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ALTERATIONS IN ELECTROPHYSIOLOGY OF ISOLATED AMPHIBIAN SMALL INTESTINE PRODUCED BY REMOVING THE MUSCLE LAYERS

JOHN F. WHITE

Department of Physiology, Emory University School of Medicine, Atlanta, Ga. 30322 (U.S.A.)

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Summary

Isolated segments of Amphiuma small intestine bathed in chloride or sulfate buffer generate a greater short-circuit current and a larger change in current in response to galactose when the serosal muscle layers are stripped from the mucosa. Intact (unstripped) segments are not apparently anoxic since stripped segments exposed to serosal N_2 for 3 h display normal short-circuit currents but a reduced potential response to galactose, while the presence of muscle layers tends to reduce the short-circuit current but does not alter the potential response to galactose. Bullfrog small intestine also generates greater short-circuit current following removal of the muscle layers. The enhancing effect of stripping appears to be related to removal of a resistance to ion flow across the tissue.

Introduction

It was noted in initial in vitro experiments with Amphiuma that the serosa and smooth muscle layers could be easily separated from the mucosa by blunt dissection and the resulting stripped segments exhibited electrical behavior consistent with greater ion transport capacity than the intact intestine. This observation had been reported previously for rabbit ileum [1] and the explanation was advanced that the muscle layers represent a significant barrier to the diffusion of oxygen. Alterations in electrical behavior produced by selective exposure of the separate faces of the epithelia to anaerobiosis tend to support this view [2,3] as do measurements of tissue oxygen consumption [4]. These approaches have led to the suggestion that the absorptive and crypt cell contributions to the overall electrical behavior are separate and identifiable [2—4].

This study documents the enhancement of ion transport activity following removal of the serosal muscle layers of Amphiuma and bullfrog small intestine and examines the response in Amphiuma in more depth from the view that stripped segments exposed to serosal N_2 should behave like intact segments if the presence of the muscle layers is a barrier to oxygen diffusion.

Methods

Adult Amphiuma obtained from Carolina Biological Supply (Burlington, N.C.) were stored in tap water at room temperature. Adult bullfrogs were obtained from Mogul-Ed (Oshkosh, Wisc.) and stored in tap water. Amphiuma were anesthetized in 0.2% chloretone (Parke-Davis and Co.); bullfrogs were decapitated. In both cases the entire small intestine was excised and the proximal one-third just distal to the attachment of the pancreas was used. The excised segment was placed in a dish of oxygenated buffer and rinsed through with buffer solution. Either or both of two protocols was performed (1) a 2 cm length of intestine was opened along the mesenteric border (an intact or unstripped segment); (2) a 2 cm length of intestine was stripped of its serosal muscle layers by dissection with fine pointed forceps (a stripped segment). The latter operation required about 1 min and served to remove the serosa and the outer longitudinal and inner circular smooth muscle as revealed by histology. The muscle removed represented 30 ± 4 (S.E.) % of the dry weight of Amphiuma small intestine and 18 ± 3% of bullfrog small intestine. Stripped and intact segments were mounted between two halves of a lucite chamber which was then filled with the appropriate buffer. These are referred to as unmatched experiments. In other experiments adjacent segments of tissue from the same animal were placed in separate but identical chambers. In some experiments both segments were stripped; in others only one of the segments was stripped. These are referred to as matched experiments. In every series an effort was made to eliminate systematic error by randomizing with regard to tissue treatment (stripped vs. intact when applicable), chamber assignment and short-circuiting electronics. The chambers were nearly identical to that used by Quay and Armstrong [5] employing an oxygen lift to continuously circulate the buffer solution. Originally 0.18 cm^2 of tissue was exposed to the buffer solution. The chamber was later altered to expose 0.32 cm^2 of tissue. All experiments were conducted at room temperature.

The buffer solutions employed are tabulated in Table I. The pH was maintained at 7.2. When galactose, glucose or valine was added at 10 mM an osmotic equivalent of mannitol was omitted from the bathing solution.

Transmural potential difference was measured to 0.1 mV with a model 268 Digitec digital millivoltmeter (United Systems, Inc.) or an Orion model 701 digital pH meter (Orion Research Inc.) in the mV mode. Current was passed from a battery in series with fixed resistors and a ten-turn 1 MΩ potentiometer.

TABLE I

COMPOSITION OF BUFFER SOLUTIONS

Buffer	Component (mequiv./l)							
	Na^+	K^+	Mg^{2+}	Ca^{2+}	$Tris^+$	Cl^-	SO_4^{2-}	Mannitol
Amphiuma, Cl^-	90	2.5	1.0	1.8	5.0	103.1	—	20
Amphiuma, SO_4^{2-}	90	2.5	1.0	1.8	5.0	—	52.2	65
Amphiuma, Na^+ free	—	2.5	1.0	1.8	95	102.0	—	20
Bullfrog, Cl^-	99	2.5	1.0	1.8	5.0	112.1	—	20
Bullfrog, SO_4^{2-}	102.6	2.5	1.0	1.8	5.0	—	60.5	70

The voltage drop across a precision kΩ resistor was displayed on a Digitec 262C multimeter and taken as the short-circuit current. Current-passing and potential-sensing electronics made contact with calomel half-cells which, in turn, made contact with the chamber solution through bridges of saturated KCl in 4% agar. To insure minimal leakage of KCl into the bath, current passage was very brief (approx. 10 s) and infrequent (every 20 min).

Prior to killing the animal one or both chambers were assembled and pre-equilibrated with the appropriate buffer solution to reduce potential offsets and minimize leakage of KCl into the bath. With the tissue in position the buffer was frequently replaced with fresh solution. The current passing through the system at zero transmural potential was taken as the short-circuit current after correction for solution resistance by the method of Clarkson and Toole [6]. The tissue resistance was calculated from the short-circuit current and the open-circuit potential. Following the experiment the chambers were reassembled and equilibrated with buffer to record the offset of the potential-sensing electrodes.

Results

Amphiuma

In vitro segments of isolated Amphiuma small intestine develop a spontaneous transmural potential (ψ_{ms}) of 1—2 mV, serosa positive when bathed in NaCl buffer. As is evident in Fig. 1 for intact segments and those relieved of their serosal muscle layers (stripped), after an initial period of equilibration the potential and the short-circuit current (I_{sc}) are stable over hours. For the unpaired observations in Fig. 1 it is also obvious that the magnitude of ψ_{ms}, I_{sc} and the tissue resistance (R_T) is related to the presence or absence of the muscle layers. When the serosal muscle layers are removed the resultant stripped segments exhibit a greater ψ_{ms}, I_{sc} and lower R_T than the intact segments. This observation is consistent with the findings of Field et al. [1] that isolated segments of stripped rabbit ileum develop greater I_{sc} than segments with intact smooth muscle layers. Also shown in Fig. 1 is the maximum electrical response of the tissue when the actively transported but non-metabolized sugar galactose (10 mM) was added to the bath after 180 min. There was no significant difference in the transmural potential response between stripped and intact segments ($P > 0.20$) but the I_{sc} response of stripped intestine significantly exceeded ($P < 0.05$) the response of the intact segments. The increased I_{sc} response in stripped segments is also in agreement with Field et al. [1]. They reported nearly 3-fold increases in current response on addition of 10 mM glucose. For Amphiuma, sugar-induced changes in tissue resistance did not occur or were small and not reproducible. For this reason effects of sugar on R_T will not be reported.

As stated previously the data of Fig. 1 is from unpaired tissues. Since it is possible that batches of animals respond differently adjacent segments of intestine from the same animal were compared, one stripped, the other left intact. The difference in ψ_{ms} and I_{sc} (stripped minus intact) is plotted in Fig. 2 for these paired observations. Obviously, over the 3 h interval the difference in ψ_{ms} was very nearly zero. In this regard there is a difference from the unpaired ob-

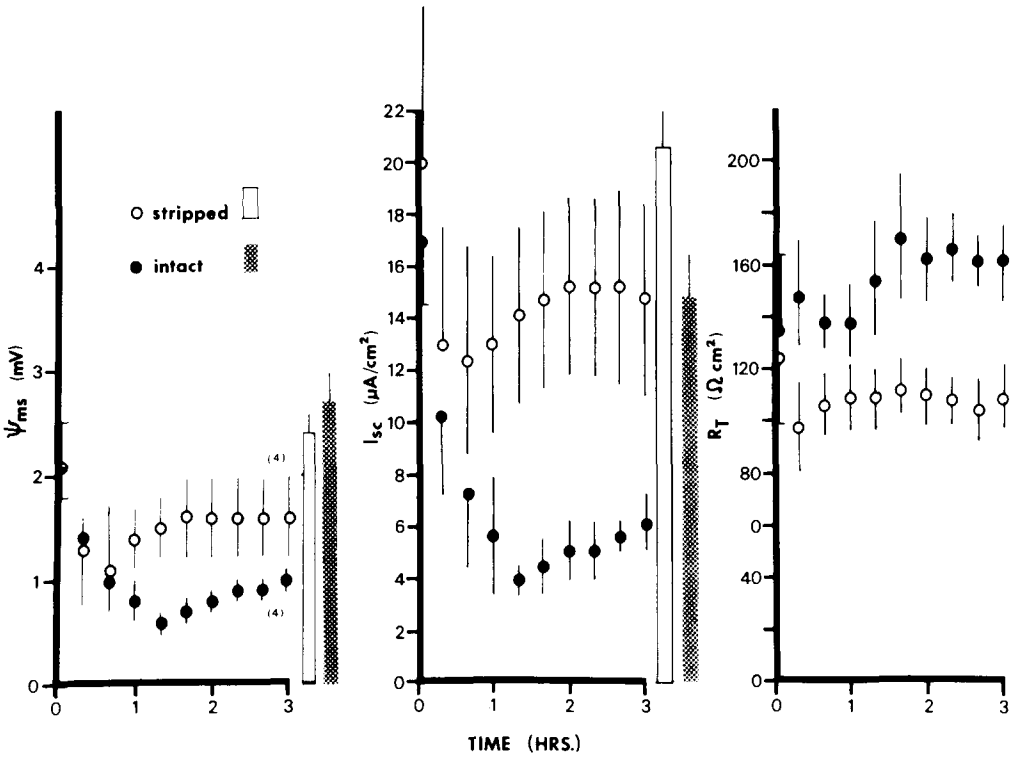

Fig. 1. Time course of transmural potential (ψ_{ms}), short-circuit current (I_{sc}) and tissue resistance (R_T) in unpaired segments of Amphiuma small intestine bathed in chloride buffer with (●) or without (○) attached serosal muscle layers. Bars indicate peak response to 10 mM galactose ±S.E. The number of experiments is in parentheses.

servations of Fig. 1. The I_{sc}, however, is much higher in the stripped segments over 3 h and the I_{sc} response to galactose is also greatly increased. Finally, the transmural potential response to galactose was identical in the adjacent segments. On these points there is agreement with the unpaired experiments that the removal of serosal muscle layers elevates the basal, unstimulated I_{sc} and the galactose-induced ΔI_{sc}.

To determine whether the effect depended on chloride this ion was replaced with sulfate without effect on the response. For the unpaired experiments in Fig. 3 the polarity of the intact segments was serosa negative and was reversed by stripping. The direction of the I_{sc} was reversed accordingly but the magnitude of the current was much below that observed in chloride buffer. The resistance of the tissue was quite high in intact segments (Fig. 3) and considerably reduced by stripping. The galactose-induced $\Delta\psi_{ms}$ was not different ($P > 0.2$) in the two series, however, the ΔI_{sc} was different ($P < 0.01$). In summary, the removal of the serosal muscle layers elevates the basal I_{sc} and the galactose-induced ΔI_{sc} in chloride or sulfate buffer. Since the short-circuit current is a measure of the net transport of ions by the tissue it follows that stripped segments have a greater ion transport capacity.

It has been proposed that the increased transport capability of stripped intestine results from enhanced oxygen availability to the epithelia from the serosal

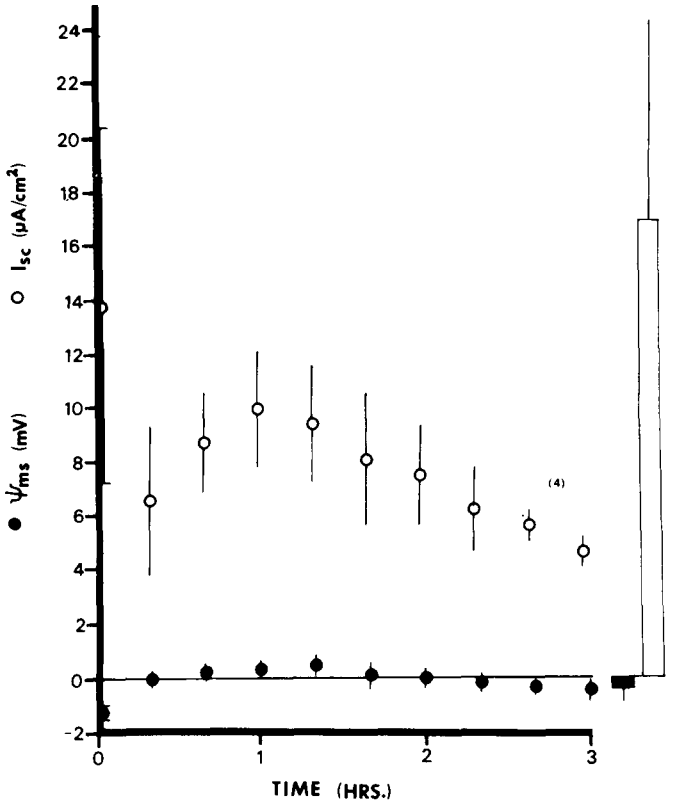

Fig. 2. Time course of the difference (stripped-intact) in ψ_{ms} (●) and I_{sc} (○) between paired segments of Amphiuma small intestine bathed in chloride buffer. The bars indicate the difference in potential (solid) and current (open) response to 10 mM galactose.

bath [1]. This implies that intact unstripped tissue is relatively anoxic and that the ion transport mechanisms, dependent on oxidative metabolism, are operating at subnormal rates. In agreement with this interpretation Frizzell et al.

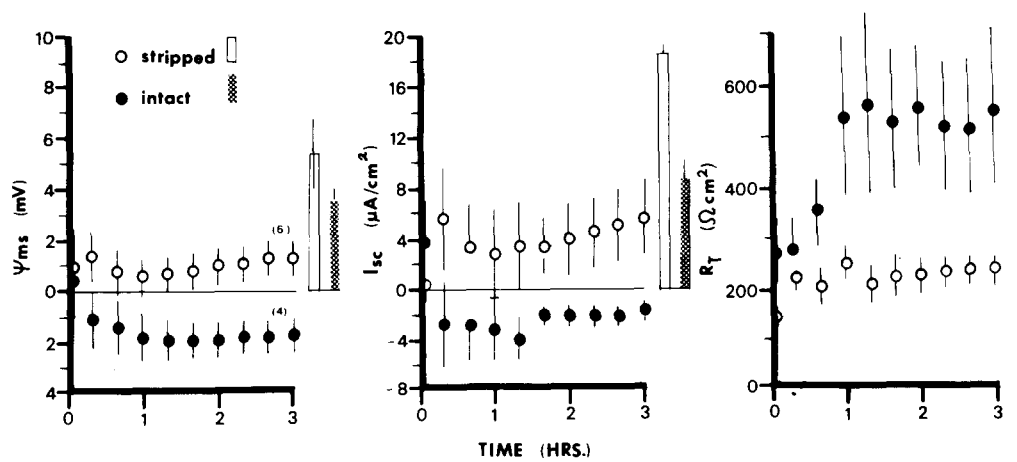

Fig. 3. Time course of elelctrical parameters in unpaired segments of Amphiuma intestine bathed in sul fate buffer with (●) or without (○) attached muscle layers.

[4] found levels of oxygen consumption in mucosal scrapes and stripped segments of rabbit ileum exceeded that of intact segments.

If the proposal is applicable to Amphiuma small intestine then replacement of oxygen in the serosal compartment of stripped tissues should reduce ψ_{ms} and I_{sc} to values observed with intact tissues. This was examined in two ways. In one series stripped segments were oxygenated normally until the electrical parameters stabilized. Then the serosal solution was gassed with N_2. As seen in Fig. 4 for four pieces of tissue incubated in sulfate buffer and one in chloride buffer, serosal N_2 produced either no change in ψ_{ms} or small, sometime transient, increases or decreases. Addition of 10 mM galactose at 180 min in the SO_4^{2-} experiments caused an increase in I_{sc} of $17.4 \pm 3.9\ \mu A/cm^2$ well within the normal response to this sugar (e.g. Fig. 3). Therefore, exposing the serosa to N_2 for 30 min does not mimic the intact segment with regard to its basal or sugar-stimulated electrical characteristics. Since it is possible that the different behavior of stripped an intact segments results from more prolonged anoxia, stripped segments were incubated in Cl^- buffer and exposed to oxygen on the mucosal side and N_2 on the serosal for 3 h (Fig. 5). Comparing the basal ψ_{ms} and I_{sc} with tissue exposed on both sides to O_2 (Fig. 1) there is no difference in these electrical parameters as a result of the serosal N_2 exposure. On the other hand there is a significant reduction in the potential response ($P < 0.01$) although not the current response ($P > 0.05$) when galactose is added at 180 min. Serosal N_2 did not apparently alter the basal electrical characteristics but reduced the normal effect of the sugar galactose. Comparison of these unpaired experiments may be misleading. Therefore, the basal potential and its response to galactose was examined in paired tissues from the same animal, one exposed on both sides to oxygen, the other exposed to serosal N_2. In Table II (a) the response to galactose is listed after 3 h of serosal N_2 for the two conditions. In this most important series the paired tissues matched very well, i.e. the basal ψ_{ms} at 180 min in the individual experiments did not vary by more than 0.1 mV. Thus galactose response was significantly reduced ($P < 0.01$) by serosal N_2 in agreement with the previous data. It is also demonstrated in Table II (b, c

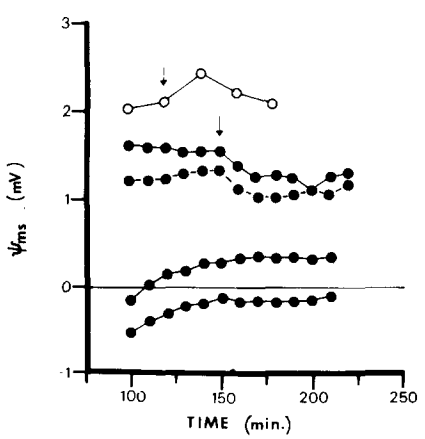

Fig. 4. response of ψ_{ms} to serosally applied nitrogen added at 150 min to tissues bathed in sulfate buffer (●) or at 120 min in chloride buffer (○).

TABLE II

EFFECT OF MUCOSAL OR SEROSAL N_2 ON THE TRANSMURAL POTENTIAL (ψ_{ms}) AND THE RESPONSE TO GALACTOSE ($\Delta\psi_{ms}$) IN PAIRED TISSUES

n is the number of experiments.

Experimental protocol	Control		Experimental			
	ψ_{ms}	$\Delta\psi_{ms}$	ψ_{ms}	$\Delta\psi_{ms}$	*n*	*P*
(a) Serosal N_2, 3 h	1.4 ± 0.1	3.5 ± 0.2	1.5 ± 0.1	2.3 ± 0.2	4	<0.01
(b) Serosal N_2, 2 h	1.8 ± 0.5	2.7 ± 0.5	1.7 ± 0.5	2.1 ± 0.8	4	>0.25
(c) Mucosal N_2, 3 h	1.3 ± 0.2	3.0 ± 0.5	1.4 ± 0.3	2.3 ± 0.5	6	>0.10
(d) Mucosal N_2, 2 h	1.2 ± 0.2	2.4 ± 0.3	1.3 ± 0.4	2.0 ± 0.3	4	>0.25

and d) that no significant decline in response is produced by a 2 h N_2 exposure in either chamber or a 3 h N_2 exposure in the mucosal chamber. It is also obvious from Table II that serosal N_2 did not alter the basal ψ_{ms} in agreement with previous data (Fig. 5), and so would not be expected to alter I_{sc}.

Only when both mucosal and serosal compartments are gassed with N_2 is there a dramatic effect on the basal and sugar-stimulated potential and current. This is illustrated in Fig. 6 with two typical experiments in Cl^- buffer. In the first (open circle) both mucosal and serosal compartments were gassed with N_2. The addition of galactose produced a steady, elevated potential difference which was rapidly reduced when the mucosal compartment was also gassed with N_2. These results indicate the mechanisms responsible for maintaining the

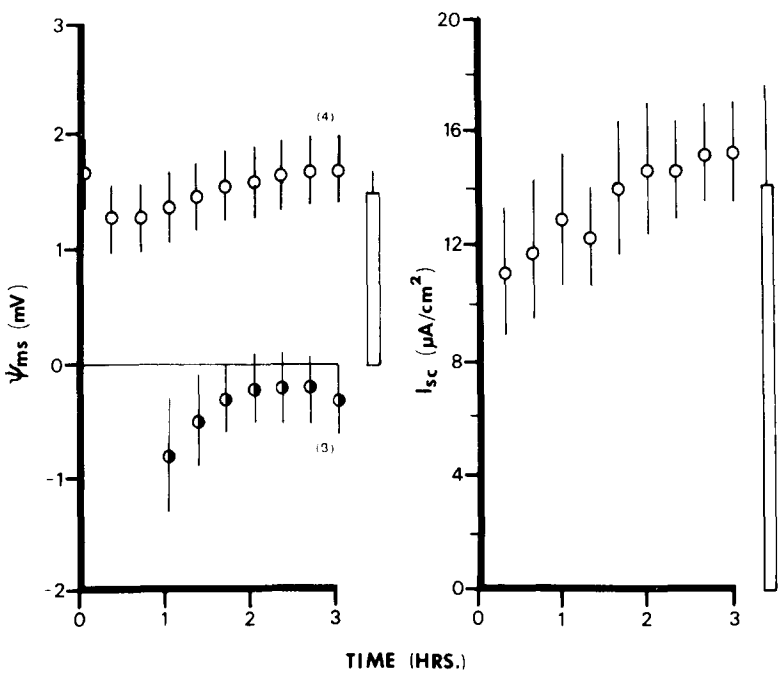

Fig. 5. Electrical parameters of Amphiuma intestine bathed in chloride buffer and exposed to serosal N_2 (○) or bathed in oxygenated Tris · chloride buffer (●). The bars indicate the response to galactose of the tissues exposed to N_2.

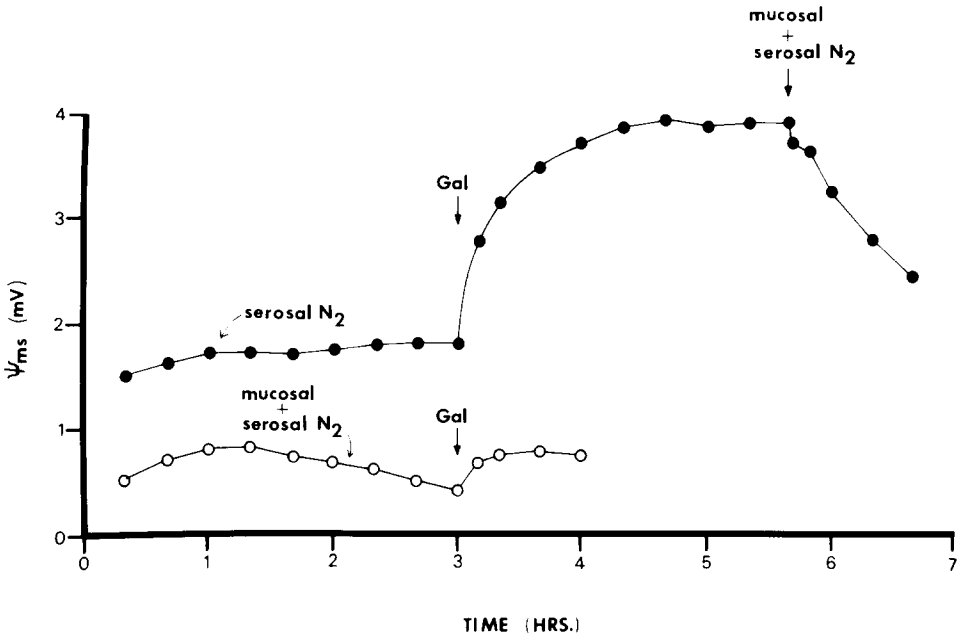

Fig. 6. Time course of response of potential in two separate experiments in Amphiuma in which the tissue was exposed on one or both sides to N_2 and the response to 10 mM galactose recorded.

basal potential difference and for the galactose-stimulated potential difference are oxidative in nature.

Taken together these results contradict the proposal that the presence of the muscle layers produces a relative hypoxia in the transporting cells of Amphiuma since gassing with serosal N_2, which should also produce anoxia in the same loci, does not lower the basal I_{sc}, but does lower the ψ_{ms} response to galactose. The presence of muscle layers has the opposite effect, lowering the basal I_{sc} but not altering the galactose-induced potential. The effect of serosal N_2 on Amphiuma intestine is opposite that reported in rat jejunum. Baker et al. [2] and Munck [3] have reported that gassing intact segments with serosal N_2 lowers the basal ψ_{ms} and I_{sc} but does not influence the response to actively transported solutes. It was suggested that the basal potential difference may be dominated by crypt cell ion transport processess more susceptible to serosal N_2 by virtue of their proximity to that compartment; more distant villous absorptive cells responsible for sugar transport would not be exposed to N_2 and the electrical correlates of sugar absorption would occur in full. Clearly this explanation would not apply to Amphiuma since the villous sugar absorptive process is affected by serosal N_2 at least as deduced by the electrical measurements while the basal transport processess are not. It is more likely that for Amphiuma and rat the basal and sugar-stimulated processess are separate, distinct and fully exposed to N_2 but differentially sensitive. The results with Amphiuma suggest the need for caution in the assignment of basal electrical behavior to one or another cell type in the epithelium.

These data do confirm the conclusion of Baker et al. [2] that the basal

potential difference and sugar-stimulated potential difference are manifestations of different processess since, in Amphiuma, the former is not influenced by serosal anoxia while the latter is reduced.

In further comparison with rat jejunum, mucosal anoxia for 3 h fails to significantly reduce the response to galactose in Amphiuma as seen in Table II while short exposures of the mucosal surface of rat jejunum seriously depresses the electrical response to sugars [2] or amino acids [3] and the uptake of galactose across the brush border [7]. Like all mammalian preparations studied, the transmural potential difference and the response to sugars depends on the presence of sodium since, as seen in Fig. 5, replacement of sodium with Tris reduced the spontaneous transmural potential to zero. Addition of galactose after 3 h in this series of four experiments, elevated ψ_{ms} insignificantly (0.1 ± 0.1 mV).

Bullfrog

The above results are not unique to Amphiuma. As seen in Fig. 7 for unpaired segments of bullfrog intestine in chloride buffer, removal of the serosa and muscle layers elevates the short-circuit current and the response to galac-

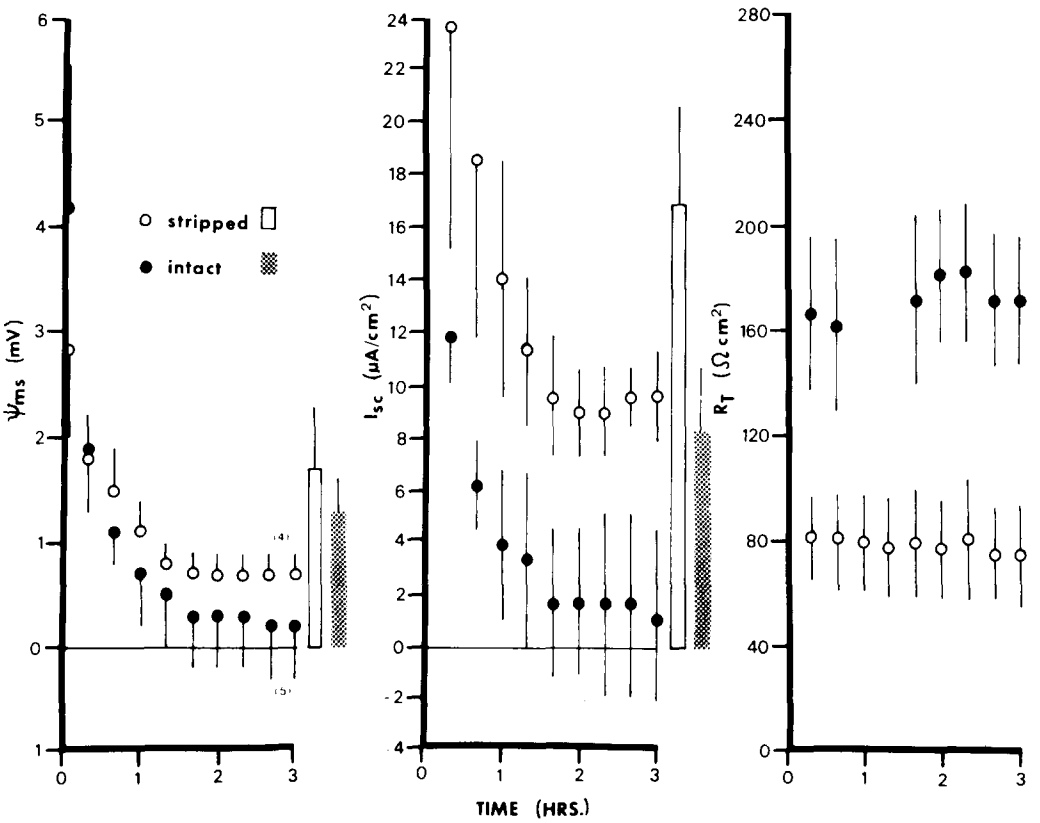

Fig. 7. Time course of the electrical parameters in unpaired segments of bullfrog small intestine bathed in chloride buffer with (●) or without (○) attached muscle layers. Bars indicate the peak response to galactose.

tose (10 mM) while lowering the tissue resistance. These results were confirmed in paired adjacent segments.

Discussion

In contrast to intact (unstripped) segments of mammalian small intestine, which suffer a dramatic decline in transport activity over 1 h, unstripped segments of Amphiuma and bullfrog small intestine are both capable of sustaining a short-circuit current over several hours. Thus it was surprising that removal of the muscle layers would enhance the ion transport capacity of the tissue. Presumably, these amphibian preparations are less dependent on oxidative metabolism and would thereby be less dependent on a supply of oxygen. It has already been demonstrated that exposing the serosal side of the tissue to N_2 does not mimic the presence of the muscle layers. Clearly, for these preparations, stripping the muscle layer must elevate the short-circuit current by another means. The most direct means would be by elevating the tissue conductance, that is, by reducing the resistance to the flow of ions that have been transported across the basolateral membrane into the subcellular tissue. A model which accounts for these observations is shown in Fig. 8 and is a form of the equivalent circuit proposed by Armstrong et al. [8] but modified to include a series resistance, R_{mu}, the resistance of the serosal muscle layers. The solution to this circuit is given in Eqns. 1 and 2 where the transmural potential (ψ_{ms}) is related to the mucosal and serosal membrane potentials (electromotive forces E_m and E_s), a junction potential (E_j) in parallel with the cellular elements, membrane resistances R_m and R_s, and the resistance of the junction (R_j). E_j is a diffusion potential across the tight junction which develops as a consequence of ion transport into the lateral intercellular spaces [8]. The transmural potential (Eqn. 1) is seen to be independent of the presence of R_{mu} in agreement

$$\psi_{ms} = [(E_s - E_m)R_j - E_j(R_m + R_s)]/R_T \tag{1}$$

$$I_{sc} = \frac{E_s - E_m + E_j[1 - (R_m + R_s + R_j)/R_j]}{R_m + R_s + R_{mu}(R_m + R_s + R_j)/R_j} \tag{2}$$

with the data from Amphiuma (Fig. 2) and bullfrog (Fig. 7). On the other hand

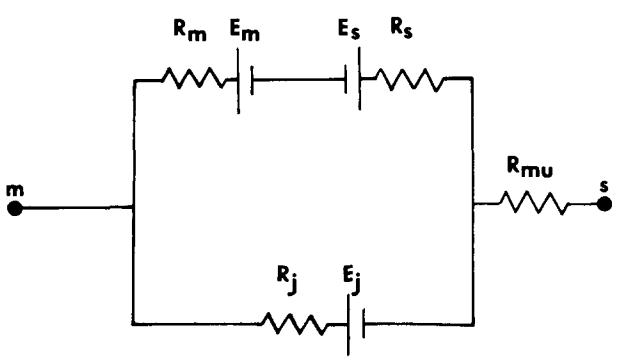

Fig. 8. Equivalent circuit for intact Amphiuma small intestine (see text for a description).

R_{mu} has a dramatic effect on the short-circuit current (Eqn. 2). If, for example, $(R_m + R_s)/R_j = 5$, approximating that reported for isolated rat [14] and rabbit [15] small intestine, then Eqn. 2 reduces to

$$I_{sc} = \frac{E_s - E_m - 5E_j}{5 + 6R_{mu}} \quad (3)$$

Since removal of the muscle layers nearly doubles I_{sc} (Figs. 1 and 7) R_{mu} must have a relative value of about 1, i.e. nearly equal to the equivalent resistance of $R_m + R_s$ in parallel with R_j.

In contrast to intact segments of bullfrog small intestine which develop a higher transmural potential in sulfate buffer [5] replacement of chloride with sulfate in stripped segments of Amphiuma has little effect (cf. Figs. 1 and 3) in keeping with observations in the turtle [9]. Since at the same time the I_{sc} is so much higher in chloride buffer it would appear that chloride facilitates net Na^+ transport. This could occur if sodium and chloride enter simultaneously into the absorptive cell by means of a carrier in the mucosal membrane. Such a mechanism has been proprosed for amphibian [5] and mammalian [10] small intestine. Active chloride accumulation by absorptive cells has been demonstrated in Amphiuma small intestine [11] which suggests that the carrier utilizes the energy of the Na^+ gradient for uphill Cl^- transport. Subsequently, the Na^+ would be tranported across the basolateral membrane by an energy-dependent mechanism.

The effect of prolonged serosal anoxia to reduce the response to galactose deserves further comment. Mucosal N_2 is without effect on the electrical characteristics of Amphiuma intestine suggesting that the sugar response does not depend on oxidative mechanisms at the mucosal membrane of the absorptive cell. Observations in bullfrog [12], rabbit ileum [13], and Amphiuma (White, J.F., unpublished) indicate that sugars depolarize the mucosal membrane. This probably occurs solely by increasing mucosal Na^+ permeability. The passive entry of Na^+ is followed by intracellular events which require metabolic energy to transport sodium from the cell. It is this latter active transport step which must be susceptible to prolonged exposure to N_2. Gradual inhibition of basolateral Na^+ transport would lead to elevation of cellular sodium and a reduction of the electrical response to galactose as the sodium gradient is dissipated.

Acknowledgements

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References

1 Field, M., Fromm, D. and McColl, I. (1971) Am. J. Physiol. 220, 1388—1396
2 Baker, R.D., Wall, M.J. and Long, J.L. (1971) Biochim. Biophys. Acta 225, 392—396
3 Munck, B.G. (1972) J. Physiol. Lond. 223, 699—717
4 Frizzell, R.A., Markscheid-Kaspi, L. and Schultz, S.G. (1974) Am. J. Physiol. 226, 1142—1148

5 Quay, J.F. and Armstrong, W.McD. (1969) Am. J. Physiol. 217, 694—702
6 Clarkson, T.W. and Toole, S.R. (1964) Am. J. Physiol. 206, 658—668
7 Baker, R.D., Lo, C. and Nunn, A.S. (1974) J. Membrane Biol. 19, 55—78
8 Armstrong, W.McD., Byrd, B.J., Cohen, E.S., Cohen, S.J., Hamang, P.J. and Myers, C.J. (1975) Biochim. Biophys. Acta 401, 137—151
9 Schoffeniels, E. (1961) in Biological Structure and Function (Goodwin, T.W. and Lindberg, O., eds.), pp. 621—631, Academic Press, New York
10 Nellans, H.N., Frizzell, R.A. and Schultz, S.G. (1974) Am. J. Physiol. 226, 1131—1148
11 White, J.F., in the press
12 White, J.F. and Armstrong, W.McD. (1971) Am. J. Physiol. 221, 194—201
13 Rose, R.C. and Schultz, S.G. (1971) J. Gen. Physiol. 57, 639—663
14 Munck, B.G. and Schultz, S.G. (1974) J. Membrane Biol. 16, 163—174
15 Frizzell, R.A. and Schultz, S.G. (1972) J. Gen. Physiol. 59, 318—346