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ZERO EXTRACELLULAR K^+ AND PROSTAGLANDIN E RELEASE IN THE GUINEA-PIG TAENIA COLI

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Summary

Prostaglandin E release rates from isolated strips of guinea-pig taenia coli increased during exposure to zero K^+ bathing fluid, from control values of 0.78 ± 0.11 ng/g per min to levels as high as 29.2 ng/per min. Release rates increased for 40—50 min and then remained constant or fell despite progressive increases in intracellular sodium [Na_i^+] or fall in intracellular potassium [K_i^+]. Readmittance of K^+ to the bathing solution resulted in rapid reversal of elevated prostaglandin E release rates. [Na_i^+] and [K_i^+] were markedly more abnormal in strips exposed to zero K^+ for 70—201 min compared to 30-min exposures. Upon the readdition of K^+ after long zero K^+ exposure, the rate of prostaglandin E release fell long before [Na_i^+] and [K_i^+] returned to control levels. After K^+ was readded to the bathing solution, the ion concentration of tissues exposed to zero K^+ for 30 min returned to normal much more quickly than did those of tissues exposed for the longer time periods, yet the exponential rate constants for fall of prostaglandin E release rate after K^+ was added were not significantly different after short or long zero K^+ exposure. Thus there was a dissociation between the return of [Na_i^+] and [K_i^+] and the fall of prostaglandin E release rate to control levels. Ouabain augmented prostaglandin E release under conditions where [K_i^+] could not fall. Addition of known neurotransmitters present in this tissue to the bathing fluid did not augment prostaglandin E release. Guinea-pig taenia coli strips that had been incubated with [^{3}H]arachidonic acid, constantly released [^{3}H]arachidonic acid and [^{3}H]prostaglandin E and a prostaglandin which cochromatographed with prostaglandin E but could not be converted to prostaglandin B by alkali and was shown to be 6-ketoprostaglandin $F_{1\alpha}$. Release of [^{3}H]arachidonic acid and [^{3}H]prostaglan-

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din E plus 6-[^{3}H]ketoprostaglandin $F_{1\alpha}$ was increased when strips were exposed to zero K^+. Data obtained in this study suggest the augmented prostaglandin E release seen during zero K^+ or ouabain is related to increased availability of unbound arachidonic acid at the site of cyclooxygenase in the cell. Augmented prostaglandin E release is apparently not related to alterations in intracellular electrolyte concentrations or release of known neurotransmitters.

Introduction

Treatment of isolated strips of guinea-pig taenia coli with ouabain, or zero extracellular K^+ markedly augmented release of prostaglandin E from the strips into bathing solution [1]. This experiment was performed because application of exogenous prostaglandin E in very small concentrations to isolated guinea pig taenia coli caused a plasma membrane effect, i.e. depolarization with increase in spontaneous action potentials, and decreased membrane resistance [2]. It seemed a likely possibility that the effector structure of the cell, the plasma membrane, might exert feedback control on prostaglandin E synthesis and/or release.

Endogenous prostaglandin-like compounds may modulate actions of various neurotransmitters on some smooth muscle cells or may modulate neurotransmitter release [3,4] and might be involved in the mechanisms controlling spontaneous myogenic contractions or smooth muscle tone [2,5]. A plasma membrane Na^+ pump has been postulated to have a role in generation of slow waves which control the rate of spontaneous myogenic contractions [6]. $(Na^+ + K^+)$-ATPase and the Na^+ pump may be involved in some effects of neurotransmitters on smooth muscle cells [7—9]. Ouabain and zero K^+ alter responses of the smooth muscle to a number of transmitters and agonists [10]. Working out the mechanism or mechanisms by which inhibition of a plasma membrane $(Na^+ + K^+)$-ATPase influences prostaglandin E release might relate to the above findings and add basic information about the role of plasma membrane in control of prostaglandin E release from cells.

Zero K^+ or ouabain effects on prostaglandin E synthesis and release may not be directly coupled to inhibition of the Na^+ pump in smooth muscle cells. Almost all of the cells in this tissue are smooth muscle [11], so it is likely that prostaglandin E synthesis occurs in smooth muscle cells; however, the possibility that neurotransmitters are released as a result of zero K^+ or ouabain treatment and that free neurotransmitters are involved in prostaglandin E release needs to be evaluated. Prostaglandin synthesis or release mechanism, or mechanisms, which are altered by zero K^+ or ouabain might involve changes in intracellular electrolyte concentrations or membrane Na^+ or K^+ gradients rather than an effect mediated directly by inhibition of the plasma membrane Na^+ pump or $(Na^+ + K^+)$-ATPase.

In the present study we further characterized temporal relationships of exposure of strips to zero K^+ and release of prostaglandin E and the reversibility of this phenomenon. We 'loaded' muscle strips with [^{3}H]arachidonic acid and studied effects of zero K^+ on release of [^{3}H]prostaglandin E and [^{3}H]-arachidonic acid. We investigated whether increases in prostaglandin E release

from strips of guinea-pig taenia coli on exposing them to zero K^+ could be caused by an effect of an intracellular electrolyte on a rate-limiting process involved in prostaglandin E release. We also studied the possibility that known neurotransmitters present in the guinea-pig taenia coli could be involved in augmented prostaglandin E release during zero K^+.

Methods

Adult white male guinea-pigs (600 g) were stunned on the back of the head and exsanguinated by cutting the jugulars and carotids. An incision was made in the abdomen and strips of taenia coli 15—25 mm in length were dissected out. Four or five strips with total mass 40—80 mg were mounted in a 5 ml volume organ bath and 0.5 g tension was applied to each strip. The isometric tension of one strip in each bath was monitored with a strain gauge (Model F103C, Grass Instruments Co.). The strips were bathed in a modified Krebs' solution which contained (mM): Na^+ 137; K^+ 5.9; Mg^{2+} 1.2; Ca^{2+} 2.5; Cl^- 134; $H_2PO_4^-$ 1.2; HCO_3^- 15.4; glucose 11.5. In experiments involving Krebs' solution without K^+ (zero K^+ Krebs), K^+ was replaced by an equal molar quantity of Na^+. The solutions were bubbled with a 3% CO_2/97% O_2 mixture which produced a pH of 7.33—7.38. All experiments were performed at 37 ± 0.1°C.

Zero K^+ experiments: measurement of prostaglandin E release. After an equilibration period in the organ bath with fresh warmed Krebs' solution for at least 1 h, control measurements of prostaglandin E release were made by measuring the increase in bathing solution prostaglandin E over a 60 min period. The bathing solution was then switched to zero K^+ Krebs for periods of time varying from 27 to 201 min and effects on prostaglandin E release into the bathing solution determined. K^+ was then readmitted into the bathing solution and several 5—10-min fluid samples were collected, followed by a final collection for about 1 h. We usually paired a 'long' (70—201 min) and 'short' (27—31 min) zero K^+ exposure in each experiment by using two organ baths each containing a set of muscle strips from the same animals.

Effects of neurotransmitters known to be present in taenia coli. Control prostaglandin E release rates were determined over a 60 min period and acetylcholine (10^{-6} g/ml) or norepinephrine (10^{-7} g/ml) were injected into the organ bath fluid and the prostaglandin release measurement repeated. We also determined effects of zero K^+ on prostaglandin E release, as described above, under conditions where atropine (10^{-6} g/ml) was present in the bathing solution.

Measurement of prostaglandin E release rate. In most of the experiments we assayed bathing solution samples directly on rat fundus [2] and gave the data in terms of prostaglandin E-like activity. With this method we can detect release of 0.5 ng into the bathing solution. Precision of the prostaglandin E release rate measurement depends on sampling time; for a 60 min collection we can detect ±0.1 ng/min per g wet wt.; for a 5 min collection ±1 ng/min per g.

In some of the experiments bathing fluid samples were acidified to a pH of 3.0, extracted with ethylacetate, which was then evaporated at reduced pressure. The residual was redissolved in Krebs' solution and assayed on rat fundus. Results were similar to those seen with direct assay.

It is established that release of contractile activity by isolated guinea pig

taenia coli strips in bathing solution, as measured on rat fundus, is entirely abolished by treatment of guinea-pig taenia coli with several different prostaglandin synthetase inhibitors [1,2]. 'Prostaglandin E activity' cochromatographs with prostaglandin E_2 [1]. In a subsequent study in our laboratory we utilized radioimmunoassay as well as biological assay found no difference in increases in prostaglandin E release during zero K^+. Thus specificity of biological assay for prostaglandin E appears to be satisfactory.

Measurement of tissue ion content. The extracellular fluid space was determined using [1,2-^{3}H]polyethylene glycol, mol. wt. 900 (New England Nuclear Corp.). Strips were exposed to the extracellular marker (approximately 1 μCi/ml bathing solution) for a 10—12 min period. They were then removed from the bath. The strip ends were transected and removed with the thread. The strips were blotted on Whatman 541 filter paper and placed on No. 5 glassine powder paper. Wet and dry weight were determined using standard methods. The extracellular marker and the tissue ion content were extracted from the tissue by immersing the muscle strips in 5 ml of 0.1 N HNO_3 for at least 20 h at room temperature (21°C). Attempts at solubilizing the residue resulted in further recovery of less than 3% of the total extracted radioactivity. After extraction a 0.5 ml aliquot of the sample was added to 4.5 ml Hydromix scintillation fluid (Yorktown Research) and the isotope activity was counted using a Packard TriCarb Liquid Scintillator Counter (Packard Instruments) and corrected for quenching. The extracellular space (ml of extracellular volume) and intracellular space were computed from these data [12]. The remainder of the sample was used to measure the Na^+ and K^+ content using an EEL spectrophotometer (Evans Electroselenium Ltd., Halstead-Essex, U.K.) with a Na^+-K^+ hollow cathode lamp (Jarrell Ash Fisher Scientific Company). Aliquots of the tissue extraction samples and of the bathing solutions were diluted with 0.1 N HNO_3 to concentrations ranging between 0.01 and 0.15 mM Na^+ or K^+ and were compared to standards. $[K_i^+]$ and $[Na_i^+]$ were computed by dividing total mmol of ions minus extracellular ions, by the cell water volume.

Determination of the K^+ content by spectrophotometry resulted in artifactually elevated values due to the presence of Na^+ and undefined ions in the solution. The artifact was removed by adding relatively large amounts of Na^+ (2 g/l) to both the sample and the K^+ standards so that the Na^+ content originally in the sample was insignificant compared to that added. A separate aliquot of each sample was analyzed for Na^+ and an aliquot used for measurement of K^+ after addition of Na^+. The K^+ content of extraction samples which were analyzed in this manner were lower by approximately 4% as compared to samples diluted only with HNO_3 and analyzed using K^+ standards without benefit of added Na^+. For those measurements of tissue K^+ content which had been measured without the added Na^+ procedure, a correction curve was made as a function of the Na^+ content and the values were adjusted accordingly.

Nine experiments were performed to assess the use of polyethylene glycol-900 as an extracellular marker in guinea pig taenia coli. In four experiments there was no significant difference in the computed extracellular space between runs where the polyethylene glycol incubation was 10 min and runs where the incubation time was 16, 32 or 60 min. This suggested equilibrium between the bathing fluid and extracellular space is achieved within 10 min and that the

marker does not leave the extracellular compartment in as long a time as 60 min. We compared polyethylene glycol and sorbital spaces in five experiments. Sorbital runs gave data which averaged 115 ± 8% of polyethylene glycol values.

Incubation of strips with [^{3}H]arachidonic acid. Strips of guinea-pig taenia coli were prepared as above and after equilibration in 37°C Krebs' solution for at least 60 min transferred to a flask containing [^{3}H]arachidonic acid in Krebs/albumin solution. This solution was prepared by slowly adding [^{3}H]arachidonic acid (greater than 98% purity, New England Nuclear, Boston, MA, 61 Ci/mmol) dissolved in hexane to a solution of fatty acid-free bovine serum albumin (Sigma Chemical Co., St. Louis, MO) at 60°C. Albumin-[^{3}H]arachidonic acid was then added to normal Krebs' solution giving an albumin concentration of $2.05 \cdot 10^{-7}$ M and radioactivity of $1.5 \cdot 10^{-4}$ dpm/ml. Strips were incubated in this solution for 30 min and then washed by transferring to flasks containing Krebs/albumin solution. After release of ^{3}H from tissue became constant, half of the strips were placed for 30 min in zero K^+ Krebs/albumin solutions and half in normal Krebs/albumin solution. These solutions were extracted in acid ethylacetate, dried with $MgSO_4$, evaporated to dryness, redissolved in chloroform and plated on silica gel 'H' plates (Analtech, Newark, DE). Plates were developed in chloroform/methanol/acetic acid (16 : 1 : 1, v/v/v) or diethyl ether/methanol/acidic acid (90 : 1 : 2, v/v/v). Zones corresponding to arachidonic acid and prostaglandin E_2 were scraped, extracted and counted in Hydromix in a liquid scintillation counter, correcting for quenching.

We characterized compounds which cochromatographed with prostaglandin E_2 with this system, by boiling with NaOH at pH 12.5 for 20 min, followed by acidification, extraction and thin-layer chromatography on 'G' plates using the same developing solutions as above. This procedure stoichiometrically converted prostaglandin E_2 to prostaglandin B_2. [^{3}H]Arachidonic acid taken from thin-layer plates was further studied by incubation with methanolic HCl, thereby converting arachidonic acid to its methyl ester, extraction in hexane and then injection into a gas chromatograph utilizing a stream splitter (Perkins Elmer Sigma III with 10% EGSS-X column). The relationship of peaks determined on the gas chromatograph and radioactivity was determined.

Results

Effect of zero K^+ on prostaglandin E release rate and mechanical tension

Baseline prostaglandin E release rate averaged 0.78 ng/g per min. ±0.11 (11 experiments). Fig. 1 shows some typical data obtained in experiments where strips of guinea-pig taenia coli were exposed to zero K^+ for the time periods of 27—200 min. In all experiments, prostaglandin E release rate increased progressively over the first 30—50 min of zero K^+ exposure. As described in a previous publication [1], increases in prostaglandin E release rate were impressive, frequently reaching peak values 50 times baseline rate (Table I). With more prolonged exposure, the results were variable in different experiments. In some preparations, prostaglandin E release rates continued to increase with time; in others it became constant, or peaked and fell spontaneously during the zero K^+ stimulus (Fig. 1).

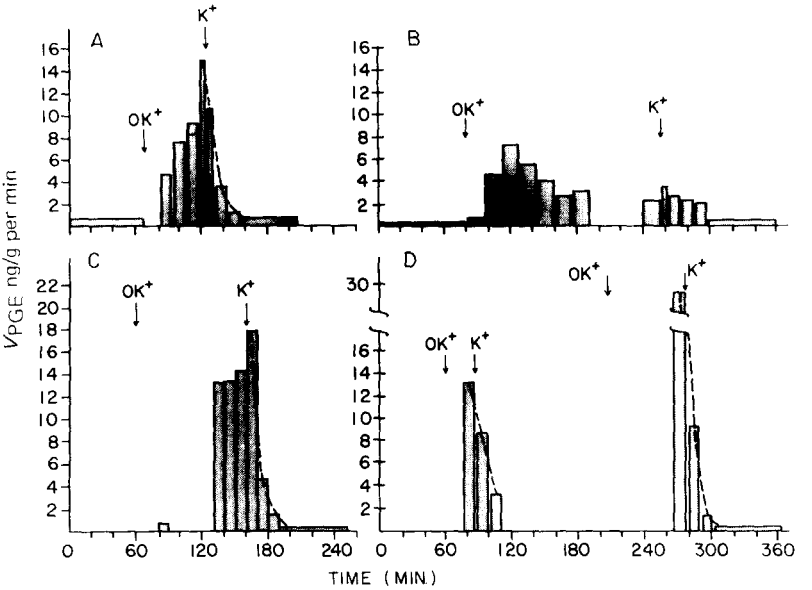

Fig. 1. Effect of zero extracellular K^+ on prostaglandin release. Typical data from four different experiments. Progressive increases in prostaglandin E release rate during zero K^+ exposure and rapid fall on readmitting K^+ to the bathing solution. B. An experiment where spontaneous fall in prostaglandin E release rate occurred during zero K^+. C. An experiment where readmittance of K^+ into the bathing solution caused transient increase in prostaglandin E release rate prior to fall to control level. D. A short (27 min) zero K^+ exposure and recovery and a long (72 min) zero K^+ exposure, performed on the same preparation. Samples were not collected during time intervals where there are no bars.

TABLE I

FALL IN PROSTAGLANDIN RELEASE AFTER K^+ WAS READMITTED TO BATHING FLUID

Isolated guinea-pig taenia coli strips were exposed to zero K^+ for either 27—32 min (short) or 70—201 min (long) and then K^+ was readmitted to the bathing fluid. Bathing fluid samples were taken every 5—10 min and prostaglandin E concentrations determined. Rates of prostaglandin E release were computed from the measurement. Initial rate constants are the fall in prostaglandin release rates ($\dot{V}_{PGE}$) determined during the first 10 min following peak $\dot{V}_{PGE}$. Method of computing this constant is illustrated in Fig. 1. Control prostaglandin E release rates in these experiments averaged 0.78 ng/g wet wt. per min.

Experiment	Duration zero K^+ (min)		Peak $\dot{V}_{PGE}$ (ng/g per min)		Initial rate constant (%/min)		$T_{1/2}$ (min)	
	Short	Long	Short	Long	Short	Long	Short	Long
1	27	72	13.2	29.2	8.3	7.5	1.8	3.6
2	31	91	1.6	2.4	9.1	9.1	1.6	1.6
3	31	75	5.4	8.4	7.6	7.3	4.2	4.7
4	31	91	4.3	11.0	6.5	6.0	6.2	7.0
5	32	78	4.7	12.5	3.3	7.4	25.0	4.5
6	31	201	5.5	11.9	10.7	9.6	2.3	3.5
7	30	70	1.7	18.0	4.9	8.1	10.0	4.2
Mean	30.4	96.9	5.2	13.3 *	7.2	7.9	7.3	4.2
± S.E.			1.5	3.2	0.9	0.5	3.2	0.6

* $P < 0.05$.

TABLE II

GUINEA-PIG TAENIA COLI K^+ AND Na^+ MEASUREMENTS

All data are means ± S.E. Numbers in parentheses indicate number of measurements. PEG, polyethylene glycol.

(A) At end of zero K^+	Control	30 min	90 min
Na^+ content (mmol/kg wet wt.)	85.9 ± 9.3 (4)	116.6 ± 6.6 * (3)	148.9 ± 3.5 * (4)
K^+ content (mmol/kg wet wt.)	93.0 ± 1.9 (4)	67.1 ± 1.4 * (3)	24.0 ± 5.3 * (4)
[^{3}H]PEG space (ml/kg wet wt.)	348.0 ± 19.1 (4)	383.2 ± 13.1 (2)	389.6 ± 8.5 (4)
Intracellular space (ml/kg wet wt.)	461.7 ± 17.2 (4)	418.7 ± 17.1 * (2)	397.6 ± 5.6 (4)
Dry/wet (%)	19.0 ± 0.9 (4)	20.5 ± 1.0 (3)	21.3 ± 0.8 (4)
Apparent [Na_i^+] (mM)	80.8 ± 20.2 (4)	130.8 ± 28.1 * (2)	237.9 ± 13.4 * (4)
Apparent [K_i^+] (mM)	196.7 ± 9.4 (4)	151.1 ± 7.8 * (2)	53.3 ± 14.4 * (4)

(B) 10 min after K^+ is readmitted	Control	Length of exposure to zero K^+ Krebs' solution		
		30 min	75 min	90 min
Na^+ content	79.2 ± 2.8 (21)	85.4 ± 4.3 (5)	117.3 ± 3.1 * (4)	166.1 ± 2.6 * (5)
K^+ content	90.2 ± 1.0 (21)	87.0 ± 2.4 (5)	63.1 ± 1.0 * (4)	58.9 ± 1.4 * (5)
[^{3}H]PEG space	318.7 ± 8.1 (21)	340.9 ± 18.4 (4)	370.8 ± 5.3 * (4)	353. 1± 14.0 (5)
Intracellular	492.0 ± 14.0 (21)	464.5 ± 19.8 (4)	415.0 ± 8.5 * (4)	440.5 ± 8.9 (5)
Dry/wet	19.3 ± 0.2 (21)	19.9 ± 0.5 (5)	21.4 ± 0.8 (4)	20.7 ± 0.3 (6)
Apparent [Na_i^+] (mM)	74.0 ± 5.4 (21)	87.4 ± 8.8 (5)	161.8 ± 9.6 * (4)	150.6 ± 7.8 * (4)
Apparent [K_i^+] (mM)	181.0 ± 3.4 (21)	183.3 ± 4.2 (5)	148.0 ± 5.9 * (4)	128.2 ± 2.6 * (4)

* $P < 0.05$ compared to control data.

Spontaneous contractions were always seen in our preparations under control conditions, which were identical to those described previously in many laboratories which have used this muscle. With zero K^+ treatment there was an initial excitatory response followed by cessation of active tension and spontaneous mechanical activity, as illustrated previously [1].

Changes in total tissue Na^+ and K^+ content as a function of exposure time to zero K^+ Krebs' buffer are listed in Table II. The values after 'short' and 'long' exposure to zero K^+ are markedly different from one another and from control levels.

Reversal of zero K^+ effects

In Table II are also listed the Na^+ and K^+ total tissue ion contents and other data observed after the addition of K^+ to the bathing solution. Data are listed for a time 10 min after K^+ readmittance to the organ bath, since at this time [Na_i^+] and [K_i^+] have returned to control levels in 'short' zero K^+ experiments. The 'long' values were still markedly abnormal illustrating the differences seen in recovery in the 'short' and 'long' experiments. The return of computed [K_i^+] toward normal levels after the addition of K^+ is also shown in Fig. 2 for preparations exposed to zero K^+ Krebs. Recovery of mechanical activity of guinea pig taenia coli after K^+ was added to the bathing solution was also markedly prolonged after 'long' zero K^+ exposure, compared to 30-min exposures, probably reflecting a longer period of abnormal intracellular electrolyte concentrations.

On returning K^+ to the bathing solution, prostaglandin E release rate fre-

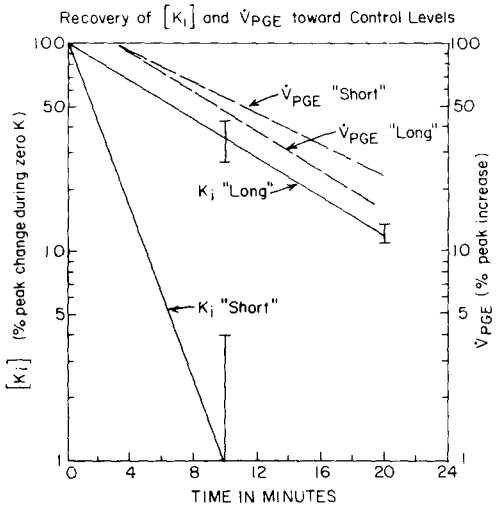

Fig. 2. Return of $[K_i^+]$ and prostaglandin E release rate $\dot{V}_{PGE}$ toward control levels after addition of K^+ to the bathing solution. (K^+ was added at time zero.) $[K_i^+]$ data are given in percent of peak change during zero K^+ and prostaglandin E release rate in percent of peak increase. Average changes in $[K_i^+]$ and prostaglandin E release rate are compared in short and long zero K^+ runs in five experiments. Note that peak prostaglandin E release occurred on the average 3.5 min after K^+ was added to the bathing solution, reflecting the finding that prostaglandin E release was initially stimulated in some experiments by K^+ addition. Prostaglandin E release rate plots were determined from the initial rate constant assuming fall is exponential. However, falls in prostaglandin E release rates from measured points without the exponential assumption give similar plots. Bars indicate ± S.E.

quently increased during the next 5 min and then fell rapidly to control levels (Fig. 1). It is not clear if this initial rise on adding K^+ was reflecting progressive increase during zero K^+ and time delay prior to reversal effect of K^+, or another process. In computing falls in prostaglandin E release rate, we plotted average values for the 5 or 10-min collection periods. The 'rate' change for the initial fall was determined from the slope of the plot drawn from peak prostaglandin release rate, as illustrated in Fig. 1.

Table I gives data in 'long' and 'short' experiments. Initial falls in prostaglandin E release rate in ng/g per min were much larger after 'long' zero K^+ exposure than after 'short' exposure. Calculating the data in terms of percent fall/min (i.e. a rate constant) or half-times indicated there was no difference in 'long' and 'short' data (Table I). The 201 min zero K^+ experiment gave data which most impressively showed no change in the rate constant compared to 'short' data since changes in $[Na_i^+]$ and $[K_i^+]$ were the most extreme (should have become nearly equal to $[Na_o^+]$ and $[K_o^+]$) [14]. In some of the 'long' experiments, K^+ was introduced at a time when prostaglandin E release rate was falling spontaneously. The rate constant for spontaneous fall was very small compared to that measured following addition of K^+, and if the processes were additive it would have only a small effect on the overall rate constant.

In comparing the fall in prostaglandin E release rate to $[K_i^+]$ measurements we selected data from five experiments where we either monitored prostaglandin E release during exposure to zero K^+ and knew that it was either still increasing or had reached a constant state, or where peak prostaglandin E

TABLE III

ACETYLCHOLINE, NOREPINEPHRINE AND 'HIGH' K^+ EXPERIMENTS

Strips of isolated guinea-pig taenia coli were mounted in organ baths. Release of prostaglandin E into bathing solution was determined by biological assay. In acetylcholine (ACh) and norepinephrine (NOR) experiments, control measurements were made over a 40—60 min period, ACh or norepinephrine injected into the bathing solution, and the measurement of prostaglandin E release determined again over the succeeding 30 min. In the high K^+ experiments, control prostaglandin E release was determined with Krebs' solution in paired experiments. Solutions were then changed to 140 mM K^+, 1.4 mM Na^+ in one organ bath and 140 mM K^+, 1.4 mM Na^+, 10^{-5} M ouabain in the other organ bath.

	ACh (10^{-7} g/ml, $N = 5$)	NOR (10^{-7} g/ml, $N = 3$)	140 mM K^+, 1.4 mM Na^+ ($N = 3$)	140 mM K^+, 1.4 mM Na^+ ($N = 3$)
$\dot{V}_{PGE}$ (ng/g per min)	0.34 ± 0.18	0.78 ± 0.22	0.53 ± 0.12	1.7 ± 0.30
% control	88 ± 32	121 ± 21	333 ± 102 *	731 ± 151 *

* $P < 0.05$.

release rate was very high suggesting that spontaneous fall had not occurred. The plot of these data is shown in Fig. 2. It was not possible to compare time for complete reversal of elevated prostaglandin E release rates in the 'long' and 'short' experiments since as prostaglandin E release rate falls to near normal it is necessary to collect bathing fluid samples for long time periods in order to be able to detect prostaglandin E activity on assay. Our comparison is limited to initial rate constants and the early part of the fall in prostaglandin E release rate after adding K^+.

High extracellular K^+ experiments: Na^+ pump inhibition under conditions where K_i^+ could not increase

Data from experiments where ouabain was added to the bathing solution under conditions where bathing fluid K^+ was 140 mM are shown in Table III. In experiments where the strips were exposed to 140 mM K^+ without ouabain there was augmented prostaglandin E release, but release rates were significantly higher when ouabain was included in the high K^+, low Na^+ bathing solution.

Prostaglandin E release during acetylcholine and norepinephrine

Data are shown in Table III which indicate that neither of these transmitters caused an increase in prostaglandin E release from isolated guinea-pig taenia coli strips. Mechanical effects of acetylcholine and norepinephrine were as expected; acetylcholine caused a phasic contraction and norepinephrine inhibited spontaneous mechanical activity.

[^{3}H]Arachidonic acid and [^{3}H]prostaglandin E release from loaded strips

Table IV compares release of [^{3}H]prostaglandin E and [^{3}H]arachidonic acid with the strips in Krebs/albumin and zero K^+ Krebs/albumin solutions. In these experiments between 30 and 50% of [^{3}H]arachidonic acid originally in the incubating solution was incorporated into the tissue. In every experiment, strips in zero K^+ Krebs/albumin solution showed an increased release of both compounds compared to control strips in Krebs/albumin. Indomethacin treat-

TABLE IV

[^{3}H]ARACHIDONIC ACID LOADING EXPERIMENTS

Guinea-pig taenia coli strips were incubated with [^{3}H]arachidonic acid in Krebs/albumin solution for 30 min. Following this, the strips were placed in Krebs/albumin solutions until release of ^{3}H was constant. Half of the strips were then placed in zero K^+ Krebs/albumin solution and the other half in K^+ Krebs/albumin solution, for 30 min. The solutions were then analyzed for [^{3}H]arachidonic acid and [^{3}H]prostaglandin E as described in the text. Data below are means from 11 experiments. Following exposure to zero K^+ Krebs/albumin, the strips were incubated with indomethacin $5 \cdot 10^{-6}$ g/ml for 20 min after which release of [^{3}H]arachidonic acid and [^{3}H]prostaglandin E was determined. Data are given as percent of dpm found in control incubation solutions ± S.E. The large S.E. reflects the large variation in total [^{3}H]-arachidonic acid used for loading. '[^{3}H]Prostaglandin E' also includes 6-[^{3}H]ketoprostaglandin $F_{1\alpha}$.

	% control	
	Zero K^+	Indomethacin
[^{3}H]Arachidonic acid	145 ± 27 *	193 ± 73 *
[^{3}H]Prostaglandin E	297 ± 45 *	60 ± 15 *

* Means are significantly different from control as determined by paired *t*-test ($P < 0.05$).

ment had no effect on the arachidonic acid increase seen with zero K^+ but markedly decreased release of [^{3}H]prostaglandin E. The finding that indomethacin did not completely abolish release of radioactivity in compounds which cochromatographed with prostaglandin E_2 suggests the presence of radioactive non-prostaglandin-like compounds which cochromatograph with prostaglandin E_2. The amount of ^{3}H release in the 30 min collection period was, for the case of arachidonic acid, 4—5% of total tissue radioactivity and for prostaglandin E, approximately 0.3—0.5% of total tissue radioactivity. Large amounts of [^{3}H]prostaglandin E were released during the incubation with [^{3}H]-arachidonic acid. Over 95% of ^{3}H radioactivity which cochromatographed with arachidonic acid on thin-layer chromatography was associated with arachidonic acid ester peaks measured with gas chromatography. An average of 32.8 ± 7.62% of the [^{3}H]prostaglandin E recovered on the H plates in zero K^+ experiments was converted by alkali to a compound which cochromatographed with prostaglandin B_2 (seven experiments). Similar findings were seen in three control (Krebs) experiments. However, there was complete conversion of authentic prostaglandin E_2. Dr. Pace-Asciak studied the nature of prostaglandin compounds in our organ bath fluid, using gas chromatography-mass spectrometry [13]. In each of two experiments organ bath fluid collected during zero K^+ runs contained two principal compounds. Of these, 86 and 84% were 6-keto-prostaglandin $F_{1\alpha}$ and 14 and 16% prostaglandin E_2. There was no evidence of thromboxane B_2 and prostaglandin $F_{2\alpha}$ was not measured. Thus it appears that the fraction of chromatographed 'prostaglandin E' that was not converted to prostaglandin B by alkali may be 6-ketoprostaglandin $F_{1\alpha}$ which is known to cochromatograph with prostaglandin E in our system. These data suggest that zero K^+ appears to augment prostacyclin release as well as prostaglandin E release.

Discussion

The Na^+ and K^+ contents found in the present study are larger than found by Casteels and coworkers [12,14,15] and by Brading and Jones [16] (although

some earlier studies gave Na^+ or K^+ content values similar to ours [17—20]. This 10—15% discrepancy results in a more than doubling of calculated $[Na_i^+]$ and also higher calculated $[K_i^+]$. Using the higher sorbital extracellular space determined in our laboratory in this calculation resulted in a lower computed $[Na_i^+]$ than found using polyethylene glycol data, but this produced an increase in calculated $[K_i^+]$.

A high $[Na_i^+]$ might be explained by deterioration, however the high computed $[K_i^+]$ could not be explained on this basis. Our preparation appeared normal in terms of mechanical activity under control conditions. Practically all published measurements of $[Na_i^+]$ and $[K_i^+]$ give much higher sums than expected by osmotic considerations and commonly used activity coefficients, so our high $[Na_i^+] + [K_i^+]$ sum is not unusual in this sense. We could find no technical error in the Na^+ and K^+ analyses of our tissue. Our guinea-pigs were larger and probably older than those used by previous investigators, but whether this is a factor is unknown. Assuming our values are 10—15% in error, this would not have significant effects on our conclusions since measured changes in $[Na_i^+]$ and $[K_i^+]$ during zero K^+ were very similar to those found by other workers. Previous data on effects of zero K^+ on intracellular electrolytes [14] and our present data are in agreement about the magnitude of differences in $[Na_i^+]$ and $[K_i^+]$ after 30 and after 90 min zero K^+ exposure.

The present data show dissociation between $[Na_i^+]$ and $[K_i^+]$ and prostaglandin release under conditions where we were looking at reversal after readmittance of K^+ to the bathing solution. There was no relationship between $[Na_i^+]$ and $[K_i^+]$ at the time of K^+ replacement on the exponential rate constant for fall of prostaglandin E release. Initial decreases in prostaglandin E release rate (in ng/g per min) were actually greater in strips where $[Na_i^+]$ and $[K_i^+]$ were much more abnormal. Measurements of $[K_i^+]$ and $[Na_i^+]$, of course, reflect all cells in the tissue. There is probably heterogeneity of $[Na_i^+]$ and $[K_i^+]$ in different cells in smooth muscle and heterogeneity in changes under conditions of zero K^+. There may have been heterogeneity of K^+ in extracellular fluid in intercellular clefts. Certainly K^+ did not fall to zero in clefts since K^+ was steadily lost out of cells. Despite heterogeneity it seems safe to conclude that with long duration of exposure to zero K^+, $[Na_i^+]$ and $[K_i^+]$ were more abnormal in most cells in the tissue that have plasma membrane bound $(Na^+ + K^+)$-ATPase which is driven by extracellular $[K^+]$, than with short zero K^+ exposure. This should be true in prostaglandin E-synthesizing cells as well as cells which did not synthesize this compound. We conclude that it is very unlikely that changes in intracellular Na^+ or K^+ were related to the augmented prostaglandin E release seen during zero K^+.

We have previously discussed evidence against a role of intracellular H^+ or Ca^{2+} in augmented prostaglandin E release during ouabain or zero K^+ [1]. The most likely intracellular electrolyte which might alter a rate-limiting step during prostaglandin E synthesis is K^+. There is abundant evidence that K^+ in intracellular compartments can influence cell metabolism [21—25]. Our evidence that K_i^+ was not involved in augmented prostaglandin E release was strenghthened by experiments which showed that ouabain augmented prostaglandin E release under conditions where extracellular K^+ was elevated and presumably K_i^+ could not fall. Prostaglandin E release rate was increased in the control runs

where extracellular K^+ was 140 mM and Na^+ 1.4 mM, in the absence of ouabain. This could be due to fall in $[Na_i^+]$ resulting from low extracellular Na^+ causing partial inhibition of the pump [26] or an effect on Ca^{2+} binding. But regardless of the mechanism this increase was augmented by ouabain under conditions where $[K_i^+]$ should not fall.

The finding that known neurotransmitters present in this tissue did not cause augmented prostaglandin E release makes it less likely that the zero K^+ effect is on a nerve cell. In addition, it is shown in the present study that augmented prostaglandin E release persists for 50 to as long as 200 min during zero K^+ and it seems unlikely that neurotransmitters would be constantly released over this time period.

The finding that arachidonic acid is released at a higher rate from tissue exposed to zero K^+ than in control strips is strong evidence that prostaglandin E release is controlled by prostaglandin synthesis, as is currently accepted in the prostaglandin field [27,28]. Apparently zero K^+ has an effect on the cell which leads to increases in free cellular arachidonic acid, possibly by stimulating phospholipid hydrolysis. If arachidonic acid availability is the rate-limiting step, an increase in this compound would lead to an increase in synthesis of prostaglandin compounds. The finding that arachidonic acid release is increased in zero K^+ does not give evidence for or against the possibility that the zero K^+ effect is mediated at the level of the plasma membrane versus effects of intracellular electrolytes on this process.

In more recent studies [29,30] performed in our laboratory it was found that greater than 95% of taenia coli arachidonic acid is found in membrane phospholipid fractions and other prostaglandin precursors are practically absent. [^{3}H]Arachidonic acid that is released during zero K^+ originates in phospholipids [29]. This is consistent with a large body of evidence which has indicated that membrane phospholipids are the source of activating arachidonic acid in other tissues [31—33]. Ca^{2+} in extracellular fluid, but not the cytoplasm can further activate prostaglandin E release, suggesting phospholipid in the plasma membrane may be the source of activating arachidonic acid [30]. These data are consistent with the finding in the present study that changes in intracellular electrolyte concentrations resulting from inhibition of the Na^+ pump are not involved but that it is more likely an effect of inhibiting the pump mediated at the level of the plasma membrane. However, the link between activity of $(Na^+ + K^+)$-ATPase, or the Na^+ pump, and arachidonic acid relesase has not been established. A clue to this coupling mechanism could be the recent finding in heart cells that ouabain treatment alters Ca^{2+} binding to membrane phospholipid [34]. It is already known that interactions between phospholipids and $(Na^+ + K^+)$-ATPase activity occur in the plasma membrane. Phosphatidylinositol or phosphatidylserine can alter the activity of $(Na^+ + K^+)$-ATPase in isolated membrane preparations [35,36].

In conclusion, the present experiments have further characterized the augmented prostaglandin E release that is seen on exposing strips of guinea pig taenia coli to zero K^+ solutions. It is shown that prostacyclin release is also augmented during zero K^+. Evidence is obtained that augmented prostaglandin E release during zero K^+ may be related to increases in unbound arachidonic acid in the smooth muscle cell. Comparison of changes in intracellular $[Na^+]$

and $[K^+]$ and prostaglandin E release rates during recovery from zero K^+ leads us to conclude that augmented arachidonic acid and prostaglandin E release is not related to alteration in intracellular electrolyte concentrations but more likely is linked at the level of the plasma membrane. Augmented prostaglandin E release with zero K^+ is not due to release of known neurotransmitters in the guinea-pig taenia coli.

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