A comparison of methods for measurement of rectal potential difference in man: effects of rectal infusion of amiloride

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Summary

1. Two methods for measurement of rectal potential difference in man were compared. A saline-filled catheter technique gave more reproducible results and was better tolerated by patients than the solid probe technique.

2. Infusion of amiloride at a concentration of $4 \times 10^{-6}$ mol/l in saline produced a variable fall of potential difference in six normal subjects. Dose-response curves in two subjects showed that complete inhibition of rectal potential difference occurred at $4 \times 10^{-5}$ mol/l and $4 \times 10^{-6}$ mol/l respectively.

3. This finding provides additional evidence that rectal potential difference in man results from electrogenic ion transport across the mucosal epithelial cell.

Key words: amiloride, electrogenic ion transport, potentiometry.

Introduction

The colonic mucosa is electrically polarized, the luminal side being negative with respect to the serosal side (Cooperstein & Brockman, 1959). The potential difference (PD) increases when sodium transport is stimulated (Edmonds, 1967; Edmonds & Marriott, 1967). In early studies in man (Edmonds & Godfrey, 1970) subcutaneous electrodes were used and sigmoidoscopy was necessary to insert the rectal probe. Endoscopy was later eliminated (Edmonds & Richards, 1970), and saline infused into the rectum before a blunt-probe electrode was advanced blindly.

Amiloride inhibits electrogenic ion transport in animal models (Cuthbert, 1973), and our study in man was designed to throw light on the mechanism of generation of rectal PD by observing the effects of rectal administration of amiloride. In addition the original endoscopic method for inserting a dry rectal probe has been compared with a technique which eliminates endoscopy and depends upon the introduction of a saline-filled catheter into the rectum to provide electrical contact.

Methods

Informed consent was obtained from male members of the medical and laboratory staff, all of whom had normal blood pressure and ate a normal diet. Their ages ranged from 28 to 40 years. Rectal PD measurements were made at the same time of day on different days.

The endoscopic rectal PD method (Archampong & Edmonds, 1972) required a probe electrode which was built into the tip of a Perspex catheter filled with agar–saline to provide electrical contact. The reference (skin) electrode was a silver/silver chloride junction fitted into a Perspex disc containing agar–saline. The probe was introduced through a proctoscope and PD recorded on a high-impedance millivoltmeter.

The saline-filled catheter could be inserted into the rectum without prior proctoscopy. The rectal electrode (Fig. 1) consisted of a silver rod housed in polyvinyl chloride (PVC) tubing through which
isotonic sodium chloride solution (154 mmol/l; saline) was infused continuously at 0.85 ml/min. The rectal catheter was of narrower bore (internal diameter 4 mm), fitted snugly into the end of the PVC tubing which housed the electrode, and could be inserted 10 cm into the rectum. In order to avoid junctional potentials, caused by saline coming into contact with the soldered joints, the latter were housed in plastic tubing sealed with epoxy resin (Araldite). The skin electrode was a square sheet of silver (15 mm x 15 mm) with a narrow handle which was soldered to copper wire leading to the millivoltmeter, the soldered junction being insulated as for the rectal electrode. Both electrodes were coated with a layer of silver chloride by electrolysis in hydrochloric acid (0.1 mol/l) with a 1.5 V cell.

Subjects were studied in the left lateral position, without prior rinsing of the rectum. The rectal catheter was primed with isotonic saline ensuring that there were no air bubbles in the infusion system, and inserted 10 cm into the rectum. The saline flow rate was increased to 8.5 ml/min for a few seconds to eliminate any bubbles which may have formed at the time of insertion of the catheter into the rectum, and the flow rate was subsequently maintained at 0.85 ml/min. The reference electrode was wrapped in paper tissue, wetted with isotonic saline and taped over the site of an intradermal injection of 0.2 ml of isotonic saline in the skin of the buttock. In preliminary experiments rectal PD readings were taken every 30 s for 3 min. At the beginning and end of each series of measurements the two electrodes were placed together in isotonic saline to check that the asymmetry between them was no more than ±3 mV. Rectal PD was measured in six subjects, both the endoscopic technique with its dry electrode and the saline-filled catheter technique being used alternately on the same occasion.

To determine the effect of amiloride on rectal PD in six normotensive subjects a baseline was first established by monitoring rectal PD for 20 min at 30 s intervals whilst constantly infusing saline into the rectum at 0.85 ml/min. On a subsequent occasion rectal PD was measured during infusion of amiloride. There was no response to a dose of 1 x 10^{-6} mol/l (Cuthbert, 1973) but a dose of 4 x 10^{-6} mol/l in isotonic saline was used to produce a significant effect, being infused at 0.85 ml/min for about 12 min after 8 min of control observations and PD being measured at 30 s intervals. Rectal PD was also measured in two normal subjects during infusions of amiloride at concentrations ranging from 4 x 10^{-5} to 4 x 10^{-4} mol/l, different doses being infused at intervals of not less than 5 days.

Results

Comparison of rectal potential difference methods

The saline catheter technique for measurement of rectal PD gave higher values in six normal subjects (54.9 ± 15.4 mV) than the endoscopic technique (35.9 ± 12.2 mV). In six normal subjects the coefficient of variation for six to 11 successive measurements with the endoscopic technique ranged from 2.5 to 11.9% and from 1.0 to 3.4% in the same subjects when the saline-filled catheter technique was used. The saline catheter was better tolerated by the patients than the endoscopic technique because of the narrow bore of the catheter.

Amiloride infusions

In all six subjects a preliminary study using the saline-filled catheter confirmed the stability of rectal PD over 20 min. Variations in PD in the first 2–3 min resolved spontaneously and the coefficient of variation during the 20 min of rectal PD measurement was less than 5% when these initial variations were excluded. Infusion of 4 x 10^{-6} mol of amiloride/l in saline reduced the rectal potential difference by 50–100% in all six normal subjects. The log normal dose–response curve in two subjects showed that this was a dose-related
response. Complete inhibition of rectal PD occurred in subject D.W. when amiloride was infused at a concentration of $4 \times 10^{-5}$ mol/l, and in subject F.M. at $4 \times 10^{-6}$ mol/l. The points on the two log dose–response curves (Fig. 2a) were fitted by a linear least-squares regression line (Fig. 2b). The pooled data for each regression line showed that the lines were significantly different from zero response, but were not parallel.

Discussion

Determination of rectal potential difference in man

We found the endoscopic technique to be less reproducible than the saline-filled catheter technique. Similar variability of endoscopic technique has been noted by Beevers, Morton, Tree & Young (1975), who found that the coefficient of variation for 10–20 repeated measurements on eight subjects ranged from 7.7 to 23%. Factors contributing to the poor precision of the endoscopic technique included damage and contamination of the agar with faeces, since the rectum was not washed out before each measurement. To obtain reproducible results with the catheter it was necessary to ensure that it was free of bubbles, which could give rise to false or unstable values. The higher PD obtained with the catheter may reflect the wide spread of infused saline throughout the rectum, whereas electrical contact with the solid probe is confined to one point at which the mucosa may be easily damaged by the probe. The saline-filled catheter technique was better tolerated by our subjects and had the additional advantage that it could be used for pharmacological experiments requiring the infusion of fluids into the rectum.

Effect of amiloride on rectal potential difference

Amiloride hydrochloride was infused into the rectum to show whether rectal potential difference in man was generated by electrogenic ion transport. It inhibits such transport in frog skin (Cuthbert, 1973), amiloride at 1 μmol/l producing 90% inhibition of short-circuit current. In man, however, we found it necessary to use a concentration of 4 μmol/l in order to obtain a marked effect. There was a dose-dependent relationship between the concentration of infused amiloride and the extent of inhibition of rectal potential difference. The difference between slopes of the dose–response curves could be due to a combination of factors including variation in receptor sensitivity or the concentration of the amiloride at the biophase. The concentration of infused amiloride may well not be the concentration at the receptor site since the infusate may be diluted by fluid already in the rectum.

Active transport of sodium occurs from the lumen of the rectum to the blood against a concentration gradient (Edmonds, 1971). This appears to be largely responsible for the observed transmucosal potential difference (Cooperstein & Hogben, 1959; Archampong & Edmonds, 1972). An increase in human rectal potential difference is associated with the increased sodium absorption which follows aldosterone and carbenoxolone administration (Edmonds & Godfrey, 1970; Tomkins & Edmonds, 1975).

Amiloride has recently been shown to cause a prompt fall in potential difference across human colonic mucosa when applied to the mucosal surface in vitro, and rectal infusion of amiloride (1 mol/l) in vivo reduced PD by 33–52% (Rask Madsen & Hjelt, 1977). Our study confirms this finding and also shows a dose-dependent relationship between rectal potential difference and the concentration of amiloride infused into the rectum. Reduction of short-circuit current and PD across both human colonic mucosa in vitro, and also isolated frog skin, in response to amiloride is associated with reduction in sodium flux (Salako &...
Smith, 1970; Rask-Madsen & Hjelt, 1977). By analogy we suggest that the effects of amiloride on human rectal PD in vivo reflect a reduction in sodium flux across the rectal mucosal cell.

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References


