b) The glucose effect was not changed by the presence of agents known to inhibit anion transport processes (HCO_3^- removal, methazolamide, DIDS or anthracene-9-COOH) suggesting that such processes are not involved in the increased current.

The present techniques are not sufficient to discriminate which component of the tissue's current generating processes may be affected by a metabolite. Similar conclusions were drawn by Lawrence, et al. (J. Physiol. 255: 515, 1972) in their study of transmural potential difference in chiton intestine. This study was supported in part by NIH grant AM 12619 and the Whitehall Foundation.

ELECTRICAL AND SECRETORY RESPONSES OF SKATE GASTRIC MUCOSA

George W. Kidder, III and Elizabeth L. Kidder, Department of Physiology, University of Maryland School of Dentistry, Baltimore, Md.

The isolated gastric mucosa of the little skate (Raja erinacea) was mounted in a chamber designed for microelectrode experiments, under conditions giving good acid secretion (Kidder & Kidder, Bull. MDIBL 22:30, 1982). Oxygenated (90%.O_2/10% CO_2). Forster's solution was used on the serosal surface with an unbuffered similar solution on the mucosal surface. Potential (V_{ms}) was recorded between Ag/AgCl/KCl agar electrodes in the solutions adjacent to the tissue; current (I_{ms}) was passed via Ag/AgCl electrodes remote from the tissue; transepithelial resistance (R) was measured with 1 sec pulses of V_{ms} or I_{ms}, recording the other signal for calculation; acid secretion (J_H) was measured by pH-stat. V_{ms} was usually set by an electronic voltage clamp controlled by the computer which recorded the values to magnetic disk for analysis. The data reported are macroelectrode and J_H observations obtained in the course of ongoing microelectrode studies. Glucose (20 mM) and histamine (0.1 mM) were added to the serosal solution for standard conditions. Data are mean + SE.

Table 1 shows the effect of voltage clamping at +40 mV and -40 mV on J_H and R. The changes in the raw averages are not significant, so the data were normalized to the control value for that tissue, which yields a significant reduction in

Table 1.--Effect of Voltage Clamping on Skate Gastric Mucosa

Condition	Acid Secretion J_H (μEq/cm^2·hr)	J_H/J_H^c	Resistance R (Ohm·cm^2)	$R-R^c$
CONTROL (V=O)	6.51 +0.72 N=13 n=9	1.00 N=13 n=9	279 + 27 N=13 n=9	O N=13 n=9
CLAMP (V=-40)	4.64 +0.51 N=6 n=6	0.70 +0.10* N=10 n=6	264 + 36 N=7 n=7	3 +19 N=10 n=7
CLAMP (V=+40)	8.00 +1.26 N=5 n=5	1.19 +0.13 N=9 n=5	429 + 87 N=5 n=5	165 + 32** N=9 n=5

Measured values and ratio (J_H) or difference (R) with respect to control (c) condition in that tissue, for control (V_{ms} = 0) and voltage clamp at + 40 mV, for N measurements in n tissues. Since two control periods (start and end) were run in some tissues, ratios and differences were calculated for each control period; thus N > n in these cases. *indicates $p<0.05$ for difference from control, unity or zero as appropriate; **indicates $p<0.01$.

J_H upon clamping to -40 mV, while the stimulation by +40 mV is still not significant, a pattern also seen in frog (Crane et al., Biochem. J. 43:21, 1945) and sufficiently oxygenated (hyperbaric) dogfish gastric mucosa (Kidder, AJP 245:G236, 1983). This observation, and the high secretory rates observed, tend to the conclusion that this tissue is sufficiently oxygenated without hyperbaric conditions.

In four tissues, histamine was omitted and cimetidine (15 μM) used to produce a low starting J_H. The effects of readdition of histamine (0.1 mM, to produce standard conditions) and addition of thiocyanate (10 mM) are seen in Table 2. Except for the lack of a major rise in R with SCN^- addition, these results are similar to the observations in frog gastric mucosa:

Table 2.--Histamine and Cimetidine Effects on Skate Gastric Mucosa

	Parameter			
	J_H ($\mu Eq/cm^2 \cdot hr$)	J_H/J_H^c	R ($ohm \cdot cm^2$)	$R-R^c$
Cimetidine Inhibited	1.44 +1.25*	0.23 +0.21*	261 + 22*	113 + 16*
Histamine Stimulated (Cont.)	5.72 +0.98	1.00 --	147 + 12	0 --
SCN Inhibited	0.63 +0.43*	0.11 +0.07**	169 + 16	22 + 19

Stimulation by histamine (10^{-4}M) of cimetidine (15 μM) inhibited tissue, and inhibition by SCN (10 mM). All compounds added to serosal surface. Histamine stimulation taken as control condition for normalizations. Significance as in Table 1.

In four tissues, additional urea was added to raise the serosal urea concentration from 350 to 600 mM. With this number of experiments, the decreased J_H (to 60 + 23% of control) and the decreased resistance are not significant, but are consistent with the data from dogfish gastric mucosa (Kidder, Bull. MDIBL 20:39, 1980).

In most tissues examined, a current-voltage (I-V) plot was obtained, by varying the clamp voltage in 1-2 mV increments (1 sec pulse, 3 sec at steady state value) and recording the current required to maintain the clamp voltage at the end of the pulse. Usually 200 values were obtained for the I-V plot. The I-V plot can usually be resolved into straight lines separated by a sharp transition region; the intersection of these lines defines a breakpoint voltage. There were frequently more than two straight line segments in the plot, and correspondingly more than one breakpoint.

In a series of tissues under standard conditions, these breakpoints and the resistances of the lines separating them were determined. At a steady state voltage of zero, breakpoints in 58 I-V runs on 28 tissues fell into three clusters: at -45.7 + 2.4 mV (N=16); at -8.04 + 0.45 mV (N=58); and at 65.3 + 2.8 mV (N=18). Thus every tissue showed a "major" breakpoint between -20 and 0 mV, and some had "minor" breakpoints as well. V_{ms} when I_{ms} is zero (the intercept) was 0.55 + 0.40 mV for these tissues, which is a measure of the expected spontaneous potential under these conditions, and agrees with direct measurements. Clamping the tissue to +40 mV (which does not change J_H) shifts the range of the I-V plot such that for technical reasons the most negative breakpoint could not be detected, but in 17 runs on 7 tissues, a major breakpoint was found at -4.4 + 0.66 mV (N=17) and a minor breakpoint at 45.2 + 3.9 mV (N=9). The intercept V_{ms} when clamped to +40 mV was 15.1 + 1.6 mV for these 17 determinations.

Clamping to -40 mV, which inhibited J_H, resulted in an I-V relationship which was no longer a series of straight lines, but rather a single convex-upward curve. Consequently, no rational breakpoint analysis could be performed. The intercept V_{ms}, however, could be determined as -16.2 + 1.3 mV (N=16).

From these results, it is clear that voltage clamping away from the spontaneous potential (zero) has two effects. Negative clamps inhibit J_H, presumably by increasing the energy requirement H^+ transport, but also change the electrical characteristics of the tissue, as is seen in the change in intercept voltage. Among the possible changes expected from the passage of current are changes in the ionic composition of the cell cytoplasm. These changes clearly take some time, as they are not manifest during or following the 1 sec pulse used to measure resistance or the I-V plot.

The time course of the change can be followed by measuring the long time-constant transient (LTCT) response of V_{ms} to a step change in I_{ms}. Using 4 min pulses of 38 $\mu A/cm^2$, the LTCT was recorded, sampling the voltage every second. An immediate response (V_0 = IR at 1 sec) was observed, followed by a gradual change to a new steady state value of V_{ms} (V_f). A plot of ($V_{ms} - V_f$) vs. time is found to be a single exponential from 10 seconds onward. The current pulse was applied and removed in both polarities; the four transients had a magnitude $[(V_0-V_f)/V_0]$ between 0.51 and 0.87, while the exponential time constant ranged from 29 to 80 seconds. These results on a single tissue are similar to the data from frog gastric mucosa (Kidder & Rehm, Biophys. J. 10:215, 1970), but in marked contrast to the dogfish gastric mucosa, which even under sufficient oxygenation has a magnitude of zero and thus an indeterminant time constant.

SODIUM INFLUX ACROSS THE BLOOD-BRAIN BARRIER (BBB) CONTRIBUTES TO BRAIN INTERSTITIAL FLUID (ISF) VOLUME REGULATION IN THE LITTLE SKATE (Raja erinacea)

Ken Mackie, Michael DePasquale and Helen F. Cserr, Yale University School of Medicine, New Haven, Ct. and Section of Physiology and Biophysics, Brown University, Providence, R.I.

Brain ISF volume depends on the total tissue content of extracellular electrolytes, chiefly Na and Cl. Previously we have shown in response to hypernatremic dehydration the brain of the little skate loads NaCl in sufficient quantity to restore its ISF volume to normal (Bulletin, MDIBL 21:4, 1981). In this abstract we present evidence indicating that the volume regulatory NaCl flux requires inwardly directed (toward brain from plasma) [Na] and [Cl] gradients, occurs at the BBB and is partially inhibited by "loop" diuretics.

There are three possible routes of NaCl entry into brain - via the fluid around the brain (the extradural fluid or EDF), via the cerebrospinal fluid (CSF) or across the BBB. Using ^{125}I-RISA and ^{22}Na to trace EDF penetration into brain, Melton and Cserr (Bulletin, MDIBL 22:45, 1982) have shown that the EDF is not the source of the volume-regulatory NaCl.

In this study, Na influx from CSF to brain was evaluated both by direct observation of CSF volume (judged by the upward distension of the transparent meningeal roof of the third ventricle) and by measurement of the ratio of concentrations, ^{22}Na-CSF/^{22}Na-plasma, 30 min after IV ^{22}Na injection (an estimate of CSF turnover). The quantity of Na taken up by the brain during volume regulation is about the same as that in the entire volume of skate CSF (30 μl). During hypernatremia there was no obvious decrease in CSF volume. Also there was no increase in CSF turnover; the CSF plasma ^{22}Na ratios being .21±0.8 (N=3) and .24±.07 (N=6), control and hypernatremic, respectively. Thus, CSF may be excluded as a major source of volume regulatory NaCl during hypernatremia.

Na influx across the BBB was assessed by determining vascular permeability to ^{22}Na using the technique of Ohno et al. (AJP 235: H299, 1978) as modified by Cserr and DePasquale (Bulletin, MDIBL 22: 44, 1982) for use in the skate. This method yields the average unidirectional influx coefficient, k. of ^{22}Na across the BBB for the