J. Physiol. Biochem., 53 (4), 385-386, 1997
Revista española de Fisiología

Effect of dithiothreitol on mucus gel layer and electrophysiological properties in rat colon

In spite of its physiological roles (1), very different values have been reported for the actual thickness of the adherent mucus gel layer (MGL) of rat distal colon, ranging from about 16 to 163 µm (1, 10, 15, 16). For experimental work *in vitro*, it is often desirable to eliminate the MGL to allow exposure of the apical membrane of enterocytes. Since WEISER'S original suggestion (17) the sulfhydryl reducing agent, dithiothreitol (DTT), has often been used as a mucolytic in gastrointestinal research. However, its lack of effect on enterocyte electrophysiological properties has not been clearly documented. The effect of incubation with DTT on rat distal colon MGL thickness and electrical parameters is here reported.

Male adult Wistar rats, fasted for 20 to 24 h were employed to obtain either mucosa-submucosa or isolated mucosa preparations, as previously described (13). The former were fastened with pins as flat sheets on a plate covered by beewax, with the MGL facing upwards, to obtain 1-mm thick slices with two parallel razor blades, as described for gastric MGL thickness measurement (6, 12). Slices were transferred to a small glass chamber filled with Ringer solution either with or without previous incubation with 1 mM DTT for 10 min. MGL thickness was measured under a light microscope with a calibrated rule. From 10 to 12 measurements at evenly spaced distances were performed on each preparation.

Statistical analysis was performed by the Mann-Whitney U test for MGL thickness data; by a paired, two sided Student's *t* test for DTT effects on baseline electrophysiological properties, and by Bonferroni's method for multiple comparisons for DTT effects on the response to hypoxia and reoxygenation. Results are reported as mean ± SEM. A $p < 0.05$ was considered significant.

Normal MGL was usually continuous with a thickness of 115.4 ± 5.5 µm (216 measurements in 20 preparations). The frequency distribution was skew ranging from 0 to 390 µm; median and mode being 90.9 µm. The MGL layer of DTT-treated slices was often discontinuous, amounting to 40.5 ± 6.4 µm (101 measurements in 9 preparations; ranging from 0 to 318 µm, the mode being 0 µm, and the median 36.4 µm). The difference between both groups was significant ($t = 8.231$; $p < 0.01$).

In Ussing chamber experiments, baseline short-circuit current (Isc) and transepithelial potential difference (PD), were permanently reduced by DTT, without a consistent effect in transepithelial resistivity (Rt). Control values were Isc = 106.0 ± 8.5 $\mu A \cdot cm^{-2}$; PD = 12.1 ± 0.9 mV; Rt = 115.4 ± 4.3 $\Omega \cdot cm^2$. Values after DTT addition were Isc = 66.2 ± 5.4 $\mu A \cdot cm^{-2}$; PD = 8.5 ± 0.6 mV; Rt = 130.8 ± 8.1 $\Omega \cdot cm^2$ ($n = 9$; $p < 0.001$ for Isc and PD).

On the other hand, although starting from lower baseline values, the qualitative Isc responses to either total and selective hypoxia and reoxygenation, or to total hypoxia followed by sequential reoxygenation, were not modified by DTT (fig. 1) as compared with preparations of either normal isolated mucosa or isolated mucosa with mechanical removal of the MGL (13). In no case did Rt show a significant change, since Isc and PD were proportionally modified. Quantitatively, smaller fractional depressions of Isc and PD were observed for serosal hypoxia after DTT.

Normal mean MGL thickness is within the range of values reported by other authors (1, 10, 15,16). Different dissection techniques and tissue preparation methods no doubt play a role in the wide range of values found in the literature. Fresh mucosa-submucosa preparations retain the MGL, are easily mounted and allow ready visualization of the MGL.

DTT is an effective mucolytic employed in intestinal research at concentrations in the millimolar range (2-5, 8, 11, 14), and its use is commended for enterocyte membrane isolation procedures (9). At 1 mM no effect on Isc has been reported (3), though no values were provided. Present data show, on the contrary, that the agent does cause long-lasting depressions of Isc and PD. Since disulfide and sulfhydryl groups are ubiquitous and subserve different functions, DTT could be expected to have other effects besides the mucolytic one, as when DTT collapses the gastric pH gradient within 10 min, yet its mucolytic action takes about 1 h to be noticeable (2). Agents reacting with S-S groups are also

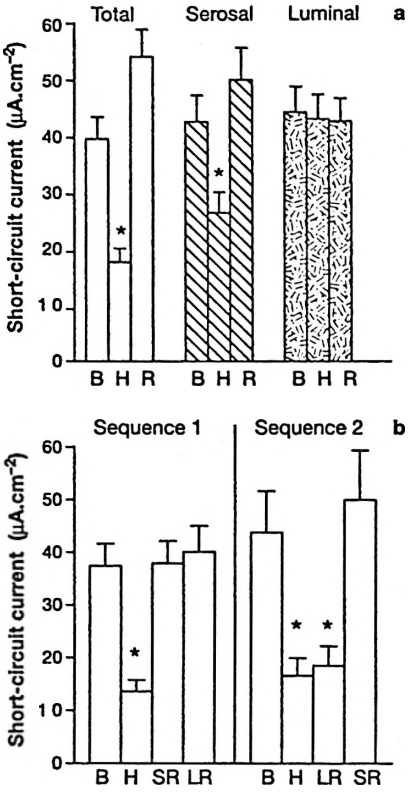

Fig. 1. *Response of rat distal colon mucosa to hypoxia and reoxygenation after incubation with dithiothreitol*

a) Isc of isolated mucosa preparations (n = 12) submitted to total, serosal or luminal 5-min hypoxia. b) Isc of isolated mucosa preparations (n = 9) submitted to 5-min total hypoxia followed by sequential serosal-luminal (sequence 1) or luminal-serosal (sequence 2) reoxygenation. B, baseline; H, hypoxia; R, reoxygenation. SR, serosal reoxygenation; LR, luminal reoxygenation. An asterisk indicates a significant difference from the respective baseline.

known to modify colonic apical membrane function (7).

In summary, 1) rat distal colon MGL thickness may be reliably measured by direct light microscopy in mucosa-submucosa preparations; 2) DTT markedly reduces MGL thickness; 3) DTT depresses Isc and PD, but 4) without affecting qualitative Isc and PD responses to hypoxia and reoxygenation. DTT should be used with care when preservation of normal electrophysiological properties of the epithelium is required.

Acknowledgements

Supported by a grant from the "Consejo de Investigación" and a scholarship to Mr. Arrieta from the "Facultad de Ciencias Médicas", both from "Universidad Nacional de Cuyo" (Argentina).

Key words: Dithiothreitol, Rat colon.

Palabras clave: Ditiotreitol, Colon de rata.

References

1. Allen, A., Hutton, D.A., Pearson, J. P. *et al.* (1990): In "The cell biology of inflammation in the gastrointestinal tract" (T. J. Peters, ed.). Corners Publications, Hull, pp. 113-125.
2. Bell, A. E., Sellers, L. A., Allen, *et al.* (1985): *Gastroenterology*, 88, 269-280.
3. Dagher, P. C., Balsam, L., Weber, J. T. *et al.* (1992): *Gastroenterology*, 103, 120-127.
4. Dagher, P. C., Behm, T., Taglietta-Kohlbrecher, A. *et al.* (1996): *Am. J. Physiol.*, 269, C899-C906.
5. Diener, M., Rummel, W., Mestres, P. *et al.* (1989): *J. Membr. Biol.*, 108, 21-30.
6. Kerss, S., Allen, A. and Garner, A. (1982): *Clin. Sci.*, 63 187-195.
7. Luger, A. and Turnheim, L. (1980): *J. Physiol. Lond.*, 317, 49-66.
8. Mircheff, A. K., Ahnen, D. J., Islam, A. *et al.* (1985): *J. Membr. Biol.*, 83, 95-107.
9 Mircheff, A. K. and van Corven, E. J. J. M. (1990): *Meth. Enzymol.*, 192, 341-354.
10. Sakata, T. and Engelhardt, W. V. (1981): *Cell Tiss. Res.*, 219, 629-636.
11. Sandberg, J. W., Lau, C., Jacomino M. *et al.* (1994): *Human Gene Ther.*, 5, 323-329.
12. Sandzén, B., Blom, H. and Dahlgren, S. (1988): *Scand. J. Gastroenterol.*, 23, 1160-1164.
13. Saraví, F. D., Saldeña, T. A., Cincunegui, L. M. and Carra, G. E. (1997): *J. Physiol. Biochem. (Rev. esp. Fisiol.)*, 53, 367-376.
14. Singh, S. K., Binder, H. J., Geibel, J. P. *et al.* (1995): *Proc. Natl. Acad. Sci. U.S.A.*, 92, 11573-11577.
15. Szentkuti, L. and Lorenz, K. (1995): *Histochem. J.*, 27, 466-472.
16. Szentkuti, L., Riedesel, H, Enss, M. L. *et al.* (1990): *Histochem. J.*, 22, 491-497.
17. Weiser, M. M. (1973): *J. Biol. Chem.*, 248, 2536-2541.

T. A. Saldeña, F. D. Saraví, O. R. Arrieta, L. M. Cincunegui and G. E. Carra

Cátedra de Física Biológica, Facultad de C. Médicas, Universidad Nacional de Cuyo, 5500 Mendoza (República Argentina)

(Received on May 4, 1997)