significant changes in the energy metabolic capacity of skeletal muscles (data not shown). The increase of citrate synthase activity, for instance, varied between 20–30% in different muscles.

Several studies suggest that in highly oxygenated tissues, such as heart and lung, vitamin E is the major cellular antioxidant[11]. In the microsomes of lung and heart the ratio of vitamin E to peroxidizable polyunsaturated fatty acids is several-fold higher than in microsomes of other tissues[11] and therefore the microsomes of these tissues are more resistant to lipid peroxidation. During inhalation of oxidants (nitrogen dioxide) vitamin E is assimilated by the lungs[5]. We observed no increase in lung vitamin E content in association with exercise-induced hyperventilation. The concentration of vitamin E was 47.8% higher in rat than in mouse lungs suggesting that the lungs of rat are more resistant to oxidant stress.

The activity of glutathione peroxidase was assayed using hydrogen peroxide as the substrate. This activity corresponds to the selenium dependent enzyme activity[12]. In lungs there exists also non-selenium dependent glutathione peroxidase activity. This activity consists 14.3% of the total activity in rat lung and 59.3% in mouse lung[12].

Table 2. Endurance training, detraining and the level of some antioxidants in rat lung

Variable	Control (n = 8)	Trained (n = 6)	Detraining 14 days (n = 5)	35 days (n = 4)
Catalase	8.31 ± 0.22	8.27 ± 0.13	7.68 ± 0.20	7.46 ± 0.27
Glutathione peroxidase	22.4 ± 1.0	20.3 ± 0.8	18.9 ± 1.2	23.0 ± 0.5
Vitamin E	29.4 ± 1.0	28.5 ± 1.3	28.7 ± 1.1	32.1 ± 1.3

Legends are as in table 1.

In oxidant stresses the activity of Se-dependent glutathione peroxidase has been increased, as has also that of catalase[4,13]. The changes are age-related, being greater in neonatal than in adult animals[4]. The activities of catalase and superoxide dismutase (not assayed) are low in the lung tissue compared to many other tissues of rats[13], and hence their role in the total protection may be slight. The lack of adaptive changes in lung antioxidants of exercised animals suggests that the pentane production during exercise[7,8] does not originate in the lungs.

1 This study was supported by the Academy of Finland and the Research Council for Physical Education and Sport (Ministry of Education, Finland). We thank Mr Matti Virtanen for skillful technical assistance.

2 Crapo, J.D., Peters-Golden, M., Marsh-Salin, J., and Shelburne, J.S., Lab. Invest. *39* (1978) 640.
3 Turrens, J.F., Freeman, B.A., and Crapo, J.D., Archs Biochem. Biophys. *217* (1982) 411.
4 Hoffman, M., Stevens, J.B., and Autor, A.P., Toxicology *16* (1980) 215.
5 Sagai, M., Ichinose, T., Oda, H., and Kubota, K., J. Tox. envir. Hlth *9* (1982) 153.
6 Sjöström, K., and Crapo, J.D., Lab. Invest. *48* (1983) 68.
7 Dillard, C.J., Litov, R.E., Savin, W.M., Dumelin, E.E., and Tappel, A.L., J. appl. Physiol. *45* (1978) 927.
8 Gee, D.L., and Tappel, A.L., Life Sci. *28* (1981) 2425.
9 Burri, P.H., and Weibel, E.R., in: Development of the lung, p. 215. Ed. W.A. Hodson. Marcel Dekker Inc., New York 1977.
10 Salminen, A., and Vihko, V., Acta physiol. scand. *117* (1983) 109.
11 Kornbrust, D.J., and Mavis, R.D., Lipids *15* (1980) 315.
12 Tappel, M.E., Chaudiere, J., and Tappel, A.L., Comp. Biochem. Physiol. *73B* (1982) 945.
13 Matkovics, B., and Novak, R., Experientia *33* (1977) 1574.

0014-4754/84/080822-02$1.50 + 0.20/0

Somatostatin and 3-oxy-methyl-D-glucose (3-OMG) uptake in isolated chicken intestinal epithelial cells

M. Abalde, C. Taboada and P. Fernández

Departamento de Fisiologia Animal, Facultad de Farmacia, Universidad de Santiago, Santiago de Compostela (Spain), 7 July 1983

Summary. The direct effect of somatostatin on the absorption of 3-oxymethylglucose in epithelial cells isolated from the small intestine of chicken was studied. The presence of somatostatin in the incubation medium at concentrations of 3.5×10^{-8} M and 7×10^{-8} M produced significant dose-dependent increases in the accumulation of sugar in the enterocytes. This effect might be due to an increase in the cell membrane permeability caused by hormone action.

Unger et al.[1] have proposed the hypothesis that somatostatin plays an important physiological role in the homeostasis of nutrients by controlling the entry of ingested substances into the blood stream. Also, Wahren and Feling[2] observed that the administration of somatostatin to diabetics after the oral administration of glucose reduces the rise in the blood glucose level without affecting i.v. glucose tolerance, which suggests the action of somatostatin in the small intestine.

However, whether SRIF (somatotropin release inhibiting factor or somatostatin) acts directly on the absorption of nutrients by modifying the transport process in the wall of the digestive tract[3] or whether it acts indirectly by altering the splanchnic blood flow[2,4], intestinal motility or gastric emptying[5], is still open to question. For this reason the aim of the present work is to study the direct effect of somatostatin on the uptake of 3-oxy-methyl-D-glucose by isolated chicken intestinal epithelial cells.

Methods. Intestinal epithelial cells were isolated from 3–6-week-old male broiler chickens by the method reported by Kimmich[6]. The cells were separated from the medium by centrifugation. Somatostatin-14 was purchased from Sigma Chemical Co. and was added directly to 3 ml of the incubation medium containing 1 ml of the cell suspension, in concentrations of 3.5×10^{-8} M and 7×10^{-8} M. The standard incubation medium contained 80 mM NaCl, 100 mM mannitol, 20 mM Tris-Cl (pH 7.4), 3 mM K_2HPO_4, 1 mM $MgCl_2$, 0.1 mM EGTA, 2.5 mM $CaCl_2$, and 1 mg/ml of BSA.

For the determination of sugar accumulation, 3-O-methyl-D-glucose 2 mM was added to the cellular suspension together with 0.5 μCi of 2-O-methyl-D-(U-^{14}C)glucose (sp. act.: 295 mCi/mmol)[7] purchased from Radiochemical Centre, Amersham. Samples were extraced at intervals of 1, 2, 4, 6, 8 and 10 min. Incubation was not extended beyond 10 min to avoid the possible degradation of the hormone and the loss of this effect.

Effect of $3.5 \cdot 10^{-8}$ M or $7.0 \cdot 10^{-8}$ M somatostatin in the incubation medium on uptake of 3-OMG by isolated intestinal epithelial chicken cells (number of animals: 8–10)

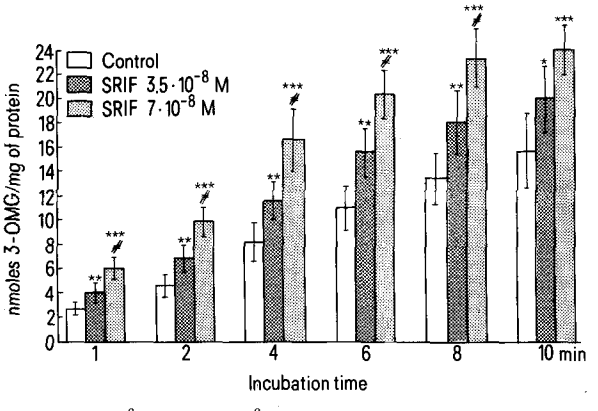
	Incubation time 1 min	2 min	4 min	6 min	8 min	10 min
Control	2.78 ± 0.23	4.62 ± 0.43	8.22 ± 0.79	11.08 ± 0.90	13.53 ± 1.04	15.68 ± 1.48
SRIF $3.5 \cdot 10^{-8}$ M	4.09 ± 0.37**	6.89 ± 0.56**	11.62 ± 0.74**	15.71 ± 1.11**	18.09 ± 1.34**	20.13 ± 1.41*
SRIF $7.0 \cdot 10^{-8}$ M	6.03 ± 0.39***#	9.94 ± 0.61***#	16.70 ± 1.22***#	20.24 ± 1.01***#	23.42 ± 1.17***#	24.16 ± 1.04***

Results are expressed: nmoles 3-OMG/mg of protein. The data are the mean ± SEM.
Statistical significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ (vs control); # $p < 0.05$ (vs somatostatin $3.5 \cdot 10^{-8}$ M).

The samples were then diluted with 2 ml of ice-cold medium and centrifuged. The pellets were washed by centrifuging 3 times with ice-cold medium to remove radioactivity adhering to the cell-surfaces. The cells were then dispersed in 0.2 ml 3% perchloric acid and centrifuged for 10 min in order to release the ^{14}C-glucose previously accumulated by the cells. 0.1-ml samples of the supernatant were added to 10 ml of the scintillation mixture.

The transport capacity is directly dependent on the quantity of cellular protein used[8], for which reason the protein content of the cell suspension was determined[6] using the method of Lowry[9]. Cell viability was estimated by determining the fraction of the population able to exclude 0.2% trypan blue[10].

Results. The table shows the effect produced on the 3-OMG accumulation in chicken enterocytes by the addition of SRIF to the incubation medium. It is seen that the presence of the hormone causes a significant increase in 3-OMG uptake. Increases above 40% were obtained with the lowest concentration when the cells were incubated for 1, 2, 4 or 6 min, decreasing to 30% when the incubation was prolonged to 8 or 10 min. At the higher concentration of SRIF, the 3-OMG accumulation is greater, being above 100% after 1, 2 or 4 min, 80% after 6 or 8 min and 54% after 10 min of incubation.



Effect of $3.5.10^{-8}$ M or $7.0.10^{-8}$ M Somatostatin in the incubation medium on the uptake of 3-OMG by isolated intestinal epithelial chicken cells. The SEM (±) is indicated. Statistical significance: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$ vs control, # $p < 0.05$ vs SRIF $3.5.10^{-8}$ M.

Discussion. Some authors have indicated that SRIF has an inhibitory effect on the absorption of carbohydrates. However, it should be borne in mind that the studies in which the hormone caused a decrease in sugar transport were carried out on humans with the somatostatin being administered i.v. Thus, the resulting decrease may be produced indirectly by gastrointestinal alterations independent of sugar transport itself such as changes in gastric emptying, intestinal motility or splanchnic blood flow[4,5,11]. The experimental technique of the present paper consists of dissolving the hormone in the medium containing the cells in which absorption is to be measured. Thus the possible occurrence of the above mentioned alterations is eliminated.

Several authors[12–15] indicate a decrease and/or delay in the increase in the blood sugar level after the administration of somatostatin. However, Marki and Miekich[16] working with everted rat jejunal segments observed a slight increase in the intestinal transport of glucose. Bearing in mind the increase in the accumulation of 3-OMG produced by SRIF in the incubation medium reported in this present paper, it would seem that the apparent discrepances in the effects of somatostatin on monosaccharide transport could be due to the different species of animals used.

Our studies indicate that SRIF can have a direct action on the tissue. Although these effects were obtained at a high, probably pharmacological dose, the possibility of a physiological effect of somatostatin cannot be excluded. In the present work the hormone had an effect on isolated intestinal epithelial cells, but it is rather difficult to prove that this effect might play a role in vivo as well.

Other studies on the influence of SRIF on baso-lateral membranes using energy-depleted cells in presence of ouabain, and tests for passive uptake of 3-O-methyl-glucose into the cell, are required to increase our understanding of the precise mechanism of the action of the hormone on epithelial transport and its possible physiological significance.

1 Unger, R.H., Ipp, E., Schusdziarra, V., and Orci, L., Life Sci. *20* (1977) 2081.
2 Wahren, J., and Feling, P., Lancet *2* (1976) 1213.
3 Pott, G., Wagner, H., Zierden, E., Hilke, K.H., Jansen, H., Hengst, K., and Gerlach, U., Klin. Wschr. *57* (1979) 131.
4 Lin, T.M., Evans, D.C., Shaar, C., and Crabtree, E., Gastroenterology *70* (1976) A 101/959.
5 Koerker, D.J., and Hansen, B.C., Metabolism *30* (1981) 335.
6 Kimmich, G.A., Biochemistry *9* (1970) 3659.
7 Kimmich, G.A., Biochemistry *9* (1970) 3669.
8 Kimmich, G.A., Envir. Hlth Perspect. *33* (1979) 37.
9 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall,R.J., J. biol. Chem. *193* (1951) 265.
10 Girardi, A.J., Michael, H.M., and Henle, W., Virology *2* (1956) 532.
11 Konturek, S., Scand. J. Gastroent. *11* (1976) 1.
12 Daumerie, C., and Henquin, J.C., Gut *23* (1982) 140.
13 Johansson, C., Wisen, O., Efendic, S., and Uvnäs-Wallenstenk, K., Digestion *22* (1981) 126.
14 Bratush-Marrain, P., Vierhapper, H., Grubeck-Loebenstein, B., Waldhäusl, W., and Nowotny, P., Horm. Metab. Res. *13* (1981) 305.
15 Krejs, G.J., Browne, R., and Raskin, P., Gastroenterology *78* (1980) 26.
16 Marki, F., Regulatory Peptides *2* (1981) 371.

0014-4754/84/080823-02$1.50 + 0.20/0