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The effect of EDTA on the electrical activity of rat jejunum

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SUMMARY

The chelating agent EDTA caused a transient increase in the potential difference across rat jejunum which was not due to a resistance change. This was followed by a gradual fall in the potential, resulting from a decrease in the resistance. Lack of Ca, but not Mg, reduced the increased potential caused by EDTA while additional Ca prevented the subsequent decline in potential. Reducing the Na gradient across the mucosal membrane or inhibiting the Na pump reduced the increased potential caused by EDTA. It is suggested that EDTA increases the permeability of rat jejunum by removing bound Ca. A resulting increase in Na influx could stimulate the Na pump leading to an increased potential. The subsequent 'loosening' of the intercellular channels may then introduce a shunt into the system, short-circuiting the potential.

The chelating agent ethylenediaminetetraacetic acid (EDTA) has been shown to influence the permeability of many epithelial tissues, including those of the alimentary tract both *in vivo*[1] and *in vitro*[2]. EDTA causes an increase in the permeability of the rat small intestine and also leads to an enhanced movement of both Na and fluid to the serosal side of the tissue[1]. Similarly EDTA has been shown to increase the passive movement of ions across rabbit stomach *in vivo*[3]. Changes in ion movement may be reflected in an alteration of the electrical activity of a tissue and the effect of EDTA on electrical parameters in rat jejunum was therefore investigated.

Electrical activity was measured in everted sacs of rat jejunum. The potential difference was determined by the method of Barry *et al.*[4] using paired sacs from the same animal, one sac acting as a control. In experiments where both potential difference and resistance were measured the technique of Barry *et al.*[5] was employed, in which control experiments were carried out separately. The tissue was bathed on both sides in Krebs bicarbonate saline[6] which was gassed throughout the experimental period with 95% O_2–5% CC
When the ionic composition of the saline was varied this was accomplished as follows:

Ca-free: the Ca concentration of both mucosal and serosal fluids was reduced from 2.5 mequiv/l to zero by replacing all the $CaCl_2$ in the medium with NaCl.

Mg-free: the Mg concentration of both mucosal and serosal fluids was reduced from 1.2 mequiv/l to zero by replacing all the $MgSO_4$ in the medium with NaCl;

Low Na: all NaCl in the mucosal fluid was replaced with KCl, reducing the Na concentration from 143 mequiv/l to 25 mequiv/l.

The Na salt of EDTA (BDH Chemicals Ltd.) was added to the mucosal fluid to give a concentration of 5 mM. Unless otherwise stated EDTA was added 10 min after setting up the preparation by which time stability had been achieved. The potential difference and resistance were recorded immediately before the addition of EDTA (initial potential difference and resistance), at the peak of the EDTA response (PD peak and R peak) and 20 min after the EDTA had been added (PD_{+20} and R_{+20}). The following parameters are used to assess the effect of EDTA:

$PD_{initial}$ = initial potential difference
$R_{initial}$ = initial resistance
$\Delta PD_{peak} = PD_{peak} - PD_{initial}$
$\Delta R_{peak} = R_{peak} - R_{initial}$
$\Delta PD_{+20} = PD_{+20} - PD_{initial}$
$\Delta R_{+20} = R_{+20} - R_{initial}$

In experiments where the effect of ouabain on the EDTA response was studied the glycoside was present in the serosal fluid at a concentration of 1 mM for 30 min before the addition of EDTA. This high concentration of ouabain is necessary as rat jejunum is resistant to the effect of this drug[7]. Initial experiments in which the potential difference was measured showed that adjacent sacs of jejunum from the same animal behaved identically in their response to EDTA. Thus paired *t* tests were used to obtain probability values. The magnitude of the response to EDTA was very variable and therefore a set of control experiments was carried out for each test condition.

When EDTA was added to the mucosal fluid there was a transient increase in the potential difference which lasted for 2–3 min and this was followed by a gradual fall in the potential (Table I). This agrees with the observations of Goldner[8] who measured changes in the potential difference across dog small intestine in response to the presence of a chelating agent. The initial rise in the potential difference was not due to a passive increase in the resistance of the jejunum since this parameter did not change significantly ($P > 0.1$) in the first few minutes following the addition of EDTA. Thus the increased potential observed is the result of a rise in the current generated by the tissue. The subsequent fall in the potential difference could be attributed to a concomitant decrease in the resistance.

The fact that a chelating agent has the ability to stimulate the potential difference across the small intestine raises the possibility that the potential change caused by ATP[9] could be attributed to the chelating ability of the ATP molecule.

EDTA chelates both Ca^{2+} and Mg^{2+} and the relative importance of these two ions in the regulation of membrane permeability is still uncertain. Tidball[10] considers both Ca and Mg to be involved while Chung *et al.*[3] believe Ca to be of primary importance. The effect of EDTA under conditions in which the Ca or Mg content of the saline was altered

TABLE I

THE EFFECT OF EDTA ON THE ELECTRICAL ACTIVITY OF RAT JEJUNUM
5 mM EDTA was added to the mucosal fluid and changes in the potential difference and resistance were determined. The potential difference is measured in mV and the resistance in Ω/8 cm sac of jejunum. Results are expressed as the mean values ± S.E. of the mean with the number of observations in brackets.

	Additions to mucosal fluid	
	None (control)	*5 mM EDTA*
$PD_{initial}$ (mV)	2.8±0.3(9)	2.9±0.4(7)
ΔPD_{peak} (mV)		1.2±0.2(7)
ΔPD_{+20} (mV)	−1.0±0.2(9)	−1.7±0.3(7)
$R_{initial}$ (Ω)	1.8±0.3(9)	1.7±0.3(7)
ΔR_{peak} (Ω)		−0.1±0.1(7)
ΔR_{+20} (Ω)	−0.3±0.2(9)	−0.8±0.2(7)

TABLE II

THE EFFECT OF Ca^{2+} AND Mg^{2+} CONCENTRATION ON THE RESPONSE TO EDTA
The effect of EDTA on the potential difference was determined under various conditions of Ca^{2+} and Mg^{2+} concentrations. EDTA was added to the mucosal fluid to give a concentration of 5 mM. Results are expressed as the mean value ± S.E. of the mean with the number of observations in brackets.

Incubation medium	*$PD_{initial}$ (mV)*	*ΔPD_{peak} (mV)*	*ΔPD_{+20} (mV)*
Normal	2.8±0.3(15)	2.5±0.4(15)	0.9±0.3(15)
Ca-free	2.4±0.2(15)	1.4±0.3(15)	−1.4±0.3(15)
Normal	2.4±0.2(8)	0.6±0.1(8)	−0.4±0.1(8)
+5 mM $CaCl_2$	1.5±0.3(3)	0.4±0.1(3)	−0.1±0.1(3)
Normal	3.3±0.4(12)	1.6±0.3(12)	−0.1±0.4(12)
Mg-free	3.1±0.3(12)	1.7±0.3(12)	−0.3±0.4(12)

was therefore investigated (Table II) and it was found that the absence of Ca significantly reduced the stimulation of the potential caused by EDTA ($0.05 > P > 0.01$) while the subsequent decrease in the potential was enhanced ($P < 0.001$). The addition of 5 mM $CaCl_2$ to normal mucosal fluid before adding EDTA prevented this fall in the potential. However, lack of Mg appeared to have no effect on either aspect of the EDTA response ($P > 0.1$ in both cases). Thus Ca^{2+} seems to be more important in regulating the permeability of rat jejunum.

The rise in the potential in response to EDTA may be due to an increase in the permeability of the mucosal membrane of the jejunal epithelial cell following the removal of bound Ca. This could result in an increased movement of Na down its electrochemical gradient into the cells, producing a rise in the intracellular Na concentration. This may then stimulate the electrogenic Na pump causing a greater extrusion of Na into the lateral channels[11] and a subsequent increase in the potential difference. After further exposure to EDTA the intercellular junctions may become 'loosened'[12]. This would introduce a shunt into the system which would allow the laterally accumulated Na to leak back into the mucosal fluid, short-circuiting the potential. This would account for the fall in potential

difference and resistance observed 20 min after the addition of EDTA. It was observed that sacs treated with EDTA deteriorated visibly more than control sacs and it should be noted that EDTA is used for producing isolated cell preparations[13]. It therefore seems reasonable to suggest that EDTA may affect intercellular junctions[1], although the possibility that shunting occurs along the luminal membrane of the epithelial cells cannot be excluded.

Conditions in which Na movement across the jejunum is altered might be expected to reduce the stimulation of the potential caused by EDTA. When the Na gradient across the mucosal membrane of the epithelial cell was decreased by reducing the mucosal Na concentration to 25 mequiv/l, the stimulation of the potential caused by EDTA was significantly less ($0.05 > P > 0.01$) than in control experiments (Table III). Inhibition of the Na pump would be expected to have a similar effect. Ouabain reduces the activity of the intestinal Na pump[14] and it was found that following treatment of the intestine with this glycoside the stimulation of the potential difference caused by EDTA was significantly reduced ($0.01 > P > 0.001$).

TABLE III

THE RESPONSE TO EDTA UNDER CONDITIONS OF ALTERED Na MOVEMENT

The Na gradient was reduced by replacing Na in the mucosal fluid with K to give a Na concentration of 25 mequiv/l. In the experiments using ouabain it was present at a concentration of 1 mM in the serosal fluid. EDTA was added to the mucosal fluid to give a concentration of 5 mM. Results are expressed as the mean values ±S.E. of the mean with the number of observations in brackets.

Conditions	$PD_{initial}$ *(mV)*	ΔPD_{peak} *(mV)*
Normal	2.6±0.3(7)	0.6±0.1(7)
Reduced Na gradient	4.7±0.3(7)	0.2±0.1(7)
Normal	2.4±0.3(8)	0.8±0.1(8)
+Ouabain	2.4±0.2(8)	0.4±0.1(8)

These results are consistent with the suggestion that Na^+ is involved in the initial potential change caused by EDTA, while the subsequent decline in the potential may be explained in terms of the development of an intercellular shunt.

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