Volume 235, number 1,2, 125-128 FEB 06126 August 1988

Monensin inhibits the expression of sucrase-isomaltase in Caco-2 cells at the mRNA level

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Received 26 May 1988

Using L-[^{35}S]methionine labeling, SDS-PAGE and Northern blot analysis of sucrase-isomaltase mRNA, two different concentrations of monensin were used to delineate in Caco-2 cells the effect of the drug on the conversion of the high mannose to the complex form of sucrase-isomaltase from its dual effect on the biosynthesis of the enzyme and on the rate of glucose consumption. At 0.1 μM the drug has no effect on the rate of glucose consumption and, although it inhibits the conversion of the high mannose to the complex form of the enzyme, it has no effect on the level of sucrase-isomaltase mRNA and on the amount of neosynthesized enzyme. At 1 μM, in addition to its inhibiting effect on the maturation of the enzyme, monensin provokes concomitantly an increase in the rate of glucose consumption and a decrease in the level of sucrase-isomaltase mRNA and in the amount of neosynthesized enzyme. All these effects are reversible within 48 h after removal of the drug.

Monensin; Sucrase-isomaltase; Biosynthesis; mRNA; Glucose; (Caco-2 cell)

1. INTRODUCTION

Monensin has been shown to block the processing of newly synthesized glycoproteins at the site of the Golgi complex in a variety of systems [1-5]. In normal small intestinal [6] and kidney cells [7] it inhibits the conversion of the high mannose to the complex form of brush border-associated hydrolases, these enzymes being known to be highly glycosylated glycoproteins [8]. In a previous investigation on the effects of monensin on the processing of two representative microvillar hydrolases in the enterocyte-like human colon carcinoma cell line Caco-2, namely sucrase-isomaltase and dipeptidylpeptidase-IV, we found that, in addition to its inhibiting effect on the maturation of both enzymes, monensin also produced two unexpected effects: a decreased expression of sucrase-isomaltase, but not of dipeptidylpeptidase-IV, and an increased glucose consumption [9]. In view of other results obtained either in Caco-2 [10], or in HT-29 cells [11,12], which suggested that, in these cells, glucose metabolism could interfere specifically with the expression of sucrase-isomaltase, it had been hypothesized that the impairment of the expression of the enzyme induced by monensin could be related to its effect on glucose consumption [9]. In this earlier work however we used a high concentration of monensin (10 μM) [9]. The purpose of the present work was to investigate: (i) by using lower concentrations of the drug, whether it was possible to delineate the effect of monensin on the processing of the enzyme from the effects on both its expression and glucose consumption; (ii) by using a cDNA clone of human intestinal sucrase-isomaltase recently isolated [13], whether the impairment of the expression of the enzyme was at the mRNA level; (iii) the reversibility of these effects.

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

2. MATERIALS AND METHODS

Culture of Caco-2 cells, treatment with monensin and L-[^{35}S]-methionine labeling were as in [9]. Preparation of cell homogenates was as in [10]. Treatment with endo-β-acetylglucosaminidase H (Endo-H), SDS-PAGE and fluorography were performed as in [9,14], except that rabbit polyclonal antibodies raised against sucrase-isomaltase from Caco-2 cells were used as in [12]. The cDNA clone for human intestinal sucrase-isomaltase pSI_2 [13] was obtained from D. Swallow (The Galton Laboratory, MRC, London). The cDNA clone for β-actin, C141 [15] was obtained from S. Alonso (Institut Pasteur, Paris). The corresponding cDNA probes were labeled with ^{32}P using multiprime DNA labeling system (Amersham International, England). Total RNA was isolated by extraction with guanidium isothiocyanate and centrifugation through CsCl gradient as in [16]. Aliquots (20 μg) of total RNA were fractionated by electrophoresis on 1% agarose gels after denaturation in 1 M glyoxal [17]. Fractionated samples were transferred to Hybond N (Amersham), prehybridized in the presence of 50% formamide at 42°C [17], and hybridized with ^{32}P-labeled probes for 24 h at 42°C in the presence of 40% formamide and 10% Dextran sulfate. Blots were washed twice in 2×SSC, 0.1% SDS at room temperature, then once in 0.1×SSC, 0.1% SDS at 50°C, and once more in 0.1×SSC, 0.1% SDS at 70°C for 15 min. Glucose consumption was measured as in [9].

3. RESULTS

In order to determine the lowest concentration of monensin responsible for an increase in the rate of glucose consumption, post confluent Caco-2 cells (day 16) were treated for two consecutive periods of 24 h with decreasing concentrations of monensin. Results reported in fig.1 show that the drug has no effect at 0.1 μM, whereas it increases the rate of glucose consumption by 40% at 1 μM. This effect on glucose consumption is reversible within 24 h after removal of the drug.

Labeling of the cells with L-[^{35}S]methionine was performed using the same protocols as in fig.1. As shown in fig.2 both concentrations of monensin inhibit the conversion of the high mannose to the complex form of the enzyme; however, while the total amount of neosynthesized enzyme is similar to the control in cells treated with 0.1 μM monensin, there is a marked decrease in the amount of neosynthesized enzyme in cells treated with 1 μM monensin. As also shown in fig.2, all these effects, including the decrease in the amount of neosynthesized enzyme, are totally reversible within 48 h.

As shown in figs 3 and 4, the effects of monensin on the expression of the neosynthesized enzyme are

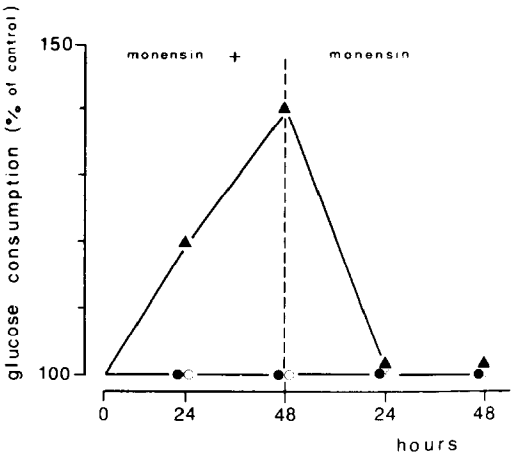


Fig.1. Effect of monensin on the rate of glucose consumption in Caco-2 cells. Time 0 is 16 days in culture. Monensin was added to the culture medium for two consecutive periods of 24 h and then removed. (○) Control cells; (●) 0.1 μM monensin; (▲) 1 μM monensin. Results are expressed as % of control. The rate of glucose consumption in control cells is 400 ± 20 nmol/h per mg protein.

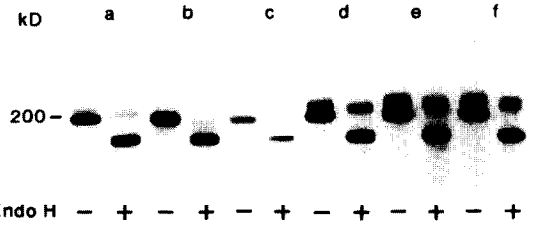


Fig.2. Fluorograms of SDS-PAGE of sucrase-isomaltase immunoprecipitated from cell homogenates of Caco-2 cells, treated or not with monensin. Cells were labeled for 3 h with L-[^{35}S]methionine. Immunoprecipitates were treated (+) or not (−) with Endo-H. As in fig.1, Caco-2 cells (day 16 in culture) were treated for two consecutive periods of 24 h and labeled at the end of this treatment: (a) control cells; (b) cells treated with 0.1 μM monensin; (c) cells treated with 1 μM monensin. Last three lanes represent the results obtained 48 h after removal of monensin; (d) control cells; (e) 0.1 μM monensin; (f) 1 μM monensin. Note in (b) and (c) the absence of the Endo-H resistant form of the enzyme and, in (c) only, a decreased staining of the band. Also note that in (e) and (f) the pattern is back to the control, including the intensity of labeling in (f). In all conditions the same amount of proteins was used for immunoprecipitation (200 μg). Since the activity of sucrase-isomaltase, and supposedly its biosynthesis, increases rapidly within the period of this experiment (i.e. between 16 and 20 days) [10], control cells were assayed at both times: note that the overall amount of neosynthesized enzyme is lower in (a) than in (d).

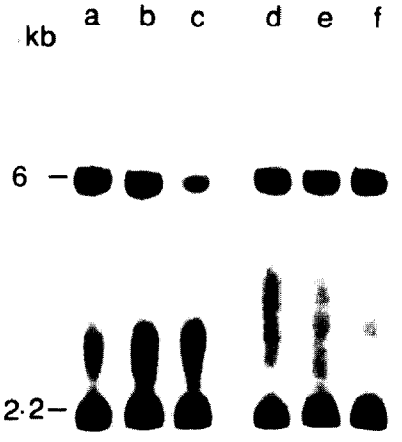


Fig.3. Northern blot of total RNA isolated from control (a,d) and monensin treated Caco-2 cells after 48 h of treatment, and 48 h after removal of the drug (same conditions and letter labeling as in fig.2). Blot was hybridized with the probes for sucrase-isomaltase (upper band) and β-actin (lower band). Note the decrease of sucrase-isomaltase mRNA level in cells treated with 1 μM monensin (c) but not with 0.1 μM (b) and the reversion to the control level 48 h after removal of the drug (f). Also note the stability of actin mRNA levels used here as a quantitative control.

associated with parallel effects on the level of sucrase-isomaltase RNA. At 0.1 μM, monensin has no effect, whereas at 1 μM there is a marked decrease in the level of sucrase-isomaltase mRNA after 48 h. This impairment of the expression of sucrase-isomaltase mRNA in cells treated with 1 μM monensin is reversible as, 48 h after removal of the drug, its level is back to the normal.

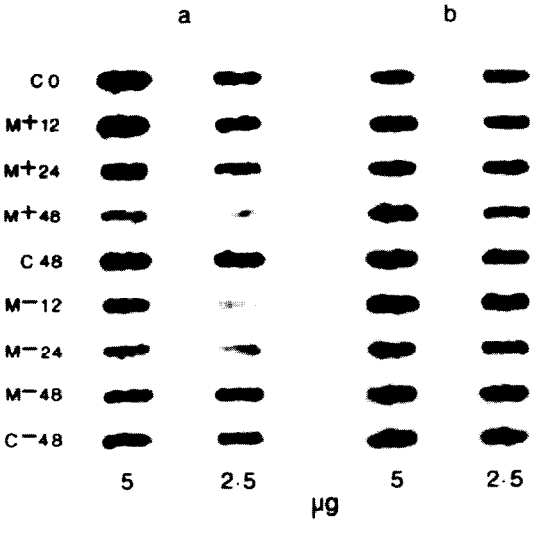
Fig.4. Dot blots of glyoxal denatured RNA from Caco-2 cells treated or not with 1 μM monensin hybridized to (6a) the probe for sucrase-isomaltase and (b) the probe for β-actin. Dot blots were performed at the indicated times of treatment (M^+) and of removal (M^-) of the drug. RNA from control cells corresponds to the beginning of the treatment (C0), 48 h of treatment (C48) and 48 h after removal of the drug (C^-48). The numbers at the bottom (μg) refer to the amounts of RNA applied. Note the progressive decrease of sucrase-isomaltase mRNA levels in cells treated with 1 μM monensin as compared to the corresponding control, contrasting with the stability of β-actin level. Also note that the sucrase-isomaltase mRNA level has returned to the control value 48 h after removal of the drug. The β-actin controls were similar under all conditions, even with shorter exposure times (not shown).

4. DISCUSSION

The present results show that if the inhibition of the conversion of the high mannose to the complex form of sucrase-isomaltase is dose-independent in the range of concentrations of monensin used here and in our previous work [9], i.e. 0.1–10 μM, the dual effect on the expression of the enzyme and on glucose consumption is dose-dependent.

That the decreased level of neosynthesized enzyme, when observed, is paralleled by a decreased level of sucrase-isomaltase mRNA, suggests that monensin provokes an overall inhibition of the biosynthesis of the enzyme. Interestingly this effect is strictly concomitant with the effect of the drug on the rate of glucose consumption: at 0.1 μM the drug has no effect on either glucose consumption or the levels of sucrase-isomaltase mRNA and the amount of neosynthesized enzyme, whereas at 1 μM, as well as at 10 μM [9], the inhibition of the biosynthesis of the enzyme is paralleled by an increased rate of glucose consumption. That there is a close relationship between both effects is further supported by the concomitant decrease to the normal rate of glucose consumption and increase to the control values of the levels of mRNA and amount of neosynthesized enzyme when monensin is removed from the culture medium.

These results raise a number of questions as to the mechanisms involved in the inhibiting effect of



monensin on the biosynthesis of sucrase-isomaltase. A first question, with no current answer, is whether the decreased level of sucrase-isomaltase mRNA is the result of an instability of this mRNA or of an impairment of its transcription. Another question, to which no direct answer can yet be given, is whether the dual effect of the drug on glucose consumption and sucrase-isomaltase mRNA level and biosynthesis is the result of a cause-effect relationship or whether both effects are independent. A last question is whether the results observed here can be generalized to other systems. In a previous study by Danielsen et al. [6] these authors had shown that monensin, at 1 μM, had no effect on the amount of neosynthesized sucrase-isomaltase in cultured explants of pig intestinal mucosa. Whether the difference of effect of monensin in Caco-2 cells, as compared to normal cells [6], is a consequence of the malignant state of the cells is questionable. It must be reminded that the metabolism of glucose in malignant epithelial cells, including in colon cancer cells [18,19], is altered. It is not unlikely that the particular effects observed in Caco-2 cells could be a consequence of the action of monensin on an altered metabolism of glucose. Such an action would not happen in cells with a normal regulation of glucose metabolism, such as normal intestinal cells.

Acknowledgements: This work was supported by the Fondation pour la Recherche Médicale, the Association pour la Recherche sur le Cancer (ARC) and the Groupement des Entreprises Françaises dans la Lutte contre le Cancer (GEFLUC).

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