Short report
Failure of glucagon to influence ion transport across human jejunal and ileal mucosa in vitro

P. E. T. ISAACS AND L. A. TURNBERG

SUMMARY Glucagon (90 to 880 pg.ml⁻¹) failed to influence electrical activity or fluxes of sodium and chloride across human jejunal and ileal mucosa in vitro. These results suggest that the intestinal secretion and diarrhoea produced in vivo in man during intravenous infusion of glucagon may be produced by changes in motility and blood flow and not directly by activating an ion secretory mechanism as is the case in cholera.

Intravenous infusions of glucagon induce jejunal secretion and watery diarrhoea in vivo in dogs, rabbits, and man (Barbezat and Grossman, 1971; Moore and Longacher, 1974; Hicks and Turnberg, 1974). In some instances a simultaneous infusion of gastrin was necessary for this effect but gastrin was not required in the human studies. Glucagon also has potent effects on intestinal motility and blood flow (Kock et al., 1967) and, in order to assess whether intestinal secretion is due to a direct effect on mucosal ion transport or is secondary to motility and blood flow changes, we have studied the influence of glucagon on human intestinal mucosa in vitro where these complicating factors are eliminated.

Methods

Ion fluxes and electrical activity were measured in stripped human intestinal mucosa from surgical specimens using the technique of Ussing as modified for mammalian intestine (Clarkson and Toole, 1964; Schultz and Zalusky, 1964).

The methods are described in detail elsewhere (Corbett et al., 1977a) but, in brief, stripped mucosa was mounted in flux chambers so that both surfaces were bathed in stirred, oxygenated, and warmed isotonic solutions. Four chambers were usually set up from one operative specimen and the exposed surface of each was 1.77 cm². The buffer composition was Na 147, K 4.2, Mg 1.2, Ca 1.2, Cl 126, HCO₃⁻ 27.6, H₂PO₄⁻ 1.2, HPO₄²⁻ 0.2 mmol.l⁻¹, glucose 15 mM, pH 7.5, and 10 ml of this solution was placed on each side of the mucosa. Normality of the tissue was subsequently confirmed by histological examination.

The mucosal potential difference (PD) was measured on a high impedance digital voltmeter via saturated KCl bridges and calomel electrodes and was nullified by passage through the tissue of a short-circuit current (SCC) through NaCl bridges and silver/silver chloride electrodes. SCC was adjusted every four to five minutes with correction made for fluid gap resistance as described by Field et al. (1971).

Mucosal resistance (R) was found by dividing PD by SCC and tissues differing in R by less than 25% of the greater value were paired for ion flux measurements. Mucosal to serosal (Jₘₐ) and serosal to mucosal (Jₘₛ) ion fluxes were measured in each tissue pair by adding 1.5 μCi ²⁵Na and 2.0 μCi ³⁸Cl⁻ to the mucosal solution of one member and to the serosal solution of the other member of the tissue pairs. Samples (1 ml) were taken from mucosal and serosal solutions at 20 minute intervals and replaced with unlabelled buffer.

After 20 minutes for tissue isotope equilibration, glucagon was added to the serosal solution of one pair of tissues and electrical and flux measurements made, usually over two, and sometimes three, additional 20 minute periods. Electrical recordings and ion fluxes were measured simultaneously in a control pair of tissues. Pancreatic glucagon (Ely Lilly & Co) was added to the serosal solutions to final concentrations of 0.2, 0.5, 1.0, or 2.0 ng.ml⁻¹. Samples were counted and ion fluxes calculated as described previously (Corbett et al., 1977a)

Statistical comparison was made between control

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and experimental tissues in the same period by Student's t-test.

In a separate series of experiments to assess the possible synergistic effect of gastrin on ion transport, frequent open circuit PD readings were taken from three jejunal mucosal preparations when glucagon (0.25 ng.ml⁻¹) and pentagastrin (0.25 ng.ml⁻¹) final concentration (Peptavlon, ICI Pharmaceuticals), or an equal volume of saline were added to the serosal solution.

Results

Although PD, SCC, and R tended to fall in all groups of tissues, there was no difference between the values in control and glucagon treated tissues at any time.

Ion fluxes in jejunal and ileal mucosa are shown in the Table. Glucagon in initial concentrations of 0.2 to 2 ng.ml⁻¹ did not apparently influence ion transport in either tissue.

In the jejunal specimen open circuit PD was uninfluenced by the addition of glucagon (0.25 ng.ml⁻¹) plus pentagastrin (0.25 ng.ml⁻¹) when compared with control tissues to which saline had been added.

In order to assess the time for which the initial concentration of glucagon was maintained in the bathing medium, a tracer dose of ¹³⁵I labelled glucagon was added to the serosal solution in three studies and the rate of disappearance was measured. The half life of labelled glucagon was 14.5 minutes, indicating a rapid removal presumably by the tissue. Thus, although the initial concentration was known to be 0.2, 0.5, 1 or 2 ng.ml⁻¹, during a 40 minute flux experiment the mean concentrations achieved can be calculated to be about 0.09, 0.22, 0.44, and 0.88 ng.ml⁻¹.

Differences between the in vitro behaviour of jejunal and ileal mucosa have been reported elsewhere and are confirmed in these studies (Corbett et al., 1977a). Net sodium absorption in the presence of glucose was greater in iliac than jejunal tissues. Net chloride transport was not significantly different from zero in jejunal tissues but there was a significant absorption of chloride in most groups of ileal tissues.

Discussion

These studies demonstrate that glucagon does not apparently influence human ileal or jejunal ion transport in vitro in calculated mean concentrations of between 0.09 and 0.88 ng.ml⁻¹. However, plasma concentrations achieved within this range have been shown to induce jejunal secretion in vivo in man when the same commercial preparation of glucagon was used (Hicks and Turnberg, 1974). There are several possible explanations for the discrepancy between the in vitro and in vivo transport responses to glucagon. Firstly, glucagon has marked effects on intestinal motility and blood flow in vivo (Kock et al., 1967; Necheles et al., 1966; Fasth and Hultén, 1971; Bowen et al., 1975), and these changes may be responsible for a secondary effect on trans-epithelial transport. For example, it is recognised that a small rise in hydrostatic pressure on the serosal side of the epithelial layer will reverse fluid absorption (Hakim and Lifson, 1969) and both blood flow and motility changes may influence pressure within the mucosa. This suggestion has also been proposed recently by Makhlouf (1977).

Secondly, glucagon in vivo could conceivably liberate a hypothetical hormone from another site which may secondarily effect ion transport.

Thirdly, it is possible, although unlikely, that the time scale of the in vitro experiments was not long enough for an effect of glucagon to be observed. Experiments continued for 60 minutes did not demonstrate any effect of glucagon in vitro, while this was sufficient time for an effect to be observed in vivo.

Table  Unidirectional and net fluxes of ions and short circuit current in μmol. cm⁻²h⁻¹ in jejunal and ileal mucosae in presence of glucagon (2 ng.ml⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>Na</th>
<th>Cl</th>
<th>Iₑₑ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Glucagon</td>
<td>Control</td>
</tr>
<tr>
<td>Jejunum (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jₑₑ</td>
<td>14.3 ± 3.4</td>
<td>13.4 ± 3.4</td>
<td>6.6 ± 1.8</td>
</tr>
<tr>
<td>Jₛₛ</td>
<td>7.9 ± 1.8</td>
<td>7.8 ± 1.6</td>
<td>9.7 ± 2.3</td>
</tr>
<tr>
<td>Jₙₙₙ</td>
<td>6.4 ± 2.4</td>
<td>5.6 ± 2.3</td>
<td>3.1 ± 0.9</td>
</tr>
<tr>
<td>Ileum (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jₑₑ</td>
<td>22.1 ± 2.8</td>
<td>19.5 ± 2.7</td>
<td>12.9 ± 1.1</td>
</tr>
<tr>
<td>Jₛₛ</td>
<td>8.3 ± 0.7</td>
<td>8.7 ± 1.8</td>
<td>8.9 ± 0.7</td>
</tr>
<tr>
<td>Jₙₙₙ</td>
<td>13.8 ± 2.2</td>
<td>10.8 ± 1.1</td>
<td>4.0 ± 0.7</td>
</tr>
</tbody>
</table>

There is no significant difference between control and glucagon results. Results from studies of similar numbers of tissue pairs with glucagon concentrations of 0.2, 0.5 and 1.0 ng.ml⁻¹ similarly showed no significant difference from simultaneously incubated control tissues.

Jₑₑ: unidirectional flux from serosal to mucosal side of tissue. Jₛₛ: flux in the opposite direction. Jₙₙₙ: the difference between these two unidirectional fluxes, the net flux. Mean value ± 1 SEM are shown.
Fourthly, it is conceivable that this in vitro technique is too insensitive to detect subtle changes in transport which may be obvious in vivo. We do not believe this to be the case as we have demonstrated in vitro mucosal responsiveness to theophylline (Corbett et al., 1977b) and acetylcholine (Issacs et al., 1976) and others have shown clear sensitivity of mucosa in vitro to cholera, VIP, and prostaglandins (Kimberg et al., 1971; Field et al., 1972; Schwartz et al., 1974; Klaeveman et al., 1975).

In several types of intestinal secretion, such as in cholera, activation of adenyl cyclase resulting in a rise in cAMP concentrations in the mucosa is an essential step (Field, 1971). It is noteworthy, therefore, that despite the intestinal secretion promoted by glucagon in vivo, adenyl cyclase activity is not influenced by glucagon (Schwartz et al., 1974), an observation in keeping with our present studies on in vitro transport and pointing towards an alternative mechanism not involving cAMP for intestinal secretion.

We conclude, therefore, that intestinal secretion may be induced in vivo in the absence of a direct effect by glucagon on primary ion transporting mechanisms. Clearly, the mechanisms for ion transport in vivo are more complex than in vitro and these studies indicate a possible role for factors, other than primary transporting processes, in the overall absorptive or secretory activity of the intestine.

References


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