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POTASSIUM TRANSPORT IN DISPERSED MUCOSAL CELLS FROM GUINEA PIG STOMACH

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Summary

Dispersed mucosal cells (approx. 70% parietal cells) prepared from guinea pig stomach maintained their cellular concentration of potassium (65—80 nmol potassium/10^6 cells) for at least 5 h in vitro. Uptake of ^{42}K by dispersed gastric mucosal cells depended on temperature, H^+ concentration and oxidative metabolism. Carbachol and, in some instances, gastrin caused a 40—50% increase in cellular uptake of ^{42}K as a consequence of the ability of these agents to increase ^{42}K influx. Ouabain reduced uptake of ^{42}K by 70% but did not alter the effect of carbachol. Cellular uptake of ^{42}K was not altered by histamine, prostaglandin E_1, glucagon, secretin, vasoactive intestinal peptide or C-terminal octapeptide of cholecystokinin. Uptake of ^{42}K was also increased by dibutyryl cyclic AMP or dibutyryl cyclic GMP but not by cyclic AMP, cyclic GMP or their 8-bromo derivatives. Theophylline caused a small (10—15%) increase in ^{42}K uptake and potentiated the increase caused by submaximal concentrations of carbachol. The increase in ^{42}K uptake caused by either dibutyryl cyclic nucleotide and carbachol was additive.

Introduction

Suspension of dispersed cells are useful for studying tissue biochemical functions at the cellular level. A suspension of dispersed cells offers the potential for enriching and isolating a particular cell type and at the same time removing regulatory influences that originate from other cell types. Preparations of dis-

Abbreviations: cyclic AMP, adenosine 3′,5′-monophosphate; cyclic GMP, guanosine 3′,5′-monophosphate; dibutyryl cyclic AMP, $N^6,O^{2'}$-dibutyryl adenosine 3′,5′-monophosphate; dibutyryl cyclic GMP, $N^6,O^{2'}$-dibutyryl guanosine 3′,5′-monophosphate; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

persed cells also offer the technical advantages of enabling one to obtain multiple, identical samples at different times during a particular biochemical reaction and of abolishing the tissue extracellular space which may constitute a substantial diffusion barrier.

Several investigators have reported techniques for preparing dispersed gastric mucosal cells [1—6]; however, these procedures have been relatively complicated and the functional integrity of these preparations has been examined only to a limited extent. In the present studies we have developed a relatively simple procedure for preparing dispersed mucosal cells from guinea pig stomach. Since stimulants of gastric acid secretion cause changes in potassium content of gastric mucosa [7,8] and since potassium-dependent ATPase has been postulated to be involved in acid secretion [9—13], we have examined the ability of various agents to modify transport of ^{42}K by these cells.

Materials

Male Hartley albino guinea pigs (200—250 g) were obtained from the Small Animals Section, Veterinary Resources Branch, National Institutes of Health, Bethesda, Md. ^{42}K (90 Ci/mol) was from International Chemical and Nuclear Co, Irvine, Calif.; 3-*O*-D-[^{14}C]glucose (50 Ci/mol) from New England Nuclear, Boston Mass.; bovine serum albumin from Armour; nylon mesh screen (No. 114T) from Nytex; carbamylcholine, theophylline, crude collagenase, cyclic AMP, cyclic GMP and both their 8-bromo and dibutyryl derivatives from Sigma Chemical Co., St. Louis, Mo.; diphenhydramine from Parke, Davis and Co., Detroit, Mich.; hexadecapeptide amide of gastrin (gastrin 2-17) from Imperial Chemical Industries Ltd., Alderly Park, Macclesfield, Chesire, England; histamine, ouabain and atropine from Calbiochem, Los Angeles, Calif. and glucagon from Eli Lilly Co., Indianapolis, Ind. Prostaglandin E_1 was gift from Dr. John Pike, Upjohn Co., Kalamazoo, Mich. Natural porcine vasoactive intestinal peptide and natural porcine secretin were gifts from Dr. Viktor Mutt, Karolinska Institutet, Stockholm, Sweden. Synthetic C-terminal octapeptide of porcine cholecystokinin was gift from Miguel A. Ondetti, Squibb Institute, Princeton, N.J. Metiamide was a gift from Smith, Kline and French Laboratories, Philadelphia, Pa. Liquid scintilation fluid used in these studies contained 15 parts toluene (J.T. Baker Chemical Co.), 5 parts Triton X-100 (New England Nuclear) and 1 part Liquifluor (New England Nuclear). Hank's buffer and Medium 199 were from GIBCO or were prepared in our laboratory. The standard buffer solution was a modification of Hank's buffer and contained: 137 mM NaCl, 5.37 mM KCl, 1.26 mM $CaCl_2$, 0.47 mM $MgCl_2$, 0.41 mM $MgSO_4$, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 5.55 mM glucose, 2.0 mM glutamine, BME vitamin solution (GIBCO), 0.1% (w/v) phenol red, 15 mM $NaHCO_3$ and 15 mM HEPES (pH 7.4). In some experiments bicarbonate was omitted and was replaced by equivalent amount of HEPES (pH 7.4). In others, bicarbonate and HEPES were replaced by 30 mM citrate/phosphate.

Methods

Cell isolation procedure. Usually three guinea pigs were used for each experiment. Each animal was killed by cervical dislocation and the gastric fundus was

removed and rinsed with iced (4°C) saline. Adherent mucus was removed by gently wiping the mucosa with paper tissue. The fundic mucosa was scraped from the muscularis, placed in standard buffer and rinsed once. The mucosa was pressed through a stainless steel grid (60 gauge) and suspended in standard buffer. The mucosal fragments were transfered to a 50 ml conical test tube, washed once with standard buffer and sedimented by centrifugation at $50 \times g$ for 7 min. The pellet was suspended in standard buffer containing 0.1% (w/v) crude collagenase, 0.1% (w/v) bovine serum albumin and 10 mM EDTA and incubated at 37°C. The incubation mixture was shaken (120 rev./min) and gassed continuously by bubbling the mixture with water-saturated 95% O_2/5% CO_2 during all stages of cell preparation. After 15 min of incubation the shaking was stopped, the mucosal fragments were allowed to settle and the supernatant was discarded. Fresh digestion solution without EDTA was added. The mucosal fragments were subsequently incubated for three 20-min periods during which the fragments were periodically passed through pasteur pipet to minimize aggregation. At the end of each incubation period the shaking was stopped, the mucosal fragments were allowed to settle and the supernatant was collected. The supernatant from the second and third incubation periods, which contained mostly single cells, was pooled and passed through nulon mesh to remove cell clumps. The dispersed cells were collected by centrifugation at $100 \times g$ for 10 min and were washed twice with fresh, iced standard buffer. When the cells were suspended in a solution other than the standard incubation solution is was introduced during the final two washes. The suspended cells (>97%) single cells) were counted in a hemocytometer using phase contrast microscopy. The cell preparation procedure required 2.5 h and 10^8 cells were obtained from the stomachs of three guinea pigs.

The suspension of dispersed gastric cells contained 70—80% parietal cells with the remainder being mucous, chief, endocrine and undifferentiated cells that could not be identified. By light and electron microscopy the parietal cells were 10—15 μm in diameter, had abundant large mitochondria and the characteristic tubulovesicular system [14].

We observed that removing the mucosa from the muscularis improved the effectiveness of subsequent tissue digestion. The optimum concentration of collagenase was 0.1% (w/v). Lower concentrations required longer incubation times and higher concentrations impaired cell viability. Other enzymes such as pronase 0.05—0.2% (w/v) or hyaluronidase 0.05—0.2% (w/v) required longer incubation times to release cells and the digestion was not complete. With Hank's buffer the time required for digestion was shorter and the yield of viable cells was greater. With Medium 199 only 20—30% of the cells obtained were viable while with Krebs-Ringer bicarbonate longer incubation times were required for digestion and cell viability was impaired. Stomachs from young, fed animals yielded more cells than did those from older or fasted animals. Finally, passing the mucosa through a stainless steel grid prior to digestion and periodically passing the mucosal fragments through a pasteur pipet during the incubation were critical for efficient digestion of the tissue.

The dispersed cells remained viable for 5—6 h as determined by their ability to exclude trypan blue (>95%), consume oxygen (2.75 μmol $O_2/10^6$ cells per h), maintain their intracellular potassium and by their response to various secre-

togagues. The rate of formation of $^{14}CO_2$ from radioactive glucose was constant for at least 2 h. Histamine, prostaglandin E_1 and theophylline caused a 12-, 3- and 5-fold increase in cellular cyclic AMP, respectively, and upon incubation with histamine or carbachol the parietal cells underwent characteristic morphological changes in the tubulovesicular system similar to those observed in intact gastric mucosa [14]. Storing dispersed cells in the standard buffer at 4°C under 95% CO_2/5% O_2 atmosphere for 16—18 h, reduced viability by 50—60%. However, the ability of these cells to respond to carbachol as measured by ^{42}K uptake, was proportional to the fraction of viable cells in the suspension.

Potassium transport. Uptake of ^{42}K was measured using the technique described previously [15]. Gastric cells ($2 \cdot 10^6$—$8 \cdot 10^6$ cells per ml) were incubated at 37°C in standard buffer containing 6 mM ^{42}K. At appropriate times triplicate 100-μl samples were taken and the cells were washed three times with 300 μl of iced (4°C) 150 mM NaCl. Washing was by alternate centrifugation (10 000 × *g* for 15 s) using a Microfuge (Beckman Instrument Co.) and resuspension. 100 μl of 10% (v/v) $HClO_4$ was added to the washed cells. The mixture was agitated, centrifuged at 10 000 × *g* for 45 s and the supernatant was dispersed into 17 ml of liquid scintillation solution. ^{42}K uptake was calculated from the cellular radioactivity, the specific activity of ^{42}K in the incubation solution, and the cell concentration.

To measure outflux of ^{42}K, cells ($6 \cdot 10^6$—$8 \cdot 10^6$ cells/ml) were preincubated in standard buffer containing 6 mM ^{42}K for 30 min at 37°C. At the end of the preincubation cells were washed three times with 12 ml iced standard buffer by centrifugation (250 × *g* for 3 min) and resuspension. Cells were incubated with standard buffer containing no radioactivity. At the beginning of and at appropriate times during the incubation triplicate 100-μl samples were taken for determination of cellular ^{42}K. Outflux of ^{42}K was calculated from the loss of cellular radioactivity during the incubation. In all experiments, variation among triplicate samples was less than 10%.

To measure cellular potassium by flame photometry, cells ($4 \cdot 10^6$ cells/ml) were suspended in standard incubation solution and incubated at 37°C. After 20 min 10 ml of cell suspension was taken and the cells were washed four times with iced, potassium-free incubation solution by alternate centrifugation (250 × *g* for 3 min) and resuspension. The cells were then lysed in a solution containing 15 mM LiCl and 0.05% (v/v) Triton X-100. The potassium concentration in the lysate was determined by flame photometry using KCl standards prepared in lysing solution. Cellular potassium was calculated from the potassium concentration in the lysate and the cell concentration.

Results

Uptake of ^{42}K by guinea pig gastric cells reached an apparent steady state after 12—14 min incubation (Fig. 1, left). Reducing the incubation temperature from 37 to 4°C decreased ^{42}K uptake by 95%. When outflux of ^{42}K was determined, the natural logarithm of the fraction of cellular ^{42}K originally present was a linear function of time for at least 12 min (Fig. 1, right). The calculated coefficient for ^{42}K outflux from gastric mucosal cells was 0.065/min, and at 4°C it was reduced by more than 90% (to 0.0046/min). This latter finding indi-

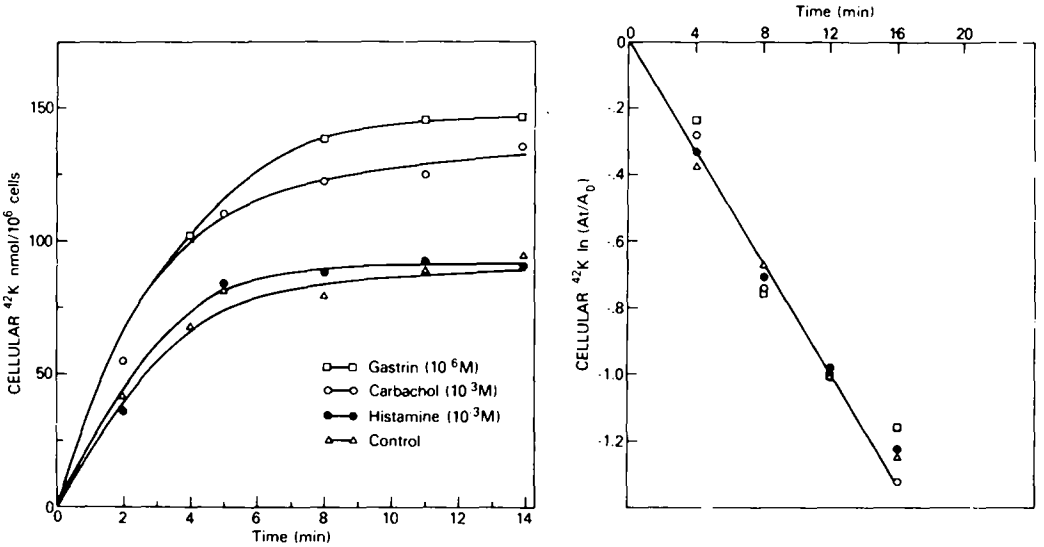

Fig. 1. Effect of gastric secretagogues on uptake and release of ^{42}K by guinea pig gastric cells. Left panel: Cells were suspended in standard incubation solution and incubated at 37°C with 6 mM ^{42}K and the indicated agents. Right panel: Cells were preincubated in standard incubation solution containing 6 mM ^{42}K at 37°C. After 30 min, cells were washed three times with iced standard incubation solution containing no radioactivity and incubated at 37°C with the indicated agents. At the beginning and at appropriate times during the incubation cellular ^{42}K was determined. The natural logarithm of the fraction of original cellular ^{42}K (A_t/A_0) is plotted as function of incubation time. Each point is the mean of triplicate determinations. This experiment is representative of two others.

cates that the loss of cellular ^{42}K during 2—3 min washing procedure required to determine cellular ^{42}K was negligible. Uptake of ^{42}K by dispersed gastric mucosal cells depended on the pH of the incubation medium. Maximal uptake of ^{42}K occurred at pH 7.0 while a decrease or an increase in the pH caused a progressive reduction in ^{42}K uptake. The reduced uptake of ^{42}K caused by changing the pH was not due to altered cell viability since returning the pH to 7.0 restored the cellular uptake of ^{42}K.

Carbachol (1 mM), a cholinergic agent which is a poor substrate for acetylcholinesterase [16] and gastrin (1 μM) but not histamine (1 mM) caused a 50% increase in uptake of ^{42}K by gastric cells (Fig. 1, left). The combination of histamine plus carbachol or histamine plus gastrin did not increase cellular uptake of ^{42}K above that observed with carbachol alone or gastrin alone. When carbachol was combined with gastrin the increase of cellular ^{42}K was the same as with either agent alone. Since outflux of ^{42}K was not altered by carbachol or gastrin (Fig. 1, right), the increase in cellular uptake of ^{42}K caused by these agents was due to increased influx of ^{42}K. In approx. 40% of our experiments ^{42}K uptake by gastric cells did not increase with gastrin but did increase with carbachol. We do not know the basis for this variation. Uptake of 3-*O*-methyl-[^{14}C]glucose by dispersed gastric cells reached apparent steady state after 5 min incubation and was not altered by 1 mM carbachol or 1 μM gastrin. Cellular potassium determined by flame photometry was 58 ± 5 nmol/10^6 cells (mean ± 1 S.D. of three experiments) and after 20 min with 1 mM carbachol was 78 ± 6 nmol/10^6 cells. In the same preparations cellular potassium deter-

mined from uptake of ^{42}K was 67 ± 5 nmol/10^6 cells and after 20 min incubation with 1 mM carbachol was 91 ± 7 nmol/10^6 cells. Uptake of cellular ^{42}K increased when carbachol was added to the incubation medium at the same time as the isotope (Figs. 1 and 2), or when carbachol was added after cellular ^{42}K had reached a steady state (Fig. 2). Increased cellular uptake of ^{42}K could be detected at 10^{-5} M carbachol (Fig. 3), was half maximal at $8 \cdot 10^{-5}$ M and maximal at 10^{-3} M. Adding 5 mM theophylline caused a small (10—15%) increase in ^{42}K uptake in control cells and enhanced the increased uptake caused by carbachol (Fig. 3). Cellular ^{42}K was not altered by prostaglandin E_1 (10^{-4} M), glucagon, secretin, vasoactive intestinal peptide or by the C-terminal octapeptide of cholecystokinin (all 1 μM).

The increase in cellular ^{42}K caused by carbachol could be inhibited by atropine but not by metiamide, a histamine H_2-receptor antagonist, or by diphenhydramine, a histamine H_1-receptor antagonist (Table I). Atropine, metiamide or diphenhydramine alone did not alter uptake of ^{42}K. Ouabain, an inhibitor of ($Na^+ + K^+$)-ATPase [17], reduced cellular uptake of ^{42}K by 70% but did not alter the stimulation caused by carbachol (Table I). Cellular uptake of ^{42}K was inhibited by 10 mM azide (87% inhibition) and 0.1 mM dinitrophenol (63% inhibition) but not by 10 mM fluoride (Table I).

Since in gastric mucosa histamine increases cellular cyclic AMP [18] and acetylcholine increase cyclic GMP [19], we tested the ability of cyclic nucleotides to alter cellular uptake of ^{42}K. Native cyclic nucleotides or their 8-bromo derivatives did not alter the uptake of ^{42}K (Table II). Dibutyryl cyclic AMP or dibutyryl cyclic GMP (both 1 mM) increased by 40—50% the uptake of ^{42}K (Table II). Butyrate (6 mM) did not alter cellular uptake of ^{42}K (not shown). For each dibutyryl cyclic nucleotide, half-maximal stimulation of ^{42}K uptake occurred at a nucleotide concentration of 1 mM and maximal stimulation

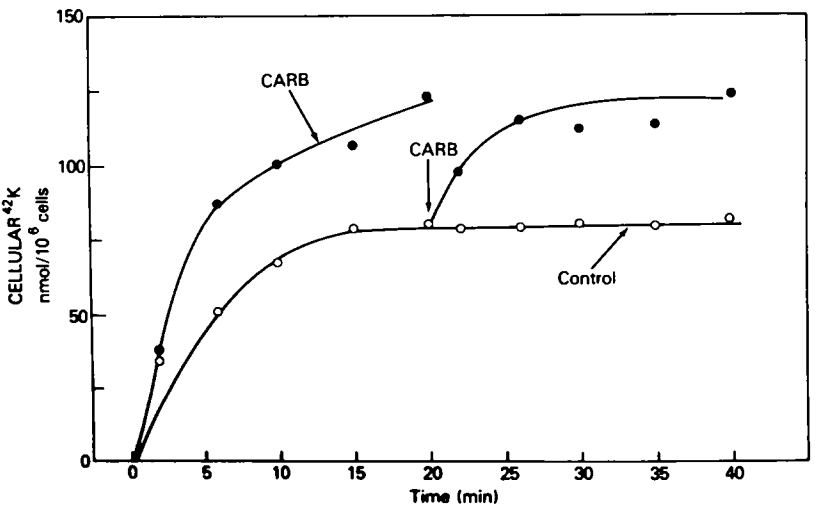

Fig. 2. Effect or carbachol on uptake of ^{42}K by guinea pig gastric cells. Cells were suspended in standard incubation solution and incubated at 37°C with ^{42}K. Carbachol (1 mM) was added at the beginning of the incubation or after 20 min. Each point is the mean of triplicate determinations. This experiment of representative of two others.

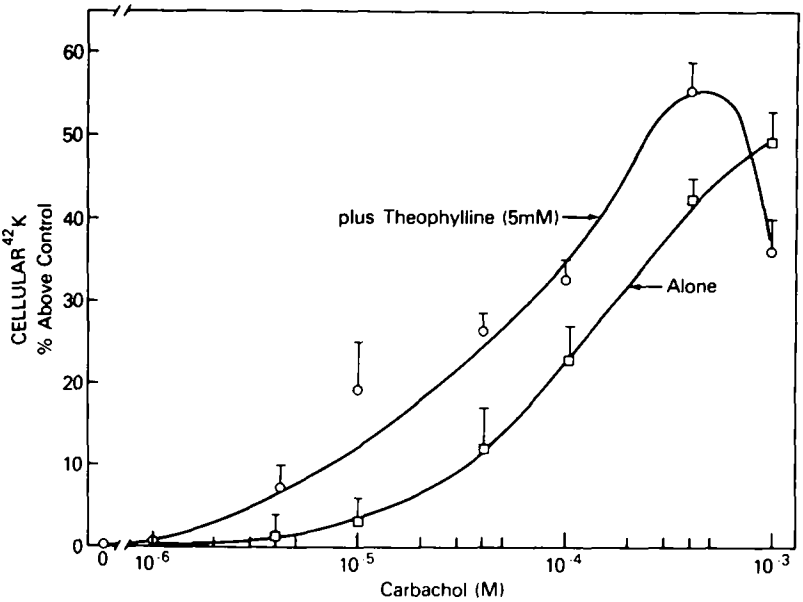

Fig. 3. Effect of carbachol and theophylline on uptake of ^{42}K by guinea pig gastric cells. Cells were suspended in standard incubation solution with or without 5 mM theophylline and incubated with ^{42}K plus the indicated concentrations of carbachol for 15 min at 37°C. Vertical lines represent 1 S.D. Each point is the mean of three separate experiments. Cellular ^{42}K in gastric cells incubated without carbachol was 70 ± 6 nmol/10^6 cells.

occurred with 5 mM (Fig. 4). When the dibutyryl derivative of either cyclic nucleotide was combined with carbachol, the increase in ^{42}K uptake was additive (Table II). Histamine did not alter the increase in ^{42}K uptake caused by dibutyryl cyclic AMP or by dibutyryl cyclic GMP.

TABLE I

EFFECT OF VARIOUS AGENTS ON UPTAKE OF ^{42}K BY GUINEA PIG GASTRIC CELLS

Cells were suspended in standard buffer and incubated with ^{42}K plus the indicated agents for 10 min at 37°C. Values are means (±S.D.) from three experiments.

Incubation	Cellular ^{42}K (nmol/10^6 cells)
Control	68 ± 5
Carbachol (1 mM)	102 ± 7 *
plus atropine (10 μM)	69 ± 10
plus metiamide (1 mM)	103 ± 10 *
plus diphenhydramine (1 mM)	100 ± 6 *
plus ouabain (0.1 mM)	56 ± 4 **
Ouabain (0.1 mM)	25 ± 3 *
Atropine (10 μM)	70 ± 6
Metiamide (1 mM)	67 ± 10
Diphenhydramine (1 mM)	65 ± 8
Fluoride (10 mM)	68 ± 4
Dinitrophenol (0.1 mM)	25 ± 3 *
Azide (10 mM)	13 ± 2 *

* Significantly different ($P < 0.01$) from control by Student's t-test.

** Significantly different ($P < 0.01$) from ouabain alone by Student's t-test.

TABLE II

EFFECT OF CARBACHOL, HISTAMINE, CYCLIC NUCLEOTIDE AND THEIR DERIVATIVES ON UPTAKE OF ^{42}K BY GUINEA PIG GASTRIC CELLS

Cells were suspended in standard buffer and incubated with ^{42}K plus the indicated agents for 15 min at 37°C. Each agent tested was present at a concentration of 1 mM. Values are means (±1 S.D.) from three experiments.

Incubation	Cellular ^{42}K (nmol/10^6 cells)
Control	70 ± 5
Carbachol	99 ± 5 *
Histamine	77 ± 4
Cyclic AMP	71 ± 10
8-Bromo cyclic AMP	66 ± 6
Dibutyryl cyclic AMP	99 ± 6 *
plus carbachol	149 ± 16 **
plus histamine	93 ± 5
Cyclic GMP	68 ± 6
8-Bromo cyclic GMP	71 ± 8
Dibutyryl cyclic GMP	103 ± 9 *
plus carbachol	135 ± 5 **
plus histamine	97 ± 4

* Significantly different ($P < 0.01$) from control by Student's t-test.
** Significantly different ($P < 0.01$) from carbachol alone by Student's t-test.

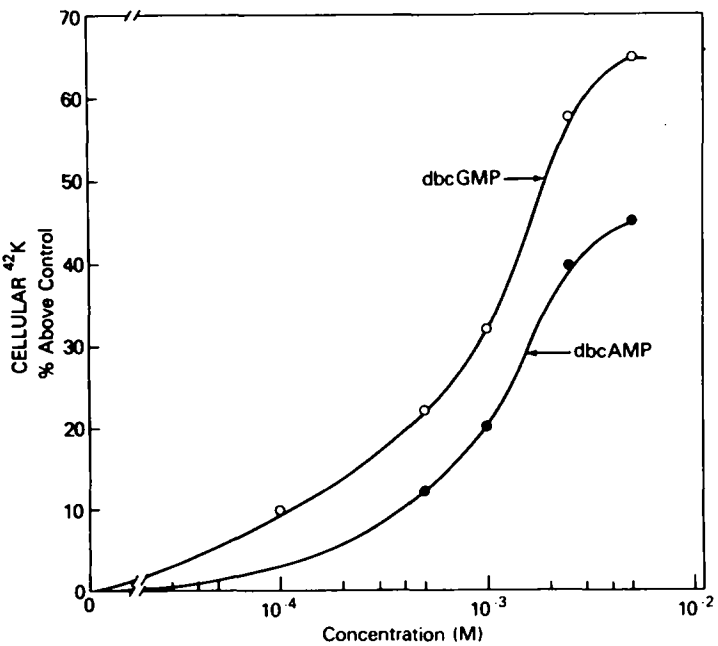

Fig. 4. Effect of dibutyryl cyclic nucleotides on uptake of ^{42}K by guinea pig gastric cells. Cells were suspended in standard incubation solution and incubated with ^{42}K for 15 min at 37°C. Each point is the mean of triplicate determinations. Cellular ^{42}K in cells incubated without cyclic nucleotides was 65 ± 5 nmol/10^6 cells. This experiment is representative of four others. dbcGMP, dibutyryl cyclic GMP; dbcAMP, dibutytyl cyclic AMP.

Discussion

We have developed a relatively simple technique to prepare dispersed gastric mucosal cells which show changes in potassium transport caused by some, but not all gastric secretagogues. Gastric mucosa was scraped from guinea pig stomach, disrupted into fragments and incubated with crude collagenase plus EDTA. Preparing small fragments enhanced digestion and as reported by others [20—22], introducing EDTA promoted disruption of cell junctions and thereby facilitated cell separation. The resulting cell suspension contained 70—80% parietal cells and compared to intact gastric mucosa, showed a 7-fold enrichment in parietal cells. Although several other methods for preparing dispersed gastric cells have been published previously [1—6], in most instances the viability and proportion of parietal cells have been variable and the response to gastric secretagogues, when reported [23,24], was not examined in detail.

Dispersed mucosal cells from guinea pig stomach maintained their cellular potassium content (65—80 nmol potassium per 10^6 cells) for up to 5 h in vitro and there was reasonably close agreement between cellular potassium determined by uptake of ^{42}K and that determined by flame photometry. Assuming that the dispersed cells are spheres 12 μm in diameter and contain 70% water, the calculated cellular potassium concentration is 100—125 mM. This value is close to that estimated for gastric mucosa in vivo [25] and indicates that in vitro dispersed gastric mucosal cells, like most other eukaryotic cells are capable of maintaining a cellular potassium concentration that is approx. 20-fold greater than that in the incubation medium. As is true of potassium transport in most other cell types in gastric mucosal cells influx and outflux of ^{42}K were reduced substantially by lowering the incubation temperature to 4°C and ouabain reduced ^{42}K uptake by 70%. Uptake of ^{42}K depended on the concentration of H^+ in the incubation medium and optimal uptake occurred at pH 7.0. Dinitrophenol or azide, but not fluoride, inhibited uptake of ^{42}K indicating that in gastric mucosal cells potassium uptake is dependent on oxidative metabolism.

Uptake of ^{42}K by dispersed gastric mucosal cells was increased consistently by carbachol, sometimes by gastrin, but never by histamine. In all preparations in which both gastrin and carbachol increased potassium uptake, the increase caused by the two agents in combination was the same as that caused by carbachol alone. We cannot explain the inability of gastrin to increase uptake of ^{42}K in approx. 40% of the experiments. Although we did not measure cell volume in the present studies, our finding that carbachol did not alter cellular uptake of 3-*O*-methylglucose suggests that the increased uptake of ^{42}K caused by carbachol did not result from an increase in cell volume. Carbachol did not alter outflux of ^{42}K from dispersed gastric mucosal cells; therefore, its effect on ^{42}K uptake reflects increased potassium influx. The increased potassium uptake caused by carbachol was inhibitable by atropine but not by either of two histamine receptor antagonists. This result coupled with our finding that histamine did not alter transport of ^{42}K in gastric cells indicates that the action of carbachol on potassium transport is not mediated by histamine. The failure of histamine to alter potassium transport also suggests that the increase in potassium uptake does not occur simply as a consequence of increased acid secretion by

these cells. Incubating gastric mucosal cells with carbachol, gastrin or histamine did not cause detectable acidification of the incubation medium. Dispersed gastric mucosal cells may be incapable of secreting H^+, or more likely, any H^+ that are secreted are neutralized by the stoichiometric liberation of HCO_3^- [26].

Ouabain inhibited cellular uptake of ^{42}K by 70% did not alter the increased uptake of ^{42}K caused by carbachol. This finding is consistent with observations by others [27,28] that membrane vesicles prepared from gastric mucosa possess a ouabain-insensitive, potassium-dependent ATPase and that these vesicles accumulate H^+ when incubated with potassium and ATP.

Theophylline, an inhibitor of cyclic nucleotide phosphodiesterase [29], caused a small increase in ^{42}K uptake and enhanced the increase in ^{42}K uptake caused by submaximal concentrations of carbachol. Since muscarinic cholinergic agents increase cyclic GMP in a number of tissues including gastric mucosa [30] and since dibutyryl cyclic GMP increased ^{42}K uptake in our cell preparation, the increase in potassium uptake caused by carbachol and the enhancement caused by theophylline may reflect actions which are mediated by cyclic GMP. On the other hand, we have not shown that carbachol increases cellular cyclic GMP in our cell preparation and uptake of ^{42}K was also increased by dibutyryl cyclic AMP. Furthermore, native cyclic nucleotides or their 8-bromo derivatives did not alter potassium transport in dispersed gastric mucosal cells.

We cannot offer a clear, unequivocal explanation for the effects of dibutyryl cyclic nucleotides observed in the present studies. In addition to the lack of effect of exogenous native cyclic nucleotides, the effects on ^{42}K uptake of carbachol and a dibutyryl cyclic nucleotide were additive. Furthermore, although dibutyryl cyclic AMP increased uptake of ^{42}K, histamine and prostaglandin E_1 each increase cellular cyclic AMP in our preparation but do not alter potassium transport. Two possible mechanisms of action of dibutyryl cyclic nucleotides do appear to be excluded by our results. Their effects do not appear to be attributable to contamination of these compounds by butyric acid since 6 mM butyrate did not alter potassium transport. Dibutyryl derivatives of cyclic nucleotides can inhibit hydrolysis of native cyclic nucleotides by phosphodiesterase [31]; however, this sort of mechanism cannot account for the effects of these agents in the present studies since theophylline, unlike dibutyryl cyclic nucleotides, did not increase uptake of ^{42}K in the presence of a maximally effective concentration of carbachol.

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