Vol. 94, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 16, 1980 Pages 960-966

SIGNIFICANCE OF THE GOBLET-CELL MUCIN LAYER, THE OUTERMOST LUMINAL BARRIER TO PASSAGE THROUGH THE GUT WALL

Fritz Nimmerfall and Joachim Rosenthaler

Pharmaceutical Division, SANDOZ LTD., Basle, Switzerland

Received April 29,1980

SUMMARY. The decisive luminal barrier to the passage of a compound through the gut wall would appear to be the mucin secreted by goblet-cells. The absorption of compounds of different chemical structures, measured in animal experiments, is directly proportional to diffusion through isolated goblet-cell mucin and inversely proportional to retention in the mucin and, with reservation also to molecular weight. The dependence of diffusion through and retention by the mucin on pH and ionic strength are discussed with reference to changes in the electrical charge and structure of the mucus and its ability to form hydrogen bridges.

After oral ingestion a compound reaching the intestinal membrane first comes into contact with a layer of hydrated goblet-cell mucin (1) before passing through further membranes to reach the efferent vessels.

Hitherto it has been assumed that there are two separate diffusion barriers (2), the unstirred water layer and the lipid membrane. However, this paper postulates that the first layer, which has hitherto been described as the unstirred water layer, is identical with hydrated goblet-cell mucin, a view supported by the fact that the unstirred water layer and goblet-cell mucin layer are similar in thickness (3,4).

A number of authors have drawn attention to the importance of the mucus secreted by the small intestine for absorption (5-9). For this reason we set out to obtain goblet-cell mucin as far as possible intact for investigations on the absorption mechanism. Tests were carried out to determine whether diffusion of certain

0006-291X/80/110960-07$01.00/0

compounds through the goblet-cell mucin or their retention by the mucin layer correlated with actual absorption measured *in vivo*. For these investigations freshly isolated goblet-cell mucin which had not been further purified (10,11) and which was free from microvilli was prepared from the small intestine of the species which was employed for the *in vivo* absorption measurements (12, 13). Mucus from different organs also varies in composition (14). The thickness of the mucin layer employed in our experiments was the same as that in the intact organism. The quantity absorbed in the *in vivo* experiments was defined as that fraction of the orally administered dose of compound which passed through the intestinal wall to the efferent vessels (15).

MATERIALS AND METHODS. The following compounds were employed: (a) [9,10-^{3}H]dihydroergotamine (16), (b) [6-^{14}C]dihydroergonine (16), (c) [9,10-^{3}H]dihydrolysergic acid m-dialkylcarbamylanilide, (d) [2-^{3}H]ergotamine (16), (e) 1-[(1,1-dimethyl[1-^{14}C] ethyl)amino]-3-[(2-methyl-1H-indol-4-yl)oxy]-2-propanol-benzoate, (f) [9,10-^{3}H]N-(6-methyl-8α-ergolinyl)-dialkyl-sulfamylamino compound, and (g) [1-^{14}C]pindolol (17). The chemical and radiochemical purity of these compounds was verified by thin-layer chromatography. The label was stable under the experimental conditions described below. The saturation solubilities of the compounds were determined and always, unsaturated solutions were employed.

Experiments to determine diffusion and retention. Goblet-cell mucin was expressed with the aid of a glass slide from intact small intestine from male rats. The mucus obtained in this way was not further purified. It was examined by electron microscopy and found to be free from microvilli. The mean protein content (18) was 62±14 (S.D.) mg per g mucus (wet weight; N=30). To measure retention of the compounds in the mucus network a 1:10 dilution of the goblet-cell mucin was prepared with McIlvaine phosphate-citrate buffer at pH 6 and ionic strength 0.1. The diluted mucus was dialysed for 4 h at 37°C against solutions of the compounds in 1 ml Teflon cells. The compartments were separated from each other by means of a cellulose membrane permeable to compounds with a molecular weight of less than 10,000. Aliquots of 500 µl were taken from each compartment, and the radioactivity of these two samples was measured in a liquid scintillation counter. The fraction retained in the mucus was determined as a percentage of total radioactivity.

In the diffusion experiments a third compartment between cellulose membranes was inserted between the two Teflon cells. This third compartment with a thickness of 500 µm was filled with undiluted goblet-cell mucin. 1 ml of buffer solution was placed in one of the outer compartments, and a solution of the test compound in the buffer was placed in the other compartment. After dialysis for 1 h at 37°C aliquots of 500 µl were taken from each

compartment and their radioactivity measured as a percentage of total radioactivity for determination of the diffusion rate. Control experiments were carried out without mucus in the middle compartment and the value obtained was defined as 100%. The relative diffusion rates were calculated from these values.

Experiments to determine intestinal absorption in the rat. These experiments were carried out as previously described (15). The compounds were administered orally as a solution in 5% glucose or 0.9% sodium chloride solution.

RESULTS AND DISCUSSION. In the control experiments (Fig. 1) with buffer instead of mucus in the middle compartment all the compounds diffused at practically the same rate. These are reference values corresponding to complete absorption (=100%) in the *in vivo* experiments. With goblet-cell mucin in the middle compartment the compounds yielded different diffusion rates which

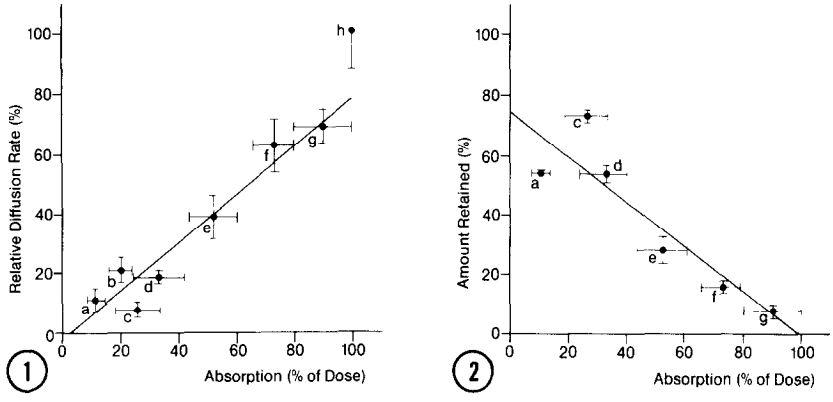


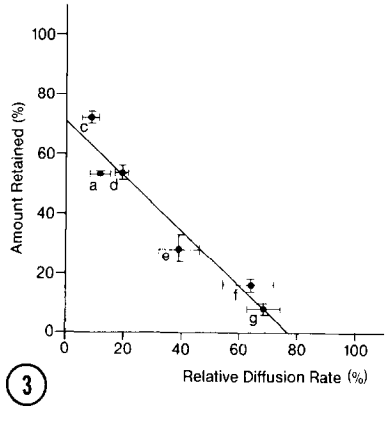
Fig. 1. Correlation between diffusion through goblet-cell mucin and absorption from the GI-tract in the rat (r=0.962, p<0.001, N=7). (a) [9,10-^{3}H]Dihydroergotamine, (b) [6-^{14}C]dihydroergonine, (c) [9,10-^{3}H]dihydrolysergic acid m-dialkylcarbamyl-anilide, (d) [2-^{3}H]ergotamine, (e) 1-[(1,1-dimethyl[1-^{14}C]ethyl)amino]-3-[(2-methyl-1H-indol-4-yl)oxy]-2-propanol-benzoate, (f) [9,10-^{3}H]N-(6-methyl-8α-ergolinyl)-dialkyl-sulfamylamino compound, and (g) [1-^{14}C]pindolol. *Diffusion experiments.* Concentrations: (a), and (c)-(g) $5 \cdot 10^{-5}$ mol·L^{-1}, (b) $5 \cdot 10^{-6}$ mol·L^{-1}. Buffer (McIlvaine), pH 6, ionic strength 0.1, t=37°C. Standard deviation of the mean calculated from results of three independent experiments. *Absorption experiments.* Concentrations: (a)-(f) $1.9\text{-}7 \cdot 10^{-7}$ mol per rat, (g) $5 \cdot 10^{-6}$ mol per rat; the solutions were given by oral route. Standard deviation of the mean obtained in experiments with 4-7 animals. *Control experiments* (h) with compounds (a), and (c)-(g) without mucus.

Fig. 2. Correlation between the amount retained by goblet-cell mucin and absorption from the GI-tract in the rat (r=-0.904, p< 0.05, N=6). For compounds (a), and (c)-(g) see Fig. 1. *Retention experiments.* Concentrations: (a), and (c)-(g) $5 \cdot 10^{-5}$ mol·L^{-1}. Buffer (McIlvaine), pH 6, ionic strength 0.1, t=37°C. Standard deviation of the mean calculated from results of three independent experiments. *Absorption experiments.* As shown in Fig. 1.

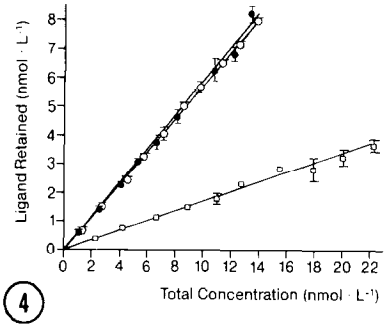
correlated with their relative *in vivo* absorption. The diffusion rate for dihydroergotamine as a function of the thickness of the mucus layer in the middle compartment roughly conformed to the hyperbolic relation for a simple diffusion process (2).

In the retention experiments in which diffusion was allowed to proceed to equilibrium, retention in the mucus was inversely proportional to the intestinal absorption of the compound (Fig. 2). This result is at variance with the findings of other authors (7,19-21) who have reported that compounds which are weakly bound to mucosa preparations or to mucus are also poorly absorbed. However, we have found that, as might be expected, retention of a compound by the mucus network is negatively correlated with diffusion through the mucus barrier (Fig. 3).

As will be seen from Fig. 4 the amount of compound retained in the mucus network bears a linear relation to the total concentration for compounds (a), (d), and (f). This relation was con-



3



4

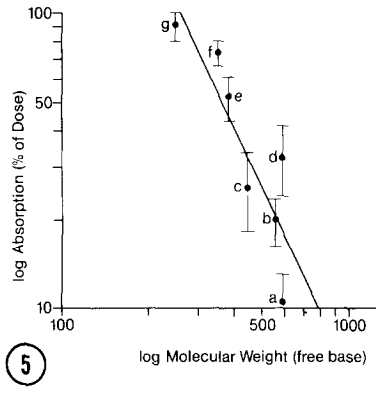
Fig. 3. Correlation between amount retained by and amount diffusing through goblet-cell mucin for compounds (a), and (c)-(g) (see Fig. 1). Values from Fig. 1 vs those from Fig. 2 yield a highly significant correlation ($r=-0.970$, $p<0.001$, $N=6$).

Fig. 4. Ligand retained by goblet-cell mucin as a function of total ligand concentration of compounds (a) (●), (d) (○), and (f) (□) (see Fig. 1). The concentration of (f) was 60 times that of (a), and that of (d). Buffer (McIlvaine), pH 6, ionic strength 0.1, t=37°C. Standard deviation of the mean calculated from results of three independent experiments.

firmed for dihydroergotamine over a wider range of concentrations ($5 \cdot 10^{-5}$ - $1 \cdot 10^{-10}$ mol·L^{-1}) in the presence of an excess of unlabelled ligand and in more dilute mucus solutions (approx. 0.5 mg protein ml^{-1}). This retention may be interpreted as unspecific binding, i.e. as a distribution of the compound between the mucus and the aqueous phase, or as a borderline case of specific binding with a large number of low-affinity binding sites. Goblet-cell mucin is a polyelectrolyte which, since it contains sialic acid ought to be fully dissociated and negatively charged at physiological pH. However, since hydrogen bridges may be formed, pK_a is assumed to be higher. The structure and rheological properties of mucus are sensitive to changes in ionic strength and pH (10,11, 22).

The diffusion of a compound through the hydrated mucus network is likely to depend on the charge, hydration radius of the molecule and ability to form hydrogen bonds (7,23,24). Intestinal absorption in the rat appears to correlate with molecular weight (Fig. 5), hence molecular weight itself seems to be a rough guide for assessing whether a compound is capable of passing through the intestinal wall (25). A further 47 compounds were found to fit such correlation.

The dependence of diffusion and retention on pH and ionic strength for dihydroergotamine (Fig. 6) may be explained by assuming that at physiological pH repulsion of the negative charges of the sialic acid gives rise to expansion, hydration and an increase in viscosity, whereas neutralisation of the charges at decreasing pH leads to contraction, dehydration and a decrease in the viscosity of the gel (11,12). One reason for the increase in the diffusion rate and decrease in retention might be reduced electrostatic interaction between mucus and dihydroergotamine. However, it is probable that reduced viscosity of the mucus and



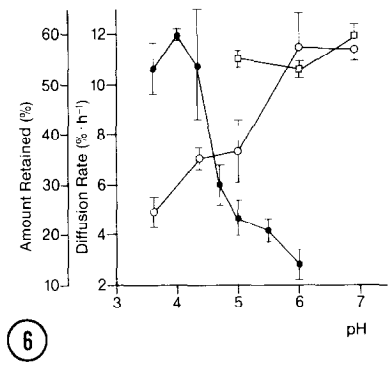


Fig. 5. Correlation of the logarithm of the relative amount of compounds (a)-(g) (see Fig. 1) absorbed from the GI-tract in the rat with the logarithm of the molecular weight of the free bases of these compounds ($r=-0.868$, $p<0.05$, $N=7$), see also (25).

Fig. 6. Dependence on pH of the amount retained by and diffusing through goblet-cell mucin. Standard deviations calculated from results of three independent experiments. *Retention experiments.* Dihydroergotamine $5 \cdot 10^{-5}$ mol·L^{-1}, buffer of various pH values (McIlvaine), ionic strength 0.1 (○), and 0.01 (□), $t=37^{0}$C. *Diffusion experiments.* Dihydroergotamine $5 \cdot 10^{-5}$ mol·L^{-1}, buffer of various pH values (McIlvaine), ionic strength 0.1 (●), $t=37^{0}$C.

cleavage of the hydrogen bridges between mucus and dihydroergotamine exert a still greater effect, since pindolol, although fully protonated passes freely through the mucus. The increased retention of dihydroergotamine at pH 5 and ionic strength 0.01 must be attributed to reduced screening of the negative charge of the mucus, so that it has a more expanded and hydrated structure and a higher viscosity.

Isolated goblet-cell mucin, since it is the outermost mucosal barrier of the intestinal wall, affords an insight into the mechanism of enteral absorption. In view of the relationship between diffusion through and retention by goblet-cell mucin and enteral absorption, suggested by the findings of the present study, it must be assumed that the lipid membrane does not perform this selective function as a barrier to the passage of compounds through the intestinal wall.

ACKNOWLEDGEMENTS. The authors are indebted to Miss K. Peter and Mr. J. Eichenberger for their technical assistance, to Dr. F.

Kalberer, Dr. J. Meier, and Dr. O. Wagner for their experiments in the rat, to Dr. E. Schreier and Dr. R. Voges for the radioactively labelled compounds and to Mr. A. Schweitzer for the electron microscopy work.

REFERENCES

1. Specht, W. (1977) in Intestinal Permeation, (Ed. Kramer, M. and Lauterbach, F.) pp. 4-40, Excerpta Medica, Amsterdam.
2. Nelson, K. G. and Miller, K. W. (1979) in Principles and Perspectives in Drug Bioavailability, (Ed. Blanchard, J., Sawchuk, R. J., and Brodie, B. B.) pp. 20-58, S. Karger, Basle.
3. Debnam, E. S. and Levin, R. J. (1975) J. Physiol. 252, 681-700.
4. Lukie, B. E. (1977) Mod. Probl. Paediat. 19, 46-53.
5. Levine, R. M., Blair, M. R., and Clark, B. B. (1955) J. Pharmacol. Exp. Ther. 114, 78-86.
6. Levine, R. R. and Pelikan, E. W. (1961) J. Pharmacol. Exp. Ther. 131, 319-327.
7. Nakamura, J., Shima, K., Kimura, T., Muranishi, S., and Sezaki, H. (1978) Chem. Pharm. Bull. 26, 857-863.
8. Meshi, T. and Tamaki, H. (1978) Chem. Pharm. Bull. 26, 379-386.
9. Nakamura, J., Muranushi, N., Kimura, T., Muranishi, S., and Sezaki, H. (1977) Chem. Pharm. Bull. 25, 851-858.
10. Forstner, J. F. (1978) Digestion 17, 234-263.
11. Lewis, R. W. (1976) J. theor. Biol. 61, 21-25.
12. Jabbal, I., Kells, D. I. C., Forstner, G., and Forstner, J. (1976) Can. J. Biochem. 54, 707-716.
13. Allen, A. (1978) Br. Med. Bull. 34, 28-33.
14. Schrager, J. (1974) Top. Gastroenterol. 2, 101-111.
15. Nimmerfall, F. and Rosenthaler, J. (1976) J. Pharmacokin. Biopharmaceut. 4, 57-66.
16. Rutschmann, J. and Stadler, P. A. (1978) in Ergot Alkaloids and Related Compounds, (Ed. Berde, B. and Schild, H. O.) pp. 29-85, Springer-Verlag, Berlin.
17. Gugler, R., Herold, W., and Dengler, H. J. (1974) Europ. J. Clin. Pharmacol. 7, 17-24.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
19. Nakamura, J., Yoshizaki, Y., Yasuhara, M., Kimura, T., Muranishi, S., and Sezaki, H. (1976) Chem. Pharm. Bull. 24, 683-690.
20. Kakemi, K., Arita, T., Hori, R., Konishi, R., Nishimura, K., Matsui, H., and Nishimura, T. (1969) Chem. Pharm. Bull. 17, 255-261.
21. Kakemi, K., Arita, T., Hori, R., Konishi, R., and Nishimura, K. (1969) Chem. Pharm. Bull. 17, 248-254.
22. Forstner, J. F., Jabbal, I., and Forstner, G. G. (1974) in Calcium Binding Proteins (Ed. Drabikowski, W., Strzelecka-Golaszewska, H., and Carafoli, E.) pp. 807-834, Elsevier, Amsterdam.
23. Braybrooks, M. P., Barry, B. W., and Abbs, E. T. (1975) J. Pharm. Pharmac. 27, 508-515.
24. Barry, B. W. and Braybrooks, M. P. (1975) J. Pharm. Pharmac. 27, 74P.
25. Stein, W. D.(1967) in The Movement of Molecules across Cell Membranes, pp. 65-125, Academic Press, New York.