nicotine acts on the presynaptic nerve terminals probably by blocking the amphetamine-stimulated release of DA.

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REFERENCES

Andersson, K., Fuxe, K., Agnati, L. F., Eneroth, P. (1981) Med. Biol. 59: 170–176

Fung, Y. K., Uretsky, N. J. (1980) J. Pharmacol. Exp. Ther. 214: 651–656

Fung, Y. K., Uretsky, N. J. (1982) Ibid. 223: 477–482

Fuxe, K., Agnati, L., Eneroth, P., Gustafsson, J. A., Hokfelt, T., Skeet, A., Lofstrom, B., Skett, P. (1977) Med. Biol. 55: 148–157

Giorguieff-Chesselet, M. F., Kemel, M. F., Wandscheer, D., Glowinski, J. (1979) Life Sci. 25: 1257–1262

Hill, E., Haley, N. J., Wynder, E. L. (1983) J. Chron. Dis. 36: 439–449

Lau, Y. S., Runce, C., Dowd, F. (1984) J. Pharmacol. Exp. Ther. 229: 32–37

Lichtensteiger, W., Hefti, F., Felix, D., Huwyler, T., Melamed, E., Schlumpf, M. (1982) Neuropharmacology 21: 963–968

Misgold, U., Weiler, M. H., Bak, I. J. (1980) Exp. Brain Res. 39: 401–409

Morley, B. (1981) J. Pharmacol. Ther. 15: 111–122

Munson, P. J., Rodbard, D. (1980) Anal. Biochem. 107: 220–239

Naquira, D., Zunino, E., Arqueros, L., Viveros, O. H. (1978) Eur. J. Pharmacol. 47: 227–229

Russell, M. A. H., Wilcon, C., Patel, V. A., Feyerabend, C., Cole, P. V. (1975) Br. Med. J. 2: 414–416

Turner, J. A. M., Sillett, R. W., McNichol, M. W. (1977) Ibid. 2: 1387–1389

Von Voigtlander, P. F., Moore, K. E. (1973) Neuropharmacology 12: 451–461

Westfall, T. C. (1974) Ibid. 13: 693–700

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The effect of sodium deoxycholate given by gavage with heparin on the histology of the intestinal mucosa of the rat

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To gain direct insight into the mechanism of sodium deoxycholate (DOC)-induced enhancement of gastroenteral heparin absorption in rats, we performed light and electron microscopic examination of the mucosa of the small intestine of animals treated orally with DOC, heparin or DOC plus heparin. The sole morphological change observed after DOC and DOC plus heparin administration was a marked reduction in the length and distribution of glycocalyx filaments on the microvilli of epithelial cells. The morphological picture had reverted to normal after 24 h, when the promotion of enteral heparin absorption by DOC is greatly reduced. Thus, we suggest that DOC may promote the enteral absorption of heparin in rats by affecting some as yet unidentified barrier mechanism requiring glycocalyx integrity.

Experimental evidence shows that certain drugs, such as EDTA, salicylic acid derivatives, ionic and non-ionic surfactants, bile acids and others, allow heparin to be absorbed (Windsor & Cronheim 1961; Engel & Riggi 1969; Davis et al 1970; Gibaldi & Feldman 1970; Nishihata et al 1981b; Ziv et al 1983; Guarini & Ferrari 1984, 1985) by the gastrointestinal tract and so produce its typical pharmacological effects, i.e. inhibition of blood coagulation and lipaemic plasma clearing activity (PC). However, the mechanism(s) of action has not, as yet, been identified. Some researchers attribute the effect to morphological disruption of the gastrointestinal mucosa (Davis et al 1970; Whitmore et al 1979; Nishihata et al 1981b), others to more subtle alterations of the biochemical mechanisms responsible for maintaining the impermeability of the mucosa to heparin (Guarini & Ferrari 1985).

Our previous work has shown that the facilitation of heparin absorption by the rat gastrointestinal tract afforded by bile acids and certain non-ionic surfactants of the polyoxyethylene-series is strictly governed by their molecular structure (Guarini & Ferrari 1984). Accordingly, we tend to support the hypothesis of a selective functional interference rather than that of a structural disruption.

To gain direct insight into the mechanism by which sodium deoxycholate (DOC) enhances heparin absorption, we examined the morphological aspect of the intestinal mucosa of rats receiving DOC, heparin, DOC plus heparin, or water, inasmuch as the DOC effect may be of clinical interest. The present paper presents the results of our investigation and shows that the activity of DOC might be attributed to its influence on glycocalyx filaments of intestinal epithelial cells.

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Materials and methods

Urethane-anaesthetized (1·25 g kg^{-1} i.p.) female Wistar rats (S. Morini, S. Polo d'Enza, Reggio Emilia, Italy), 180–200 g, were deprived of food, but not water, for 12 h before the experiments. On the basis of previous experiments the drugs, dissolved in distilled water, or distilled water alone, were administered by gavage at a fixed dose of 500 mg kg^{-1} in a volume of 5 ml kg^{-1}. DOC was administered alone, 1 or 24 h before heparin, the mucosal samples being taken 1 h after the last treatment, when heparin absorption is maximal (Guarini & Ferrari 1985), or 24 h after DOC administration.

For the purpose of light microscopy, pieces of duodenum, jejunum and ileum were fixed in 4% formalin, dehydrated, embedded in paraffin, sectioned and mounted on slides, using conventional histological methods. For the purpose of electron microscopy, small pieces of these tissues were fixed for 4 h in 3% glutaraldehyde in Sörensen's phosphate buffer (pH 7·4) postfixed in 1% osmium tetroxide in the same buffer, dehydrated through graded series of ethanol and propylene oxide, and embedded in Durcupan. Sections (50 nm) were cut with the Porter Blum MT-2 ultramicrotome and stained with uranyl acetate and lead citrate according to Reynold's method. Electron microscopic examination was performed on a Zeiss 109 electron microscope.

In the same animals heparin absorption was evaluated by measuring plasma clearance (PC) and heparin content. A blood sample was taken by heart puncture 1 or 24 h after the last treatment and PC measured by calculating the percent decrease in optical density at 650 nm of a mixture of 0·5 ml of lipostrate CB A grade (a standard emulsion, Calbiochem, San Diego, Cal., USA), 0·20 in water, and 1 ml of plasma, the mixture being incubated for 1 h at 25 °C, as suggested by Grossman (1954) with minor modifications (Ferlito et al 1980). Plasma heparin content was measured by the Boehringer (Mannheim, FRG) 'heparin low dose test' diluting the plasma where necessary with distilled water.

Heparin sodium (177 iu mg^{-1}, USP XX) was supplied by Prodotti Gianni (Milan, Italy) and sodium deoxycholate by Oxoid Italia (Milan, Italy).

Results

Duodenal, jejununal and ileal mucosa samples examined by light microscopy at a magnification of X 400 in rats receiving an aqueous solution of DOC, heparin, DOC plus heparin 1 or 24 h later, or water alone (5 rats per group), did not show any pathological change, whatever the treatment. Electron microscopy at a final magnification of X 24 000 showed that none of the treatments altered the tight junctions and desmosomes of the epithelial cells of the duodenum, jejunum and ileum. However, at a final magnification of X 100 000, electron microscopy revealed that DOC or DOC plus

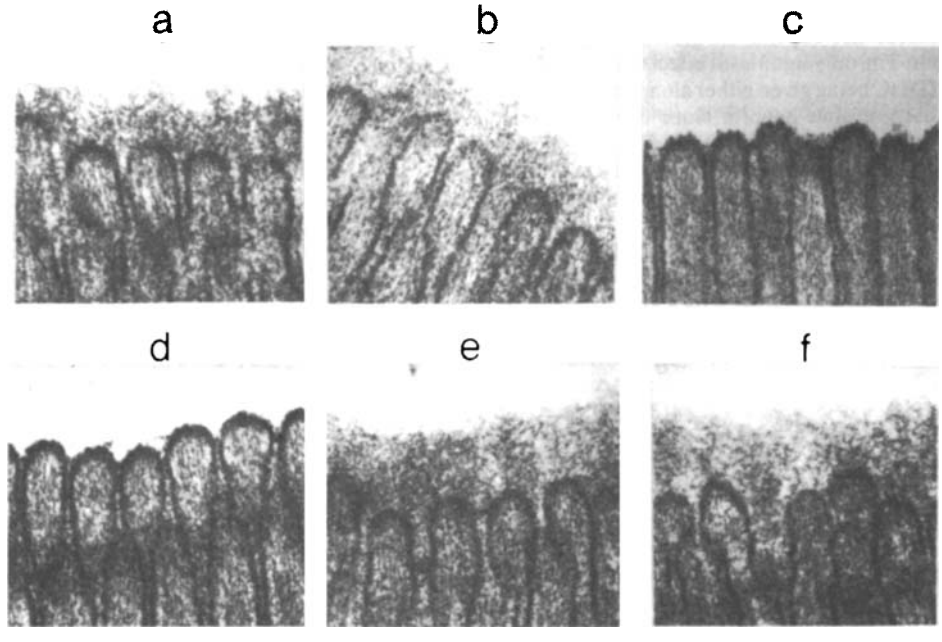

FIG. 1. Electron micrographs showing the influence of various oral treatments on length and distribution of the glycocalyx filaments on microvilli of duodenal epithelial cells, in rats (final magnification of X 100 000). (a) water; (b) heparin; (c) DOC; (d) DOC followed by heparin 1 h later; (e) DOC; (f) DOC followed by heparin 24 h later. Heparin and DOC were administered at a dose of 500 mg kg^{-1} in a volume of 5 ml kg^{-1}. The pieces of tissue were taken 1 h (a,b,c,d,f) or 24 h (e) after the last treatment.

Table 1. Influence of various oral treatments on plasma clearing activity (PC) and heparin content in rats.

Experiment	Treatment DOC (mg kg^{-1})	Heparin (mg kg^{-1})	DOC-heparin interval (h)	PC (%)	Plasma heparin (iu ml^{-1})
A	—	—	—	0	(a)
B	—	500	—	2·45 ± 1·22*	(a)
C	500	—	—	0	(a)
D	500	500	1	81·25 ± 3·35	0·30 ± 0·07
E	500	—	—	0	(a)
F	500	500	24	16·15 ± 4·22*	(a)

The animals of experiment A received only water (5 ml kg^{-1}). DOC and heparin were administered dissolved in water in a volume of 5 ml kg^{-1}. Single blood samples were taken 1 h (experiments A,B,C,D,F) or 24 h (experiment E) after the last treatment.
The values are means (n = 5) ± s.e.m.
(a) below detection threshold.
*$P < 0·001$ versus value for experiment D (Dunnett's test for multiple comparison with a control).

heparin caused a marked reduction in the length and distribution of glycocalyx filaments on microvilli of duodenal (Fig. 1), jejununal and ileal epithelial cells. The reduction was very marked 1–2 h after DOC (Fig. 1 c,d) but practically absent after 24–25 h (Fig. 1 e,f); accordingly, PC was far greater at 2 than at 25 h (81·25 ± 3·35% and 16·15 ± 4·22%, respectively) (Table 1).

Discussion

Light and electron microscopic examination of the small intestine mucosa of rats receiving DOC (500 mg kg^{-1}), heparin (500 mg kg^{-1}) or both (500 + 500 mg kg^{-1}) by gavage demonstrated that DOC-induced heparin absorption does not depend on gross damage of the mucosal cells. The only significant effect seen after DOC treatment (DOC being given either alone or followed by heparin), but not after heparin alone or water alone, was a reduction in the length and density of glycocalyx filaments on the microvilli of the epithelial cells of the small intestine, and sometimes their complete disappearance.

It is worth noting that when Sithigorngul et al (1983) exposed in-vivo rat rectal epithelium to 2% sodium salicylate at pH 7, a procedure which enhances epithelial cell permeability to trypan blue, and then subjected the tissue to light and electron microscopic examination, they did not observe any substantial structural changes, except 'a reduction in the length or distribution of glycocalyx filaments on microvilli of epithelial cells'.

Sodium salicylate has been reported to enhance the permeability of rectal epithelial cells to heparin (Nishihata et al 1981b) and other drugs (Nishihata et al 1981a). Since (i) the influence of sodium salicylate on rectal epithelium is reversible (Sithigorngul et a 1983), (ii) the dose of DOC we administered does not produce any overt signs of gastrointestinal damage (Guarini & Ferrari 1985), and (iii) the reduction of glycocalyx filaments produced by DOC is marked after 1 h but practically absent after 24 h, it seems that one possible conclusion is that DOC may promote enteral heparin absorption by affecting some as yet unidentified epithelial barrier mechanism, perhaps dependent on glycocalyx integrity.

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REFERENCES

Davis, W. W., Pfeiffer, R. R., Quay, J. F. (1970) J. Pharm. Sci. 59: 960–963

Engel, R. H., Riggi, S. J. (1969) Proc. Soc. Exp. Biol. Med. 130: 879–884

Ferlito, S., Puleo, F., Carrà, G., La Spina, E., Del Campo, F. (1980) G. Clin. Med. 61: 221–230

Gibaldi, M., Feldman, S. (1970) J. Pharm. Sci. 59: 579–589

Grossman, M. I. (1954) J. Lab. Clin. Med. 43: 445–451

Guarini, S., Ferrari, W. (1984) Arch. Int. Pharmacodyn. 270: 4–10

Guarini, S., Ferrari, W. (1985) Experientia 41: 350–352

Nishihata, T., Rytting, J. H., Higuchi, T. (1981a) J. Pharm. Sci. 70: 71–75

Nishihata, T., Rytting, J. H., Higuchi, T., Caldwell, L. (1981b) J. Pharm. Pharmacol. 33: 334–335

Sithigorngul, P., Burton, P., Nishihata, T., Caldwell, L. (1983) Life Sci. 33: 1025–1032

Whitmore, D. A., Brookes, L. G., Wheeler, K. P. (1979) J. Pharm. Sci. 31: 277–283

Windsor, E., Cronheim, G. E. (1961) Nature 190: 263–264

Ziv, E., Eldor, A., Kleinman, Y., Bar-On, H., Kidron, M. (1983) Biochem. Pharmacol. 32: 773–776