Biochemical Pharmacology, Vol. 42, No. 5, pp. 1039–1044, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00
Pergamon Press plc

N-(6-AMINOHEXYL)-5-CHLORO-1-NAPHTHALENESULFONAMIDE (W7) STIMULATION OF K^+ TRANSPORT IN A HUMAN SALIVARY EPITHELIAL CELL LINE

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(*Received* 14 *January* 1991; *accepted* 9 *April* 1991)

Abstract—Treatment of a human salivary epithelial cell line, HSG-PA, with the calmodulin antagonist *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W7; 20–70 μM) increased ^{86}Rb (K^+) influx and efflux in a manner similar to that resulting from muscarinic (carbachol; Cch) or calcium ionophore (A23187) stimulation. Unlike the Cch or A23187 responses, the W7 responses were not blocked by 0.1 mM atropine (muscarinic antagonist) or phorbol-12-myristate-13-acetate (0.1 μM). Like Cch- or A23187-stimulated ^{86}Rb fluxes, W7-stimulated ^{86}Rb fluxes were substantially blocked by the K^+ channel inhibitors quinine (0.25 mM) and scorpion venom-containing charybdotoxin (33 μg/mL), while 5 mM tetraethylammonium chloride (K^+ channel blocker), furosemide (0.1 mM; $Na^+,K^+,2Cl^-$ co-transport inhibitor) and ouabain (10 μM; Na^+,K^+-ATPase inhibitor) were ineffective. Purified charybdotoxin (10 nM) also blocked W7-stimulated ^{86}Rb influx, as well as ^{86}Rb influx stimulated by Cch or A23187. Although Quin 2 fluorescence measurements indicated that W7 increased free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), the magnitude of the increase appeared to be insufficient to solely account for the W7-stimulated increases in ^{86}Rb fluxes (i.e. K^+ channel activity). Ca^{2+} was involved in the W7 response, however, as lack of Ca^{2+} in the incubation medium reduced the W7-stimulated increases in ^{86}Rb influx and efflux. Taken together, our results suggest that W7 increased K^+ fluxes in HSG-PA cells by interacting, directly or indirectly, with the K^+ transport machinery (K^+ channels) in a manner different from that observed during muscarinic stimulation, and also in a manner not accounted for solely by the formation of a typical muscarinic- or calcium ionophore-generated calcium signal.

K^+ channels are regulated by a number of different systems, including G-proteins [1, 2], protein kinases A [3, 4] and C [3, 5–7], intracellular pH [8–11], membrane potential [8, 10, 12] and calmodulin [4, 13–21]. Utilizing ^{86}Rb flux measurements, we recently reported [22] that a human submanidibular epithelial cell line, HSG-PA [23], contains muscarinic-responsive, Ca^{2+}-activated K^+ channels which are regulated by protein kinase C modulation of the muscarinic-stimulated calcium signal [24, 25]. As it has been reported that (a) the calmodulin antagonist W7‖ inhibits muscarinic-stimulated Ca^{2+} mobilization in this cell line [26], and (b) inhibition of calmodulin function interferes with K^+ channel activity in other systems [15–21], we investigated the effect of W7 on K^+ transport in HSG-PA cells. In this paper, we provide evidence that W7 alone stimulates K^+ (^{86}Rb) channel activity (directly or indirectly), and we compare this response to that caused by muscarinic or calcium ionophore stimulation.

MATERIALS AND METHODS

Chemicals and isotopes. Hanks' balanced salt solution (HBSS; Biofluids, Rockville, MD) contained 137 mM NaCl, 5.4 mM KCl, 1.0 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.4 mM $MgSO_4$, 0.3 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 5.5 mM glucose and 0.002% phenol red, pH 7.3. *N*-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide (W7), *N*-(6-aminohexyl)-1-naphthalenesulfonamide (W5), tetraethylammonium chloride (TEA), quinine hydrochloride (QUI), ethylene glycol-bis(β-aminoethyl ether)*N,N,N′,N′*-tetraacetic acid (EGTA), sodium dodecyl sulfate (SDS), scorpion venom from *Leiurus quinquestriatis hebraeus* (venom; crude venom containing charybdotoxin), carbamylcholine chloride (Cch; carbachol), atropine sulfate (ATR), phorbol-12-myristate-13-acetate (PMA), furosemide (FUR), ouabain (OUA), *N*-(1-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (Hepes) and phloretin were purchased from Sigma (St. Louis, MO). Diethylenetriaminepentaacetic acid (DTPA) was

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‖ Abbreviations: W7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide; W5, *N*-(6-aminohexyl)-1-naphthalenesulfonamide; HBSS, Hanks' balanced salt solution; HBSS-H, HBSS containing 20 mM Hepes (pH 7.3) plus 100 μg/mL each of penicillin G and streptomycin sulfate; TEA, tetraethylammonium chloride; QUI, quinine hydrochloride; EGTA, ethylene glycol-bis(β-aminoethyl ether)*N,N,N′,N′*-tetraacetic acid; SDS, sodium dodecyl sulfate; Cch, carbachol, carbamylcholine chloride; ATR, atropine sulfate; PMA, phorbol-12-myristate-13-acetate; FUR, furosemide; OUA, ouabain; ChTX, charybdotoxin; and DTPA, diethylenetriaminepentaacetic acid.

obtained from Sigma or Calbiochem (La Jolla, CA). Quin 2/AM and A23187 were purchased from Calbiochem. 4Br-A23187 was obtained from Molecular Bioprobes (Eugene, OR). Rubidium-86 chloride (8.44 mCi/mg rubidium) was obtained from Amersham (Arlington Heights, IL). Purified charybdotoxin (ChTX) was purchased from Receptor Research Chemicals Inc. (Baltimore, MD). All other chemicals were of the highest grade commercially available.

Cell culture. HSG-PA cells ([23]; obtained from Dr. Mitsunobu Sato, Tokushima University, Japan) were grown in tissue culture dishes in Eagle's minimal essential medium supplemented with 10% newborn calf serum and 100 μg/mL each of penicillin G and streptomycin sulfate (all purchased from Biofluids). Cells were grown at 37° in a humidified 5% CO_2 atmosphere, and the growth medium was changed every 48 hr.

^{86}Rb flux measurements. ^{86}Rb was used to follow K^+ movement in HSG-PA cells as described previously [22]. ^{86}Rb flux experiments were carried out at room temperature on confluent monolayers grown in 35-mm dishes. Incubations were begun by removing the culture medium, washing the monolayers twice with 1 mL of HBSS containing 20 mM Hepes (pH 7.3) plus 100 μg/mL each of penicillin G and streptomycin sulfate (HBSS-H), and then allowing them to equilibrate with 2 mL of the same medium for 7 hr. For efflux studies, ^{86}Rb was included in the equilibration medium. ^{86}Rb efflux was obtained by removing the radiolabeled medium and immersing the dishes in 25 mL of unlabeled HBSS-H for various times. For influx studies, ^{86}Rb was added to the medium following preincubation. ^{86}Rb flux measurements were carried out in the presence or absence of effector compounds, as indicated in the figure legends. ^{86}Rb and drug stock solutions were initially made at concentrations 100-fold higher than the final concentrations tested. Incubations were terminated, and the ^{86}Rb monolayer content was determined, i.e. radioactive medium was removed and the monolayers were rapidly washed four times by sequential dipping in 100 mL/wash of ice-cold magnesium–sucrose buffer (137 mM sucrose, 100 mM $MgSO_4$, 20 mM Hepes–Tris, pH 7.5, and 0.1 mM phloretin). Phloretin was initially dissolved in ethanol such that the wash solution was 1% ethanol by volume. The monolayers were then incubated in 1 mL of 0.2% SDS, and the solubilized material plus two additional 1-mL rinses with water were transferred to glass tubes. The combined extracts were sonicated and vortexed, and 1-mL aliquots were taken for liquid scintillation counting, using ACS (aqueous counting scintillant, Amersham) and a Beckman LS-380 scintillation counter.

Measurement of $[Ca^{2+}]_i$. Estimates of $[Ca^{2+}]_i$ were made in a manner similar to that described previously [24, 25, 27]. Briefly, confluent monolayers were washed twice with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline, and then the cells were detached from the plates by incubation in the same medium containing 4 mM EGTA and 20 mM Hepes, pH 7.4, for 15 min at 37°. Next the cells were resuspended and washed twice in HBSS-H lacking phenol red, and then they were resuspended in the same medium at a concentration of 5×10^6 cells/mL. Quin 2 was loaded into the cells by incubating the suspension in 20 μM Quin 2/AM for 30 min at room temperature. Then cells were washed three times in HBSS-H lacking phenol red and resuspended in the same medium at a concentration of 1×10^5 cells/mL. Quin 2 fluorescence was measured at room temperature in an SLM-8000 spectrofluorimeter, and calibration of the signal and calculation of cytosolic Ca^{2+} were performed.

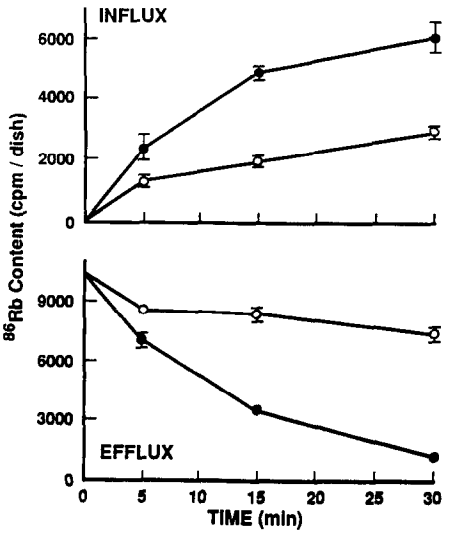

Fig. 1. W7 stimulation of ^{86}Rb fluxes in HSG-PA cells. Influx: Confluent monolayers in 35-mm dishes were incubated in HBSS-H for 7 hr at room temperature, ^{86}Rb with or without W7 was added, and then the ^{86}Rb content of the monolayers was measured after various incubation times. Efflux: Confluent monolayers in 35-mm dishes were incubated for 7 hr at room temperature in HBSS-H containing ^{86}Rb, radioactive medium was removed, and then dishes were immersed in 25 mL of HBSS-H with or without W7. In all experiments, W7 was initially dissolved as a 5 mM stock solution in 10% ethanol. No treatment incubations received vehicle (0.1% ethanol final concentration) only. Results are mean values ± SEM, obtained from three experiments, each performed in triplicate. All points contain error bars, some of which are obscured by symbol size. Symbols: (●) W7 treatment and (○) control.

Dye leakage was estimated using the *in situ* Mn^{2+}/DTPA technique [27]. The final concentrations of reagents used in this protocol were $MnCl_2$ (43 μM, DTPA (64 μM) and digitonin (50 μg/mL). Under the conditions employed, we found that 0 to 2.8% of the fluorescence signal, at the end of the 60-min incubations, was attributable to leakage of intracellular dye to the medium. Photon counting of detergent lysed samples demonstrated that no detectable dye quenching occurred during the 60-min incubations.

RESULTS

W7 stimulation of ^{86}Rb fluxes. Exposure of HSG-PA cells to 50 μM W7 (Fig. 1) increased the rates

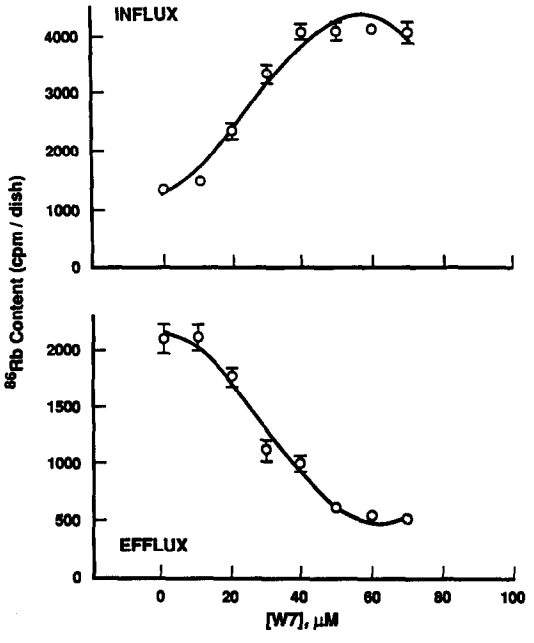

Fig. 2. Concentration-dependent W7 stimulation of ^{86}Rb fluxes. Monolayers were preincubated as described in the legend to Fig. 1. The effect of various concentrations of W7 on both ^{86}Rb influx (upper panel) and efflux (lower panel) was then measured (60-min incubations). Results are mean values ± SEM, obtained from three experiments, each performed in triplicate. All points contain error bars, some of which are obscured by symbol size.

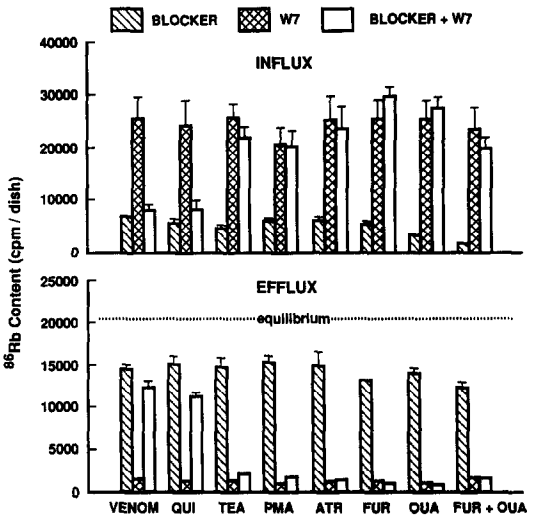

Fig. 3. Effects of various drugs on W7-stimulated increases in ^{86}Rb influx and efflux. Influx: Confluent monolayers in 35-mm dishes were incubated for 7 hr with HBSS-H at room temperature. Various drugs were then added, followed 10 min later by the addition of ^{86}Rb with or without W7. After 60 min of incubation, the ^{86}Rb content of the monolayers was measured. Efflux: Confluent monolayers in 35-mm dishes were equilibrated for 7 hr at room temperuture in HBSS-H containing ^{86}Rb, and then various drugs were added. Ten minutes later, radioactive medium was removed, and dishes were immersed in 25 mL of medium (efflux medium) with or without W7. Cells which had been treated initially with drugs also received drugs in the efflux medium. After 60 min of incubation, dishes were removed from the medium, and the ^{86}Rb content of the monolayers was measured. Final concentrations of drugs and vehicles were as follows: venom (scorpion venom containing charybdotoxin, 33 μg/mL, H_2O); QUI (0.25 mM quinine, 0.1% ethanol); TEA (5 mM tetraethylammonium chloride, H_2O); PMA (0.1 μM phorbol-12-myristate-13-acetate, 0.1% dimethyl sulfoxide); ATR (0.1 mM atropine, H_2O); W7 (50 μM *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide, 0.1% ethanol); OUA (10 μM ouabain, 0.1% dimethyl sulfoxide); and FUR (0.1 mM furosemide, 0.1% dimethyl sulfoxide). Results are mean values ± SEM, obtained from two experiments, each performed in triplicate. The control 60-min ^{86}Rb influx content was 5770 ± 70 cpm. The equilibrium value in efflux experiments was 20,500 ± 200 cpm, and the control 60-min efflux value was 14,283 ± 958 cpm. All points contain error bars, some of which are obscured by the bar line width.

of both ^{86}Rb influx (upper panel) and efflux (lower panel) as compared to untreated cells. Both increased rates of ^{86}Rb transport occurred during the first 15 min of incubation, after which the rates of both unstimulated and stimulated ^{86}Rb influx and efflux were similar. The concentration dependence of the W7 effect is shown in Fig. 2. Increases in both ^{86}Rb influx and efflux exhibited the same pattern of responsiveness to W7 over a concentration range of 10–70 μM, with detectable stimulation occurring at *ca.* 20 μM, and maximal responses occurring at *ca.* 50 μM. In parallel experiments, we found that treatment of HSG-PA cells with 100 μM W5, an analogue of W7 which is a less effective calmodulin antagonist [28], failed to elicit a detectable increase in either ^{86}Rb influx or efflux (results of three separate experiments, each performed in triplicate; data not shown).

Effects of various agents on W7-stimulated ^{86}Rb *fluxes.* In a previous study [22], we showed that muscarinic-stimulated ^{86}Rb fluxes in HSG-PA cells were inhibited by the muscarinic antagonist atropine, some putative K^+ channel blockers, and a phorbol ester. The effects of these and other agents on W7-stimulated ^{86}Rb fluxes are shown in Figs. 3 and 4. The results in Fig. 3 demonstrate that the muscarinic receptor antagonist atropine (0.1 mM), the K^+ channel blocker TEA (5 mM), the phorbol ester PMA (0.1 μM), the Na^+,K^+-ATPase inhibitor ouabain (10 μM), or the $Na^+,K^+,2Cl^-$-cotransport inhibitor furosemide (0.1 mM) failed to block W7-stimulated ^{86}Rb influx or efflux. In contrast, the K^+ channel blockers quinine (0.25 mM) and crude charybdotoxin (33 μg/mL) substantially blocked both W7-stimulated ^{86}Rb fluxes, suggesting that W7, directly or indirectly, activated K^+ channel activity in this cell line. K^+ channel involvement was further indicated by our finding that purified charybdotoxin (10 nM) inhibited W7-, as well as muscarinic (carbachol)- and direct Ca^{2+} signal (A23187)-stimulated increases in ^{86}Rb influx (Fig. 4). The ability of purified charybdotoxin to inhibit W7-stimulated ^{86}Rb efflux was not tested due to the limited amount of toxin available.

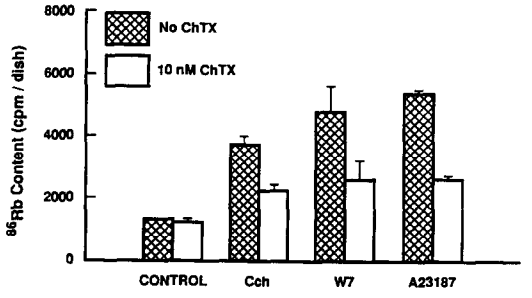

Fig. 4. Inhibition of stimulated ^{86}Rb influx by purified charybdotoxin. Confluent monolayers in 35-mm dishes were equilibrated for 7 hr at room temperature with HBSS-H. Purified charybdotoxin (10 nM ChTX final concentration in H_2O) was then added, followed 10 min later by the addition of ^{86}Rb plus one of the following: 0.1% ethanol (control vehicle for all additions), Cch (0.1 mM carbachol), 5 μM A23187, or 50 μM W7. After a 60-min incubation, the ^{86}Rb content of the monolayers was measured. Results are mean values ± SEM, obtained from two experiments, each performed in triplicate. All points contain error bars, some of which are obscured by the bar line width.

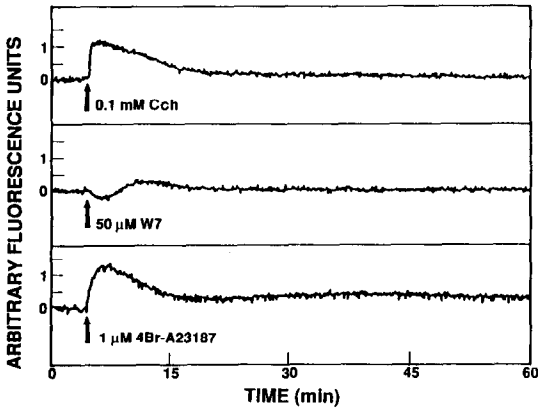

Fig. 5. Effects of various agents on $[Ca^{2+}]_i$. Cell suspensions from confluent monolayers were loaded with 20 μM Quin 2, and the change in $[Ca^{2+}]_i$ resulting from 0.1 mM Cch, 1.0 μM 4Br-A23187 or 50 μM W7 treatment was then measured (see text) as described previously [24, 25, 28]. Representative traces of at least four independent experiments are shown. Quantitative changes in peak $[Ca^{2+}]_i$ are given in the Results.

Role of Ca^{2+} *in the W7 response.* Because increases in intracellular free calcium concentration ($[Ca^{2+}]_i$) stimulated ^{86}Rb fluxes in HSG-PA cells, we compared the ability of W7, muscarinic, and calcium ionophore treatments to increase $[Ca^{2+}]_i$ above the resting $[Ca^{2+}]_i$ (60 ± 3.4 nM). Representative time-dependent changes in $[Ca^{2+}]_i$, measured by changes in intracellular Quin 2 fluorescence, are shown in Fig. 5. In direct comoparative studies, we found that 0.1 mM carbachol and 1.0 μM 4Br-A23187 stimulation resulted in rapid 285 ± 40% (mean ± SEM, N = 9) and 235 ± 69% (mean ± SEM, N = 6) increases in $[Ca^{2+}]_i$ over the resting

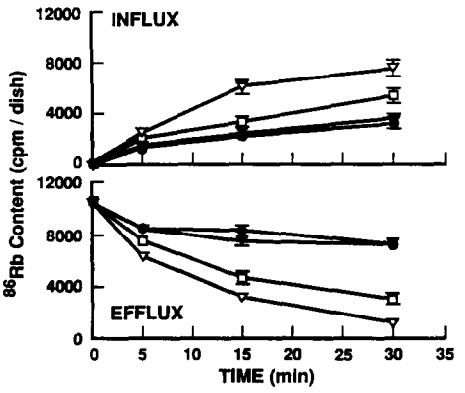

Fig. 6. Effect of $[Ca^{2+}]_o$ on W7-stimulated ^{86}Rb fluxes in HSG-PA cells. Upper panel (Influx): confluent monolayers in 35-mm dishes were equilibrated in HBSS-H for 7 hr at room temperature. HBSS-H medium was then removed, monolayers were washed twice in either HBSS-H or HBSS-H lacking Ca^{2+}, and ^{86}Rb containing or lacking W7 was then added to the dishes in 2 mL of the same medium used to wash the plates. After various times of incubation, the ^{86}Rb content of the monolayers was measured. Lower panel (efflux): confluent monolayers in 35-mm dishes were equilibrated at room temperature for 7 hr in HBSS-H containing ^{86}Rb. Medium was then removed, and dishes were immersed in 25 mL of HBSS-H with or without Ca^{2+} and W7. At the indicated times, dishes were removed from the medium, and the amount of ^{86}Rb remaining in the monolayers was measured. In all experiments, 50 μM W7 was dissolved in 10% ethanol at an initial concentration of 5 mM. Results in the upper and lower panels are mean values ± SEM, obtained from four and two experiments carried out in triplicate, respectively. Symbols: (●) control plus 1.0 mM $[Ca^{2+}]_o$ (▼) control minus $[Ca^{2+}]_o$; (▽) W7 plus 1.0 mM $[Ca^{2+}]_o$; and (□) W7 minus $[Ca^{2+}]_o$.

level, respectively. In contrast, W7 caused an initial decrease in the $[Ca^{2+}]_i$ resting level (19.2 ± 2% mean ± SEM, N = 7), followed by a gradual small increase above the resting level (23 ± 5%, mean ± SEM, N = 7). While W7 did alter $[Ca^{2+}]_i$, the results of experiments using 0.25 μM 4Br-A23187 suggested that the 50 μM W7-stimulated increase in $[Ca^{2+}]_i$ was insufficient to fully account for the W7-stimulated increase in ^{86}Rb influx and efflux. In these experiments, we found that a 50% increase in $[Ca^{2+}]_i$, caused by 4Br-A23187 treatment, resulted in increases in ^{86}Rb influx and efflux (60-min incubations) which were only 13 ± 0.1% (mean ± SEM) and 16 ± 0.2% (mean ± SEM), respectively, of those caused by a 60-min incubation with W7 (results of two separate experiments, each carried out in triplicate). Ca^{2+} did play a role in the W7 stimulation process, however, as lack of extracellular Ca^{2+} ($[Ca^{2+}]_o$) in the incubation medium (nominally Ca^{2+}-free medium) decreased the W7 stimulation of ^{86}Rb influx and efflux in this cell line (Fig. 6).

DISCUSSION

In a previous report, we partially characterized the muscarinic regulation of K^+ fluxes in a human

submandibular epithelial cell line, HSG-PA [22]. Carbachol stimulation was shown to increase K^+ influx and efflux, resulting in only a small net decrease in the intracellular equilibrium content of K^+. Both fluxes appeared to involve the participation of Ca^{2+}-activated K^+ channels as judged by inhibitor studies. In addition, phorbol ester studies indicated that protein kinase C was involved in the regulation of this response, due largely to the regulation of the Ca^{2+} signal [22, 24].

To investigate the potential role of calmodulin in the regulation of salivary epithelial K^+ transport, we examined the effect of the calmodulin antagonist W7 on ^{86}Rb fluxes in the HSG-PA cell line. W7 was utilized as it has been shown previously that (a) this calmodulin antagonist blocks muscarinic-stimulated mobilization of Ca^{2+} stores from both intra- and extracellular sources in HSG-PA cells [24], and (b) W7 [16–18], other calmodulin antagonists [4, 19], and mutations in calmodulin [15] block K^+ fluxes in other systems. Based on these observations, we anticipated that W7 would block muscarinic (carbachol)-, and perhaps Ca^{2+} ionophore (A23187)-stimulated ^{86}Rb fluxes in HSG-PA cells. When we examined the W7 effect, however, we found that W7 alone stimulated ^{86}Rb influx and efflux in this cell line. In our studies, we found that the increased ^{86}Rb flux rates occurred for approximately 15 min, which was longer than the 5-min stimulations caused by carbachol or A23187 treatment [22].

To better characterize the nature of the W7 effect on K^+ (^{86}Rb) fluxes in HSG-PA cells, we tested the ability of several agents to inhibit the W7-stimulated increases in ^{86}Rb influx and efflux. The results of our inhibitor studies suggested that the W7-stimulated increases in ^{86}Rb fluxes were not mediated directly via Na^+,K^+-ATPase or $Na^+,K^+,2Cl^-$-cotransport activity, but rather via quinine- and charybdotoxin-sensitive K^+ channels. Although patch clamp studies were not performed, sensitivity to quinine and charybdotoxin, but not to tetraethylammonium chloride, suggested that the W7 response involved channels of intermediate conductance [29].

The mechanism by which W7 stimulated ^{86}Rb influx and efflux was clearly different from that caused by muscarinic stimulation. Although W7 has been reported to interact with the muscarinic receptor [26], our studies showed that atropine (a muscarinic antagonist) failed to block the W7 effects, suggesting that the W7-stimulated ^{86}Rb fluxes were not mediated by the muscarinic receptor. In addition, the phorbol ester PMA failed to inhibit W7 stimulation of ^{86}Rb fluxes, suggesting that this response was not subject to regulation by protein kinase C. This finding contrasted with the effects of PMA on carbachol-stimulated ^{86}Rb fluxes, which were inhibited markedly by a PMA reduction of the Ca^{2+} signal [22, 24]. W7 may have competitively interfered with the ability of PMA to activate protein kinase C, as occurs with some naphthalenesulfonamides. In rat liver epithelia, however, a W7 concentration substantially greater than 50 μM was required to significantly inhibit protein kinase C activity *in vitro* [30].

The W7 stimulation of ^{86}Rb fluxes was Ca^{2+}-dependent inasmuch as removal of extracellular Ca^{2+} from the incubation medium substantially inhibited the W7 effect. The Ca^{2+} dependence may have been due to several factors, including the generation of an intracellular Ca^{2+}-signal. Although W7 increased $[Ca^{2+}]_i$, this increase alone did not appear to be of sufficient magnitude to fully account for the observed increases in W7-stimulated ^{86}Rb fluxes. Plishker has reported, however, that calmodulin antagonist (phenothiazine) inhibition of Ca^{2+},Mg^{2+}-ATPase increases K^+ efflux in red blood cells via a substantial increase in $[Ca^{2+}]_i$ [21]. In our study, some of the W7-stimulated increase in ^{86}Rb fluxes likely arose from the small increase in $[Ca^{2+}]_i$, and in this respect, we could not rule out the possibility that W7 may have caused localized increases in $[Ca^{2+}]_i$ of sufficient magnitude to totally account for the stimulated increases in ^{86}Rb flux.

Some of our findings were consistent with the suggestion that calmodulin is involved in the regulation of quinine- and charybdotoxin-sensitive K^+ channels in HSG-PA cells. In this regard, high concentrations of W5, a chlorine-deficient analogue of W7 with a markedly reduced affinity for calmodulin [28], failed to stimulate ^{86}Rb fluxes. In addition, the 20–50 μM W7 concentrations eliciting increases in ^{86}Rb flux were similar to the half-maximal concentrations reported to inhibit calmodulin-dependent processes *in vitro* [30]. If calmodulin is involved in regulating K^+ fluxes in HSG-PA cells, our findings suggest that calmodulin may normally restrict the opening of specific K^+ channels, and antagonism of this control (either by interaction with W7 or the muscarinic-generated Ca^{2+} signal) allows the channels to remain open for a longer period of time. While this interpretation may prove correct, caution must be exercised as W7 has been reported to interact with proteins other than calmodulin [26, 31].

To the best of our knowledge, our results provide the first evidence that W7 stimulates K^+ channel activity, directly or indirectly, in human epithelia. Although we do not yet know the extent to which this response occurs, we have found (data not shown) that W7 has identical effects in the human parotid epithelial cell line, HSY [32]. Thus, our overall findings suggest that W7 (a) is a useful tool to investigate mechanisms by which K^+ channels are regulated in human epithelia, and (b) may be of clinical interest as K^+ channel modulation is considered to be an important mode of therapeutic intervention in some diseases [33–35].

Acknowledgements—The authors thank Dr. Bruce J. Baum for his constant support and encouragement, Mrs. Beverly Handelman for excellent technical assistance, and Drs. Ingrid Valdez, Chih-ko Yeh, and Phil McKavity for constructive reviews of the manuscript.

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