gradient is inward: the Na^+ and K^+ gradients are in opposite direction but of about equal magnitude; whereas the Cl^- gradient is inwardly directed. Thus the cells will tend to swell. Little change in cell volume occurs upon exposure of CHO cells to gramicidin Ref. 11 and also Fig. 1 this paper) because the Cl^- conductivity of these cells is relatively low. The same is true for lymphocytes [9], and other cell types. When gramicidin and cetiedil are added simultaneously, however, the cells undergo time dependent swelling. The initial swelling rate is concentration-dependent and saturable with respect to cetiedil (Fig. 2). These observations would be expected if cetiedil caused an increase in the Cl^- conductance of the cells. The absence of substantial volume changes with cetiedil alone indicates that its effects are specific to anions and that it does not increase Na^+ or K^+ permeability.

Since the swelling response is directly dependent on the major anion in the medium, ion substitution permitted the analysis of the anion selectivity of the cetiedil induced pathway (Fig. 2). Some specificity was noted, with the permeability sequence $NO_3 > Cl^- = Br^- \gg$ isethionate $= SO_4^{2-}$. This order is roughly similar to the one found by the same method for hypotonically induced Cl^- pathways in lymphocytes [9]. The cetiedil pathway may be somewhat permeable to SO_4^{2-} and isethionate, since the cells did not shrink in media containing these anions, as might be expected if they were highly impermeant to those anions relative to Cl^-.

Substitution of K^+ for Na^+ in the medium did not affect the response to cetiedil. The swelling obtained in conjunction with gramicidin was consistent with enhancement of permeability of both cations. The fact that valinomycin could substitute for gramicidin in KCl medium indicates that the action of cetiedil is independent of the type of cation ionophore used.

Substitution of the impermeant cation, *N*-methylglucamine, results in cell shrinking rather than swelling (Fig. 3). The reversal of direction is expected because in this case the net gradient for permeant ions is outward. As expected, cetiedil, in the presence of gramicidin, induced a rapid rate of shrinkage. A slow rate of shrinkage was also observed in control cells that was moderately enhanced by gramicidin. These observations are in contrast to NaCl medium (Fig. 1), in which little volume change occurred except in the presence of gramicidin plus cetiedil. The enhanced shrinking in NMG-Cl medium produced by gramicidin may be due to the large outward K^+ gradient, leading to a large hyperpolarization as shown in Fig. 5. In NaCl medium, in contrast, addition of gramicidin leads to substantial depolarization.

The effects of cetiedil on chloride permeability could also be measured as an increased $^{36}Cl^-$ flux (Fig. 4). The isotope measurements, however, include non-electrogenic components. Cl^-/Cl^- ex-

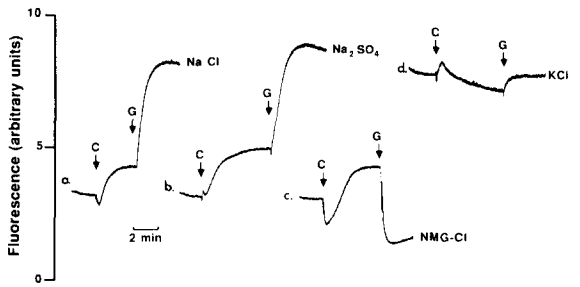

Fig. 5. The effect of cetiedil on the membrane potential in different media. CHO cells were suspended in NaCl Na_2SO_4, *N*-methylglucamine chloride or KCl medium, bisoxonol was added and the fluorescence of the cell suspension was recorded until the signal became stable. Cetiedil (10 μM) was added where indicated by 'C'. When the signal became stabilized, 40 nM gramicidin was added to the cuvette where indicated by 'G'. The traces shown are from a representative experiment in a series of four.

change was minimized by use of Na_2SO_4 medium, but some level of sulfate/chloride exchange may occur. Cotransport of $K^+/Na^+/2Cl^-$ is also reported in many cells. These pathways probably contribute to the relatively rapid rate of $^{36}Cl^-$-efflux in control cells. Nevertheless, cetiedil induced a substantial increase (42%).

As expected from its cation specificity, gramicidin alone had no effect on $^{36}Cl^-$ efflux. It did, however, enhance the cetiedil effect. It seems likely that with cetiedil-enhancement of conductive Cl^- permeability, the K^+ permeability becomes rate-limiting to KCl efflux. The maximal Cl^- efflux could only be attained in the presence of gramicidin, under circumstances where the K^+ conductance was not limiting.

In order to ascertain that the observed ^{36}Cl efflux was not due to cell lysis or nonspecific changes in permeability, the cells were simultaneously loaded with 2-[^{3}H]dGlc. The efflux of this non-metabolisable sugar was minimally affected by cetiedil and gramicidin.

The effects of cetiedil were also reflected in changes in the membrane potential (Fig. 5). In each situation as noted in the Results section, cetiedil induced changes in potential that were in the direction of the Cl^- equilibrium potential. It was also noted that small initial transient changes in potential also occurred in the opposite direction. The cause is not known, but the effect could be explained by a transient increase in basal K^+ conductance. Cetiedil is an inhibitor of Ca^{2+}- and volume-activated K^+ conductances [1,2], but it has not effect on the basal K^+ permeability [2].

The cetiedil effect on Cl^--conductance was observed in CHO cells and L6H9 cells (Table I) but not in several other cell types. Thus, it is cell-specific and not a general phenomenon.

Clinically cetiedil is known as a peripheral vasodilator [3] and has been proposed as an anti-sickling agent [4,14,15]. It has been reported to inhibit a number of membrane processes such as: (i) K^+ conductive pathways activated by Ca^{2+} [1] or by hypotonic shock [2]; (ii) superoxide generation in neutrophils [16]; (iii) aggregation of neutrophils [17]; and (iv) stimulation of phosphodiesterase and Ca^{2+}-aTPase by calmodulin [18]. Cetiedil was not found to have any effect on Cl^- conductance in human erythrocytes [1]; however, partial stimulation would not be readily detectable in these cells because of their high basal Cl^- conductivity. On the other hand, cetiedil appears to be an inhibitor of the Cl^- conductive pathways activated by hypotonic shock in lymphocytes [2] and CHO cells [11]. These particular pathways must therefore be different form those activated by cetiedil in isotonic media.

The volume of gramicidin treated CHO cells does not change significantly in response to inhibitors of calmodulin such as chlorpromazine and trifluoperazine, elevation of cytoplasmic cAMP (by 1 mM di-b-cAMP), Ca^{2+} loading (by ionophore A23187) or by inhibitors of K^+ (quinine) and Ca^{2+} (verapamil) channels. Hence, agents which might be expected to mimic some of the effects of cetiedil to not appear to increase the Cl^- conductance. Cetiedil is a hydrophobic molecule with one positive charge due to a tertiary amine, and is likely to be highly membrane-permeant, especially in the uncharged form. As such it could conceivably intercalate into the plasma membrane and form anion channels, or alternatively, activate quiescent anion channels in some cells.

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