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Correlations between electrolyte content and spontaneous electrical activity in intestinal muscle

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JOB, DONALD D., WILLIAM E. BLOOMQUIST, AND JACQUI BRIDGEFORTH. *Correlations between electrolyte content and spontaneous electrical activity in intestinal muscle.* Am. J. Physiol. 226(6): 1502–1509. 1974.—Spontaneous in vitro electrical activity was recorded from five different regions of cat small intestine. The electrolyte content was determined in these same pieces of tissue. Gradients in slow-wave frequency (SWF) and "apparent" slow-wave conduction time (SWC) were observed down the intestine. Correlations between ion levels and electrical parameters and correlations between electrical parameters themselves were computed. Slow-wave amplitude (SWA) was negatively correlated with both tissue sodium and calcium. Spike amplitude (SpA) was negatively correlated with tissue calcium and sodium and positively correlated to potassium. The latter was the strongest of the three. Spike frequency (SpF) was negatively correlated with chloride. Slow wave and spike conduction were not highly correlated to any ions. SWF was not correlated with any ion which exhibited a significant gradient, indicating that the gradient in intestinal motor activity was not primarily ionic in origin. A positive correlation between SWA and SWF was the most prominent interaction among electrical parameters. Most interactions between electrical parameters were insignificant. Most correlations between ion levels and electrical parameters were below .50, suggesting that the bulk-phase ion distribution is not the primary determinant of the spontaneous electrical activity.

intestinal slow waves; ionic content; smooth muscle electrophysiology; intestinal gradients; conduction parameters

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NUMEROUS STUDIES have been reported on the ionic content of intestinal smooth muscle (cf. 10). Also, numerous studies have been made of the ionic requirements for various electrical parameters (see below). Despite the abundance of these two types of studies, there has been no systematic study correlating the two. In the hope of learning more about the important control factors involved in spontaneous electrical activity in the intestine, we undertook such a study.

Since the intestine is a complex system, potentially having multiple interactions between ions, the usual method of inferring the origin of electrical currents by altering one ion at a time in the extracellular medium could be misleading. To assess this possibility, we determined all possible simple relationships between electrical parameters and tissue ion levels. We made correlations between parameters whose values fell in the range of normal variability between animals, tissues, and regions. The resulting correlations, therefore, should be better indicators of the ionic factors operative in vivo than the correlations obtained with changes in extracellular ion concentrations over ranges which are obviously beyond normal in vivo conditions.

The expected correlations between electrical parameters and ions based on ion-substitution studies are the following. Slow-wave amplitude (SWA) is markedly reduced in the absence of extracellular sodium ions (13, 14, 16). Spike amplitude (SpA), on the other hand, is not reduced in the absence of sodium. Spikes are reduced, however, in the absence of calcium ions. Slow waves are not very sensitive to calcium compared to spikes (13). We would expect SWA to be correlated primarily with sodium and SpA to be correlated primarily with calcium.

Both SWA and SpA are diminished when extracellular potassium is increased five- to sixfold (13). The relationships may not, however, be linear. SWA is decreased both in the presence of high extracellular potassium (low membrane potential) and also under the influence of hyperpolarization pulses (unpublished observations). Potassium ions, therefore, might be quite important and yet show only a weak linear correlation with SWA. Likewise, since in smooth muscle the membrane potential does not behave like a simple potassium electrode, the linear correlation between K and SpA is likely to be lower than its true value.

Substitution of sodium propionate for sodium chloride has been found to reduce spikes somewhat, without any appreciable effect on slow waves (14). Daniel (4) also found slow waves to be insensitive to changes in extracellular chloride concentration. From these observations, tissue chloride would not be expected to be correlated to either SWA or SWF, but it could be correlated with spike activity (amplitude or frequency). Deletion of magnesium from the medium or addition of magnesium to a calcium-free medium has been shown to have no effect on either slow waves or spikes (14).

The effects of various ions on slow-wave and spike frequency (SpF) have not been extensively studied. Job (8) has suggested that SWF is a system property which is dependent on interactions between metabolism and membrane processes, but not directly attributable to any one ionic process (8). If this view is correct then we would not expect to find high correlations between SWF and the ions. SpF is also not likely to be related to ion levels in a simple way since it would be dependent on thresholds, membrane potential, and recovery processes, each of which is likely to exhibit different dependencies on ions.

Very little information has been obtained on the effect that various ion substitutions have on conduction times.

RESULTS

Regional differences in electrical activity. Table 1 summarizes the means of various electrical parameters measured in different regions of the intestine. SWF and apparent slow-wave conduction time (SWC) were the only two parameters which exhibited differences from duodenum using the Dunnett t test with a significance level of $P < .05$. SWF and slow-wave conduction velocity tended to decrease in a parallel fashion down the intestine suggesting a positive correlation between the two. The slow-wave frequency gradient corresponds to the well-documented Alvarez gradient in mechanical activity (1). A gradient in both SWF and SWC has also been observed in the dog small intestine (2, 4). Spike frequency (SpF) and spike amplitude (SpA) tend to increase (though not linearly) as one moves toward the ileum. Due to high variability, a significant gradient was not observed, although, SpA in *region 5* was significantly greater than in *regions 2* and *3* ($P \leq .05$). These observations support earlier contentions that the frequency gradient in mechanical activity is set by the slow-wave frequency and not by the spikes (2, 5, 6, 15). There were not enough tissues from each region showing "conducted" spikes to permit a meaningful comparison of mean values.

Correlations between ions and electrical activity. In the accompanying paper (10), we found regional differences in ions in the intestine. We now find regional differences in electrical activity. Is the latter based on the former? Are there underlying mechanisms for spontaneous electrical activity which can account for the correlations to ionic content? Table 2 summarizes the linear correlation coefficients obtained between ions and electrical parameters. For each electrical parameter a new data matrix was formed within which there were no missing values. This procedure reduced somewhat the number of observations used, but eliminated possible erroneous correlations resulting from correlations between mismatched parameter values or between a parameter and zero.

The values for the correlations are not as high as one expects when accustomed to computing regression coefficients for a dependent variable and an independent variable. In the present case, however, all variables are "wandering around" with multiple and complex interactions. For this reason it is difficult to know what constitutes a physiologically meaningful correlation. We have somewhat arbitrarily designated a "good" correlation as anything over .50 and an unacceptable correlation as any below .30. Correlations greater than .30 were generally statistically significant. For most parameters there were at least 26 or 27 observations and there was at least one correlation having a confidence level (indicated in parentheses in Table 2) of over 95% ($P \leq .05$).

TABLE 1. *Summary of regional differences in spontaneous electrical parameters*

Electrical Parameters	SWF, hz	SWA, mV	SpA, mV	SpF.	SWC
Region					
1	.187	.707	.292	8.85	1.21
2	.163	.642	.150	9.05	1.63
3	.148*	.728	.104	11.75	2.45
4	.153	1.040	.217	15.42	2.17
5	.129*	.943	.510	12.52	2.76*

Regions 1–5 refer to portions of the small intestine taken from duodenum to distal ileum, respectively. Adjusted means are shown for all but the "apparent" slow-wave conduction (SWC) which is unadjusted. The spike frequency (SpF) is expressed as number per 10 slow waves. SWC is given in units of seconds of time delay per centimeter electrode separation (the reciprocal of conduction velocity). SWF and SWA are the slow-wave frequency and amplitude, respectively. SpA is the spike amplitude. * Significantly different from the value for that same parameter in the duodenum.

TABLE 2. *Correlation coefficients between electrical parameters and ions using only complete matrices*

	SWA	SWF	SpA	SpF	SWC	SpC
Na-*SI*	−.57 (.0009)	0	−.34 (.05)	0	0	0
Na-*SII*	−.53 (.002)	0	0	0	0	0
Na-*SIII*	−.69 (.0001)	0	−.34 (.05)	0	0	0
K-*SI*	.55 (.001)	.51 (.003)	.48 (.005)	0	0	0
K-*SII*	.59 (.0006)	0	.34 (.05)	0	0	−.35 (.11)
K-*SIII*	.62 (.0003)	0	.41 (.02)	0	0	−.31 (.15)
Ca-*SI*	−.43 (.01)	0	−.32 (.07)	0	0	0
Ca-*SII*	−.61 (.0004)	0	0	0	0	0
Ca-*SIII*	−.61 (.0004)	0	0	0	0	0
Mg-*SI*	0	.45 (.009)	0	0	0	0
Mg-*SII*	0	0	0	0	0	0
Mg-*SIII*	0	.41 (.02)	0	0	0	0
Cl-*SI*	0	0	0	−.37 (.03)	0	0
Cl-*SII*	0	0	0	−.44 (.01)	0	0
Cl-*SIII*	0	0	0	−.34 (.05)	0	0

Correlation coefficients were computed in each case for tissues in which all ions had been determined. The number of observations was 31 or 32 for all but SpC, in which there were only 21 or 22. The numbers in parentheses are the probabilities of the magnitude of the correlation equaling or exceeding the computed value by chance alone. The probabilities are based on an expected value of the correlation being zero. Correlations indicated by 0 were less than .30.

Slow-wave amplitude. SWA was negatively correlated with both sodium and calcium and positively correlated with potassium. Plots of each pair of parameters suggested that the data for Na vs. SWA and Ca vs. SWA could have been fit much better using an exponential curve of the form: SWA = $a \exp(-b[\text{ion}])$, where a and b are the intercept and slope of the line, respectively. To fit the data for very low slow-wave amplitudes, an even better approximation of the data for sodium and calcium would be to use two exponentials back-to-back, so that the SWA values for each cross at about 50 and 2.2 μmol/g wet wt for sodium and calcium, respectively. The maximal SWA's occurred within a range

This is partly attributed to the fact that there has been considerable debate as to whether slow waves and spikes are even conducted. The evidence for conduction of slow waves has been reviewed recently (17). Arguments against conduction are that not every section of muscle from the same animal exhibits "phase-locked" waves. Furthermore, a section in which conduction appears to occur is not always stable in time (16). These observations suggest a system of loosely coupled oscillators. Such a system implies some sort of interaction between regions, even if that interaction is not conduction in the usual sense of passive electrotonic spread. It is this interaction that we seek to measure. For lack of a better word we will refer to it as slow-wave conduction (SWC). The preponderance of evidence suggests that spikes are conducted only over very short distances (a few millimeters). If there is no interaction between the two recording sites, one would expect low correlation between SWC or spike conduction (SpC) and all other parameters.

In a preliminary report (9) the authors correlated electrical parameters with ionic content in a muscle preparation having circular and longitudinal layers combined. In the accompanying paper (10) evidence is presented showing that the two layers are dissimilar with respect to the type of interactions between ions and also with respect to tissue concentrations. The latter group of differences was more prominent. Differences in electrical properties of the two layers have been studied by Prosser and his colleagues (12, 14, 16, 19). The slow waves are believed to originate in the longitudinal layer, whereas the spikes originate in the circular layers (5, 6, 12, 16). Due to these differences between the ionic and electrical parameters in the two muscle layers, correlations were computed between the parameters in each of three sections: the longitudinal section, the circular section, and, for a check on between-the-layer interactions, a combination of sections.

MATERIALS AND METHODS

The tissue was obtained according to the procedure detailed in the accompanying paper (10). The small intestine was divided into five regions. From each region, 2- to 3-cm lengths of intestine were taken; they were stripped of their mucosal and submucosal layers and placed in warm Tyrode solution for equilibration. Near the end of the equilibration period (60–90 min), the spontaneous electrical activity in the combined layers was recorded using pressure electrodes, as in previous communications (8, 14). Usually at least two electrodes were used, separated by either 5 or 10 mm, so that the phase relations could be determined. The phase differences between several successive repetitive signals from the two electrodes were meaned and divided by the electrode separation distance to arrive at an "apparent" conduction time (the reciprocal of conduction velocity). It should be understood that whenever conduction times are referred to we cannot be certain that the signal appearing under both electrodes has, in fact, been propagated from one to the other. In the case of slow-wave conduction, 36 % (12 of 33) of the observations made on conduction times were made from signals which exhibited identical slow-wave frequencies, which suggests "real" conduction. All observations were included in the correlations tabulated so interaction (both conduction and coupling) was measured. Our justification for including all the data was that our correlations should not be biased toward ionic conditions which favor tight coupling. So as not to compromise the possibility of gaining the best information on conduction itself, we also ran the correlations on the data set in which conduction via electronic spread was most likely to occur, i.e., the two regions that exhibited equal SWF's.

The ion analysis was performed as described in the accompanying paper (10). Correlations were made using the standard method for computing correlation coefficients (18). Mean values were adjusted by a least-squares technique to minimize the influence of wide deviations on the mean of the population (10). The Dunnett *t* test was used in comparing the electrical parameters in the various regions to a control. Duodenum was used as the control in that series. Significance levels are given at the 95 % level of confidence (7). Stepwise linear regression used in modeling the data for electrical and ionic interactions was done using the computer program CORR from the Statistical Analysis System (SAS) package. This program was developed by A. J. Barr and J. S. Goodnight of the Statistics Department at North Carolina State University. This procedure assumes that each electrical parameter can be accounted for by a linear combination of the concentrations of the five ions. We searched, by means of this computer program, for the ion that accounted for most of the variability in each of the electrical parameters. The parameters of a least-squares line were then computed. We then searched for the ion with the next strongest partial correlation and computed its slope. By repeating this procedure in a stepwise fashion, a linear equation could be derived, which quantitatively ascribed relative importance to each ion when accounting for the variability in each of the electrical parameters. Only those components that were significant at the 90 % level of confidence were considered. This procedure gave additional information on the quantitative relationships between ions and electrical parameters. However, it suffers from the same defect as the correlation procedure in that it assumes a linear relationship. To examine the possibility that nonlinear relations occur, we also made plots of each pair of parameters for direct visualization of the patterns of the data.

The electrical parameters used were obtained manually from a Honeywell Visicorder chart record. The frequency response of the recording system was well above that required for faithful reproduction. DC amplifiers were used to ensure that the slow components were also recorded without distortion. SWF was determined by counting the number of slow waves (and fraction thereof) in a 20-s period. Thus, the mean of three to eight slow waves was determined. Spike frequencies were obtained by adding the total number of spikes over a period of 10 slow waves. Thus, a low slow-wave frequency would not necessarily imply a low spike frequency, as we have defined it. In essence, the correspondence between slow-wave frequency and spike frequency has been factored out, at least with respect to the known correlation between spikes and the peaks of slow waves (5).

of tissue calcium from 1.7 to 3.3 μmol/g wet wt. The data plot for SWA vs. K appeared to be best accounted for when a linear model was used.

The value for the correlation between sodium of *section I* (Na-*SI*) and SWA of .57 would indicate, from statistical arguments, that about 32.5% (.57 × .57) of the variation in SWA is determined by sodium. Stepwise linear regression yielded the parameters of the linear equation: SWA = 3.15 − .02 (.0002)Na-*SI*. These parameter estimates gave a relation which accounted for 58% of the variability of the data. The occurrence of correlations between SWA and sodium in *sections II* and *III* can be accounted for by the high correlation observed between the sections for sodium (10). The correlations to K-*SII* and K-*SIII* and Ca-*SII* and Ca-*SIII* cannot be as readily explained since the layer similarities are fewer for these ions. Correlations between any of the electrical parameters and ions in layers other than the longitudinal layer have uncertain implications since the pressure electrodes were placed on the serosal side of the tissue. They are included, however, in Table 2 to indicate possible interactions between layers.

Slow-wave frequency. SWF was positively correlated to potassium and magnesium in the longitudinal layer and to magnesium only in the combined layers. All pairs exhibited considerable scattering when plotted, suggesting that the relationships were not primary ones. Stepwise linear regression for SWF yielded parameters for the equation SWF = .09 + .0008 (.003)K-*SI*, which accounted for 38% of the variability. The number in parentheses is the significance level of the preceding parameter. The fit of data for magnesium, but not for potassium, could be improved using an exponential curve of the form: SWF = $a \exp(+b(\text{Mg-}SI))$, where a and b are the intercept and slope, respectively. Even this modification, however, would not likely account for much more of the variation.

Spike amplitude. SpA was negatively correlated with both sodium and calcium levels in the longitudinal layer. The larger correlation, however, was with potassium. The positive correlation with potassium suggests that, assuming the membrane potential is determined by potassium, the more negative the potential (higher intracellular potassium), the greater the spike amplitude. This is what one would expect for a spike which was determined by electrochemical forces.

Stepwise linear regression on SpA yielded the following relationship: SpA = −.13 + .005 (.04)K-*SI*. This relation accounted for only 19% of the variability. Inclusion of sodium and calcium in the model did not improve the fit appreciably. These results do not lend support for the current view that the inward current for spikes is carried by calcium rather than by sodium (14). On the other hand, neither do they rule out that possibility. The negative correlation between SpA and Ca suggests that the chemical gradient is directed inwardly and could contribute to calcium influx. Furthermore, the plot of Ca vs. SpA indicated that a considerable improvement in fit of the data might be obtained if two exponential curves were placed back-to-back so that they formed a peak in SpA centered within a range of calcium tissue concentrations of 2.0–3.2 μmol/g wet wt. Since the relationship between SpA and Ca does not appear to be linear, the relatively low correlation value shown in Table 2 with calcium should not be weighed too heavily. Furthermore, the absence of a high correlation with sodium (unlike SWA) still leaves the calcium-spike hypothesis as an attractive possibility.

Spike frequency. SpF was not correlated with any of the cations studied. Furthermore, no patterns were observed which would suggest there were important nonlinear relationships. We did observe a negative correlation between SpF and chloride ions. This finding parallels the studies in which propionate was substituted for chloride (4, 14). It suggests that the inhibition of spikes observed in those studies was related to the change in chloride itself and was not an effect of the substituted ion.

Slow-wave conduction. SWC was correlated positively to chloride in the set of data which included all the tissues and both the magnitude and direction of the conduction. The scatter in the plot of SWC vs. Cl-*SI* using this data, however, was even worse than that in the plot of SpF vs. Cl-*SI*. Furthermore, the relatively small number of negatively directed conductions (moved orally) appeared to heavily bias the results. As a check, we redetermined the correlations using only the magnitudes of conduction. These are the results indicated in Table 2 which show there were no significant correlations above .30. Elimination of all the tissues which exhibited dissimilar slow-wave frequencies in adjacent regions and rerunning the correlations did not alter the picture presented in Table 2. Stepwise linear-regression techniques on SWC revealed that in the longitudinal layer, calcium levels could fit in a linear relation which would account for about 15% of the variability. This fitting was significant at the confidence level of .07 even though the correlation coefficient was very low. From these data, calcium could not be considered to play a primary role in conduction, nor could any other single ion.

Spike conduction. SpC was similar to SWC in that both showed a correlation with chloride levels in the data which included the direction of conduction, but not in the data with magnitude only. In the latter case, there were no significant correlations, nor were there significant contributions to a stepwise linear-regression model with any ion in the longitudinal layer. Potassium was the only significant factor (statistically) which related to SpC in the other sections, but even there the best regression model could account for no more than 20% of the variability.

Correlations between electrical parameters. Table 3 shows the correlations between the electrical parameters themselves. The significance levels are indicated in parentheses where .01, for example, means there is only 1 chance in 100 that the correlation value could occur by chance alone. Slow-wave amplitude and spike amplitude are positively correlated. This observation is in keeping with the statement by Daniel et al. (5) that the presence of spikes is most probable when the slow-wave amplitude is greatest. It should be noted, however, that the SWA-to-SpA correlation was reduced to below .30 when only data from tissues exhibiting synchronous slow waves were used. SWA was also significantly correlated with SWF.

We included the value for the weak negative correlation between SWF and SpF since the confidence level was acceptable. Also, this correlation was increased substantially when data were used from only those tissues exhibiting synchronous slow waves. The relation between SWF and

SpF implies that the number of spikes occurring in a burst near the slow-wave peak decreases as SWF increases. This interpretation is a consequence of the way in which spike frequency has been defined, i.e., the number of spikes per 10 slow waves. The relationship between SWF and SpF appeared to be linear although there was considerable scatter. SpA was weakly correlated with SpF, just as SWA was correlated with SWF.

No significant correlations were obtained with either SWC or SpC. This was true in all the following cases: *1*) using only conduction magnitudes and only data from tissues with frequency-coupled slow waves; *2*) using the preceding with direction included; *3*) using data that also included, in addition to direction and magnitude, all degrees of interaction between slow waves.

Ion content of electrically inactive tissues. A question related to the present study is, "What changes, if any, in the tissue ion concentrations are associated with lack of spontaneous activity?" Another way of asking that same question is, "How much can the ions vary before the electrical activity is disrupted"? Eight cats, out of more than 30, had negligible spontaneous electrical activity in their small intestines. The sodium and chloride levels in the jejunums from these animals were in the normal range. The potassium, calcium, and magnesium, however, were all significantly lower than the normal values. These results are indicated in Fig. 1. The Ca and Mg values have been multiplied by a factor of 10. The standard errors are indicated at the top of the bars. Of the three cations affected, potassium was changed most, being less than half the normal value. The ionic shifts could suggest the animals were "sick" and that the changes in electrical activity were due to the disease rather than to the different ion distribution; however, the animals all appeared normal in behavior and in intestinal contents. The incidence of intestinal parasites seemed normal. Furthermore, in at least one animal, segments from various regions of intestine showed marked differences in the level of spontaneous activity. The segment having low and irregular activity had substantially lower levels of both potassium and magnesium. Given these considerations, we believe the ionic changes (especially those in K and Mg) were causally related to the absence of spontaneous electrical activity.

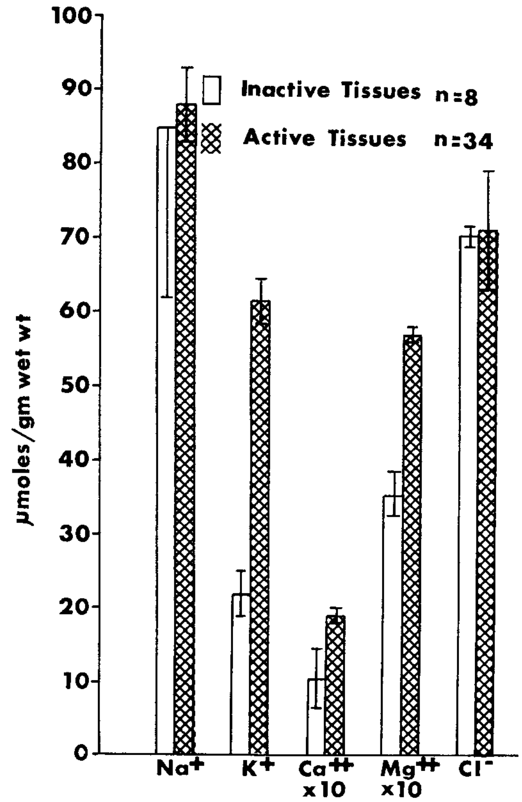

FIG. 1. Comparison of tissue ions in normal and inactive pieces of cat jejunum. Height of clear and hatched bars indicates mean ion concentrations found in electrically inactive and spontaneously active tissues, respectively. Vertical lines through mean indicate range of 2 SE.

TABLE 3. *Correlation coefficients between electrical parameters*

	SWA	SWF	SpA	SpF	SWC
SWA	1.0				
SWF	.45 (.008)	1.0			
SpA	.37 (.03)	0	1.0		
SpF	0	−.28 (.12)	.32 (.07)	1.0	
SWC	0	0	0	0	1.0
SpC	0	0	0	0	0

Correlation coefficients were computed from 31–32 observations with the exception of correlations with SpC in which only 20–22 observations were included. The numbers in parentheses are the probabilities of the magnitude of the correlation equaling or exceeding the computed value by chance alone. The probabilities are based on an expected value of the correlation being zero. Correlations indicated by a 0 were generally well below .30 and never above .30.

Examination of tissue ion content and electrical activity after cold storage indicates that high tissue sodium levels may have an inhibitory affect, at least on slow-wave amplitude. In the 1st h of warm-up, after overnight storage at 6°C, the primary shift in ion distribution was a loss of sodium. The loss of sodium was accompanied by an increase in SWA. The potassium was near normal (fresh tissue values) and was not increased substantially, nor were any other cations. Thus, electrical inactivity may be caused by at least two different factors: high intracellular sodium and low intracellular potassium. The two conditions do not have to occur simultaneously. These conclusions are in agreement with the correlations between SWA and Na and K indicated in Table 2.

DISCUSSION

It was the aim of the present study to do a systematic investigation of the correlations between ion content and spontaneous electrical activity in cat small intestine. Generally, our results were as predicted from ion substitution studies. However, some new relations were also found. One problem in comparing our results with those from ion-substitution studies is that we used pressure electrodes which measure the summed activity from many cells. Thus, such things as the energy input into the cells, the degree of coupling between cells, electrode-leakage resistances and injury, and conduction parameters could all modify the observed response regardless of ionic gradients.

Of the six electrical parameters measured, only two (SWA and SpA) are very susceptible to the drawbacks of the pressure-electrode technique. The disadvantage of not having absolute values and single-cell values for SWA and SpA were, in our opinion, outweighed by the advantages offered by the technique. Aside from the ease in use of pressure electrodes, it was possible to study response of the system as a whole.

The variability in behavior of pressure electrodes has been no greater in our experience than that of microelectrodes used for intracellular recording from single cells. Certainly the variability in electrical parameters from one cell to another when intracellular techniques are used is high. Furthermore, our system seemed to measure at least qualitative differences in transmembrane activity, since positioning of the electrodes gave a large negative DC voltage and spikes were positive. Since we used the same recording technique throughout, any differences in electrical activity observed were most likely related to some change in electrical properties of the tissue itself.

The fact that we were able to obtain significant correlations between the electrical parameters and changes in tissue ion levels also suggests the existence of causal relationships between the two. In particular, SWA was correlated with Na, which was predicted from the ion-substitution studies referred to in the introduction (13, 14, 16). Also, SpA was correlated with tissue Ca, again in agreement with results from other methods (14). The sign of the correlation is consistent with a model for spike depolarization, which depends on the influx of calcium down a concentration gradient.

The frequency measurements are not likely to be subject to variabilities resulting from the electrode technique. SWF was correlated with both potassium and magnesium. When coupling was tight, the primary correlation was with Mg; whereas, when coupling was loose, K was primary. Both primary correlations were higher than most others, were less variable, and accounted for more of the variability than any other relations except those with SWA. We conclude that these relations are most likely to be of functional importance.

The correlation between SpF and chloride ions is consistent with the reported studies in which propionate was substituted for chloride in the extracellular medium (14). Since chloride is not correlated with any other ions in longitudinal muscle (10), its relationship with SpF is probably a primary one.

SWF was also of interest because of its gradient down the small intestine. In the accompanying paper (10) it was suggested that perhaps sodium would be correlated to SWF since they both exhibited a linearly decreasing gradient. The correlation between the two, however, was seen to be very low. Correlations with magnesium and potassium were higher. The correlation with magnesium, sodium, and potassium, and lack of correlation with calcium and chloride suggests a possible relationship to a Na-K-Mg-ATPase. A magnesium-activated pump for sodium and potassium was suggested earlier to be involved in slow-wave activity (4, 14).

One aim of these studies was to examine the ionic basis for intestinal gradients. One problem raised was the possibility that changes in the ECS are responsible for variations in ion content in different regions of the intestine. If the ECS were different, differences in ion content of the tissue could occur without any changes in intracellular concentrations of ions. This possibility was explored in an accompanying paper in which it was concluded that there is no difference in ECS between regions (10). We did observe a substantial gradient in both SWF and SWC. Neither of these parameters, however, were primarily correlated with ions that exhibited a similarly large gradient. We conclude that ion distribution is not likely to be the basis for the Alvarez gradient in the small intestine.

Conduction phenomena present a set of problems different from amplitude and frequency measurements. We have pointed out that conduction per se may not be appropriate terminology for slow waves since the system may consist of numerous loosely coupled oscillators, each a potential driver. Despite this theoretical difficulty, we found that correlations between parameters for tissues that exhibited identical frequencies at an electrode separation up to 1 cm (referred to as a situation of tight coupling) did not differ appreciably from correlations for tissues that exhibited a full range of slow-wave interaction. This finding counters arguments that our correlations with conduction parameters are invalid because there is no conduction. Arguments with respect to SWC became academic when we considered only magnitudes of conduction since SWC did not correlate with any other parameter.

Interpretation of spike conduction is troubled by the difficulty in identifying a particular spike in two separate traces. The delay measured could be a function of the delay in the "conducted" slow wave that reaches the threshold level for spikes, rather than a function of spike conduction itself. The absence of correlation between SWC and SpC, however, tends to discredit this argument. The correlations between SpC and K and Ca are seen to be low and the confidence levels are also low. Even when a significant regression line could be found, the percent of variability accounted for was low. We conclude that ion distribution is not a primary determinant for either SWC or SpC.

One of the stated objectives of the present study was to examine the interactions between ions and electrical parameters in a complex system and to see whether these interactions were different from those expected from ion-substitution studies. The linear-regression technique gave the best confirmation of predictions from previous studies; i.e., sodium is involved in SWA and calcium is involved in SpA. Even in these two cases, however, the correlations were not particularly high. One might draw the conclusion that ion distribution is only one of several factors involved in the electrogenesis of spontaneous electrical activity.

Another factor which accounts for the poor correlations is that the important relationships may not be adequately describable by straight lines. It is known, for example, that when increasing extracellular calcium ions from a low level, one first gets an increase in slow-wave amplitude followed by an optimum and then a diminution of SWA (personal communication with Dr. C. L. Prosser). We observed similar nonlinear relationships between intracellular calcium and SWA and SpA. To assure ourselves that deviant data or nonlinearities were not misleading, either by indicating correlations which were not there or by hiding legitimate complex relationships, we plotted all the relationships between pairs of parameters. Those that deviated widely from a relationship which could be approximated by a straight line were noted. Correlations were also redetermined in some instances after erratic data were removed to check the sensitivity of the calculations. If the correlation coefficient changed substantively after removal of one to three data points, these latter coefficients were given more credence. To check further for nonlinearities, correlations between each of the ions and electrical parameters were run against various ratios or combinations of ions. The ratios of Na/Ca^2, Na/K, and Na/Mg^2, for example, were correlated with all other parameters. There were no substantial improvements in the values of the correlation coefficients. We concluded that examination of data plots was sufficient.

Yet another factor contributing to low correlations between ions and electrical parameters is that the tissue ion levels we determined are comprised of both free and bound ions. Only the free intracellular ion concentrations are likely to be direct determinants of electrical activity. The extent to which binding phenomena account for the generally low correlations is not determinable without knowing the percent of binding of each of the ions to anionic sites. The binding effect is likely to be significant only for the divalent cations, calcium and magnesium. Binding could explain the low correlations between the electrical parameters and the divalent cations. The sparsity of correlations with magnesium is especially suspicious since, in the inactive tissues, Mg was one of the ions most different from normal. Magnesium is generally bound more strongly to anionic groups found in membrane systems than is calcium; therefore, one would expect its correlation to be most affected. Taking into consideration all factors likely to influence correlations, such as binding, nonlinearities, possible trauma to the tissue, and tissue variability, we believe that even correlations as low as .30 could be meaningful in terms of indicating causal relationships. At the same time, however, our general conclusion must be that ion distribution alone cannot fully account for any measure of spontaneous electrical activity in small intestine. It might also be concluded from the foregoing study of interactions between parameters and from the reported interactions between the ions themselves (10), that it is not likely that the concentration of any one ion may change without at least one other ion changing its concentration. Thus, ion-substitution studies are apt to be misleading unless they are accompanied by determination of all the tissue ions.

Another objective of the present study was to determine whether there was an ionic basis for the observed differences in electrical behavior of the longitudinal and circular muscle layers. It is obvious from Table 2 that there are differences in the interactions between electrical and ionic activity in the two layers. Unfortunately, however, it is not obvious which interactions are causal and which ones can be explained through secondary or tertiary relationships. In an attempt to circumvent this problem, we have constructed an integrated view of the several different types of information as follows. We know that the only ion which is consistently different in the two layers, regardless of regions, was calcium. The mean level of calcium in longitudinal tissue was 3.15 (±.33), compared to 2.12 (±.12) in the circular layer. This represents a statistically significant difference. We suggest, therefore, that either calcium levels or the nature of the calcium relationships to the electrical parameters may explain the different electrical behaviors. Along these lines, we have shown that SWA was dependent primarily on sodium and secondarily on calcium; whereas SpA was dependent primarily on calcium and secondarily on sodium. Furthermore, the relationships between calcium and SWA and SpA were nonlinear, having optimum levels of calcium. The optimum tissue calcium level in longitudinal muscle for SWA fell in the range from 1.7 to 3.3 μmol/g wet wt, and for SpA, in the range from 2.0 to 3.2 μmol/g wet wt. These ranges cannot be distinguished from one another. The functional differences between longitudinal and circular layers cannot be explained, therefore, from differences in the pattern of interactions between the electrical parameters and calcium in the longitudinal layer. It is significant, however, that the mean level of Ca in longitudinal muscle is at the top of the optimal range for SpA.

Correlations with circular muscle are less meaningful since the electrical activity was recorded from the longitudinal layer. The ranges for optimum activity tended to be lower in circular muscle than in longitudinal muscle, which corresponded to the lower mean tissue concentrations. No differences, however, were observed which could account for the functional differences in the two layers. Our conclusion, from the foregoing argument, is that there is no obvious ionic difference between circular and longitudinal muscle which would explain the differences in the preferred electrical activity of the two. This is true whether one looks for differences in pattern of interaction (as shifted optimums, or different relations), or whether one looks for differences in levels of particular ions.

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