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CORTICOSTEROID-DEPENDENT CYCLOHEXIMIDE-EVOKED INCREASE OF SUCRASE AND MALTASE IN THE JEJUNUM OF SUCKLING RATS

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SUMMARY

Cycloheximide (20–60 μg/100 g body wt), a posttranslational inhibitor of protein synthesis, evoked a precocious increase of sucrase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) and maltase (α-D-glucoside glucohydrolase, EC 3.2.1.20) activities in the jejunum of suckling rats 48 and 72 h later.

Adrenalectomy prevented completely the cycloheximide-evoked increase of maltase activity and substantially blocked the evoked increase of sucrase activity.

Simultaneous administration of a low dose of cortisone acetate (0.5 mg/100 g body wt) and cycloheximide (30 μg/100 g body wt) to adrenalectomized suckling rats produced a higher increase of specific and total activities of sucrase than did the administration of cortisone alone. Maltase was affected only in specific activity. Cycloheximide (30 μg/100 g body wt) was without effect when used with a high dose of cortisone acetate (2 mg/100 g body wt).

Actinomycin D (25 μg/100 g body wt) did not inhibit the cycloheximide-evoked increase of sucrase and maltase; the cortisone acetate (1 mg/100 g body wt) evoked increase was inhibited by actinomycin D.

INTRODUCTION

Sucrase, a hydrolytic enzyme localized in the microvilli of enterocytes, can be induced precociously by corticosteroids toward adult values in suckling rats and mice[1–5]. This increase can be inhibited in rats by actinomycin D[6] which suggests a mechanisms of *de novo* protein synthesis. On the other hand, actinomycin D, cycloheximide and several other inhibitors of protein synthesis evoked a precocious increase of this enzyme in mice[3].

Various reports have accumulated during recent years showing that the activity of many enzymes could be stimulated by inhibitors of protein synthesis. Recently, Tomkins *et al.*[7] have reviewed this socalled paradoxical effect of inhibitors

of macromolecular synthesis on various enzymes. In our preliminary experiments we have observed that cycloheximide evoked a substantial increase of sucrase and maltase in the jejunum of suckling rats. This finding led us to perform the experiments reported below, where a relationship between the cycloheximide effect and adrenals was shown.

MATERIALS AND METHODS

Pregnant Charles River rats were obtained and gave birth in our own animal house. On the second day after birth, the size of the litter was reduced to 8–9 pups. At the start of the experiment, animals in each litter were divided into three of four groups depending on the type of the experiments, *i.e.* 1 control group and 2–3 experimental groups. Animals were sacrificed by decapitation and the small intestine was excised. The duodenum was discarded. The remaining small intestine was divided along its length into three segments[8], the first called the jejunum was used for present studies. After flushing the segments with ice-cold 0.9% NaCl, the segments were homogenized in bi-distilled water in a Potter–Elvehjem homogenizer using a Teflon piston. Homogenates from cross-sectional serial tissue sections of jejunal wall were prepared as described previously[4].

Assay of sucrase and maltase activities was performed according to Dahlqvist[9] in a slightly modified form. In each assay the reaction mixture contained 1 vol. of homogenate and 1 vol. of substrate buffer mixture (0.056 M solution of sucrose in 0.1 M sodium maleate buffer, pH 5.8). The reaction was stopped by placing the samples in the boiling water bath for 2 min. The liberated glucose was determined with Tris–glucose oxidase reagent prepared according to Dahlqvist[9] from Glucostat (Worthington). When low sucrase activity was encountered in the mucosal homogenates, it became necessary to use concentrated homogenates which, in turn, inhibit the glucose oxidase reaction. To compensate for this inhibition, boiled homogenates with the same protein concentration as used in the assay were added to the glucose standards[10]. Enzyme activities were assayed during linear conditions in respect to time and amount of protein homogenate. Protein was determined according to Lowry *et al.*[11]. Actinomycin D and cycloheximide (Calbiochem, Los Angeles, Calif.) and cortisone acetate (Upjohn, Kalamazoo, Mich.) were given freshly diluted in 0.9% NaCl (actinomycin D; cortisone) and 0.2 M Tris buffer pH 7.4 (cycloheximide) in amount 0.5 ml of solution/100 g body wt. Control animals received the same amount of solvent. Further details about their administration are given in the legends to the tables and figures. Student's *t* test was employed to test the significance of the results.

RESULTS

Fig. 1 shows the increase of sucrase and maltase at different time periods after the injection of cycloheximide (40 μg/100 g body wt). During the first 24 h no change was detected in the specific activity but a substantial increase of activities was observed on the second and third day. The time pattern of increase is similar to that evoked by injection of corticosteroids[1,4].

Previously[4,12], it was found that cortisone administration evoked the increase in cells initially at the bottom of the villus only, and that sucrase activity appeared

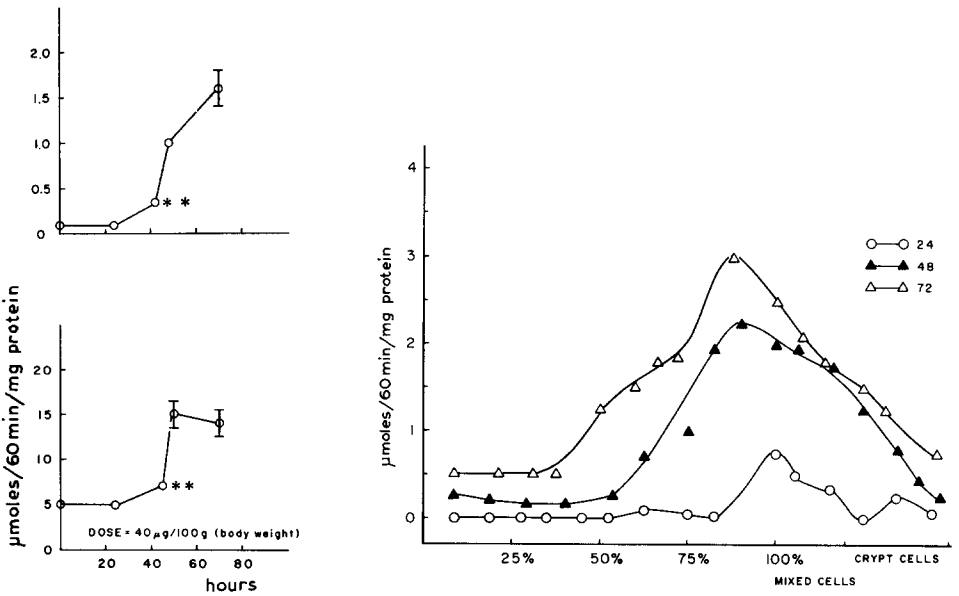

Fig. 1. Effect of cycloheximide on activity of sucrase (upper part) and maltase (lower part) in homogenates of jejunum of suckling rats. Cycloheximide was injected subcutaneously in a dose of 40 μg/100 g body wt on Day 11 and the rats were killed after different time periods (abscissa). Activity is expressed as μmoles of substrate split/60 min per mg of protein (ordinate). Circles denote mean of at least 10 determinations, short vertical lines denote 2 S.E. and are not given if smaller than symbol used. Asterisks denote the first significant difference ($P<0.02$) from 0 time values. Values of control (non-injected rats) are not given; they were the same as at 0 time.

Fig. 2. Effect of cycloheximide on sucrase activity in the jejunum of suckling rats. Activity was determined in serial tissue sections of villi and crypts[4]; sections were cut at 8 μm. Since there was some variation in the number of sections obtained from different animals, the activities of sucrase in serial samples were related to an idealized crypt–villus unit so data from various animals could be easily compared. Distances along the villus were calculated as a percentage of the length of the villi proceeding from tip (0%) to base (100%) of villus (abscissa). Mixed cells: homogenate containing mixture of villus cells and cells from the area of villus–crypt junctions. Crypt cells: homogenates containing predominantly crypt cells. Ordinate denotes activity of sucrase expressed as μmoles/60 min per mg protein. Each point represents the mean of determinations performed on 3 rats. Cycloheximide (40 μg/100 g body wt) was applied subcutaneously to 11-day-old rats; they were killed 24 (○), 48 (▲) and 72 (△) h after the injection.

later at the higher portion as the cells migrated. The question was then asked whether cycloheximide also causes a similar effect, *i.e.* whether its effect has similar time and topographical characteristics.

Activity of sucrase was determined in serial horizontal sections of the entire jejunal wall 24, 48 and 72 h after the injection of cycloheximide (Fig. 2). After the first 24 h the increase of activity could be detected only in a small area at the bottom of the villus (no activity was detected in control rats). The activity increases substantially 48 h later and appears in cells at half the height of the villus. Three days later there is a further increase and shift of activity towards the top of the villus. Thus, this pattern of cycloheximide-evoked increase resembles events after the injection of cortisone[4].

In further experiments the relationship between the increase of enzyme activity and dose of cycloheximide was studied (injections of cycloheximide preceded

the killing of animals by 72 h). Data expressed both as activity per mg of protein and per total jejunum per animal gave practically the same result. The small dose (20 μg/100 g body wt) evoked already a substantial increase of both hydrolases. Sucrase increase was highest with the 30-μg dose; maltase increased further with higher doses, but the difference between the values obtained with 40 μg and 60 μg was significant only at $P < 0.05$.

The striking parallelism between the pattern of increase evoked by cortisone and cycloheximide, as well as various data in the literature indicating stimulation of the adrenals in adult rats by cycloheximide[13–15] suggested the pertinence for further experiments on adrenalectomized suckling rats. On account of the higher sensitivity of the adrenalectomized rats to cycloheximide only the lower doses (20 and 30 mg) were used. A small but definitive increase of sucrase was observed (both in specific and total activities); maltase was affected only very little, and only when results were expressed as specific activity. Compared to the increase evoked in intact animals, the observed increase in adrenalectomized animals was very small (3–6 times smaller; data not shown).

In the preceding experiments with adrenalectomized animals the operation was performed in the morning hours, and injection of cycloheximide was performed later (between 2–3 p.m.) that same day. There was a possibility that remaining corticosteroids released during the operation by its stress effect would influence the results. Thus, in additional experiments we tried to minimize this effect by performing the operation one day before the injections, *i.e.* on Day 11 postnatally and injecting the rats on Day 12. Results given in Table I show that maltase was not influenced but sucrase activity was significantly increased again.

TABLE I

EFFECT OF CYCLOHEXIMIDE ON THE ACTIVITY OF MALTASE AND SUCRASE IN JEJUNUM OF SUCKLING ADRENALECTOMIZED RATS

Suckling rats (11-day-old) were adrenalectomized[8] on day 11 during the morning hours and then injected with cycloheximide (30 μg/100 g body wt, subcutaneously) between 8–9 a.m. the following day. They were killed 72 h after injection.

	μmoles/60 min per mg protein		*μmoles/60 min per jejunum*	
	Control	*Cycloheximide*	*Control*	*Cycloheximide*
Maltase	5.11 ± 0.35*	5.66 ± 0.316	186.3 ± 10.4	185.0 ± 8.75
Sucrase	0.068 ± 0.019	0.27 ± 0.044**	2.53 ± 0.76	9.18 ± 1.56**

* Activity is expressed as μmoles ± S.E. The control group consisted of 14 rats, and there were 16 cycloheximide-treated rats.

** The difference between control and cycloheximide injected rats is significant for $P < 0.001$.

Experiments performed on adrenalectomized rats thus clearly demonstrate the role of the adrenals in the mechanism of the cycloheximide-evoked increase; hence additional experiments were performed where the role of corticosteroids was further studied. Adrenalectomized 12-day-old rats were injected with cortisone acetate (0.5 mg and 2 mg/100 g body wt, respectively) and several received simultaneously cycloheximide. They were killed 65 h later. In agreement with previously published

data in intact animals[4] the lower dose of cortisone acetate caused a smaller increase in both hydrolases than the higher one in adrenalectomized suckling rats (Table II). Simultaneous or delayed 16 h (not shown) injection of cycloheximide with the low cortisone acetate dose caused a significant increase in specific activities of both hydrolases, but the total activity was increased only in the case of sucrase. Simultaneous injection of cycloheximide together with the higher cortisone dose did not influence the cortisone-evoked increase; on the other hand, it caused a significant decrease in the total maltase activity.

TABLE II

EFFECT OF CORTISONE ACETATE AND CYCLOHEXIMIDE ON SUCRASE AND MALTASE ACTIVITIES IN THE JEJUNUM OF ADRENALECTOMIZED RATS

Rats were adrenalectomized[8] on Day 12 postnatally between 9 and 12 a.m., and on the same day (between 2 and 3 p.m.) were injected with cortisone acetate intramuscularly. Controls received 0.9% NaCl. Part of the cortisone-treated animals received also cycloheximide (30 μg/100 g body wt, subcutaneously). Animals were killed 65 h after the cortisone injections. Mean ± S.E. are given. All values of cortisone-treated rats are significantly ($P<0.01$) higher than values found in control rats.

	Control	*Cortisone-treated animals*			
		0.5 mg/100 g body wt		*2 mg/100 g body wt*	
Number of animals:	*25*	*Only* *20*	*+ Cycloheximide* *18*	*Only* *11*	*+ Cycloheximide* *9*
Maltase (μmoles/60 min)					
per mg protein	3.8 ± 0.19	9.9 ± 0.66	12.5 ± 0.47*	32.1 ± 1.69	28.8 ± 2.22
per jejunum	268 ± 33.3	524 ± 35.5	614 ± 40.8	1683 ± 85.7	1190 ± 171.7***
Sucrase					
per mg protein	0.044 ± 0.01	0.47 ± 0.058	1.03 ± 0.093*	2.7 ± 0.41	2.94 ± 0.496
per jejunum	2.8 ± 0.49	24.1 ± 3.4	54.7 ± 3.4*	144.8 ± 20.7	119.1 ± 24.8

* Denotes the level of significance of the difference between animals treated with cortisone only and cortisone + cycloheximide, $P < 0.01$.
** Same as above, $P < 0.05$.
*** Same as above, $P < 0.02$.

Whereas the lack of response to cycloheximide administered with the higher dose of cortisone acetate can be explained by a possible limitation of the sucrase-producing system (in a broad sense), the effect of cycloheximide injected with the lower dose shows further the involvement of the corticosteroids in the cycloheximide-evoked increase of studied hydrolases, especially sucrase. Injection of cortisone acetate only increased the specific activity of sucrase by 0.43 μmole/60 min per mg protein and the total activity by 21 μmoles/60 min (Table II); the administration of cycloheximide only increased the specific activity by 0.2 μmole/60 min per mg protein and the total activity by 6.6 μmoles/60 min (see Table I and Fig. 1B). The increase evoked by both substances injected simultaneously (1.0 μmole/60 min per mg protein and 51.6 μmoles/60 min, respectively) is higher than the arithmetical sum of their effect when applied independently.

In previous experiments we have found that actinomycin D inhibited the cortisone-evoked increase in suckling rats[6]. Therefore, it was asked whether the application of actinomycin D will inhibit the cycloheximide-evoked increase of

sucrase and maltase in intact suckling rats. For comparative purposes we have also determined the effect of actinomycin D on the cortisone-evoked increase. The cortisone dose used in these experiments was 1 mg/100 g body wt in order to induce a quantitatively similar increase of both hydrolases to that evoked by the used dose of cycloheximide.

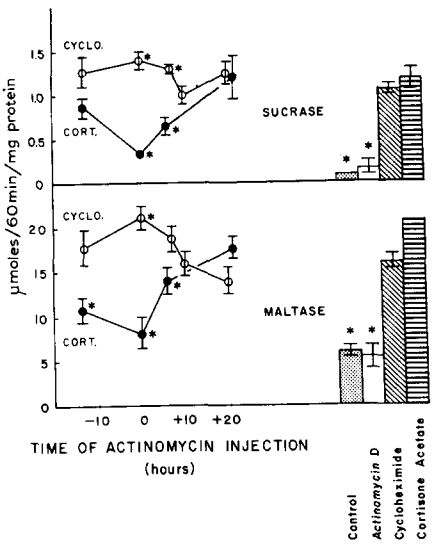

Fig. 3. Effect of actinomycin D on cortisone acetate (●) and cycloheximide (○) induced increase of sucrase (upper part) and maltase (lower part) in jejunum of 12-day-old rats. Symbols denote mean values, each group was composed of 5–15 rats. Short verticle lines denote 2 S.E. and are not given if smaller than symbols used. Asterisks indicate that values are significantly different ($P<0.01$) from values found in rats injected with cycloheximide (diagonally shaded columns) or cortisone acetate (horizontally shaded columns). For comparative purposes the other two columns denote values found in control rats (14-day-old) and in rats (14-day-old) injected 48 h before with actinomycin D. Cycloheximide (40 μg/100 g body wt, subcutaneously) or cortisone acetate (1 mg/100 g body wt, intramuscularly) was injected to 12-day-old rats at time 0 and they were killed 48 h later. Actinomycin D (25 μg/100 g body wt, subcutaneously) was injected either before or after the cycloheximide or cortisone acetate injections (abscissa). Activity is given as μmol substrate split/60 min per mg protein (ordinate).

Results of these experiments are demonstrated in Fig. 3. Injection of actinomycin D simultaneously or 6 h after cortisone acetate injection inhibited the cortisone-evoked increase of specific activity of both hydrolases. Administration of actinomycin D 13 h before or 21 h after the cortisone acetate injection did not influence the induction of sucrase. Maltase was not influenced with the delayed injection of actinomycin D, but was still inhibited by the preceding 13-h injection. On the other hand, actinomycin D never inhibited the cycloheximide-evoked increase. Simultaneous and 7 h delayed injection caused an increase of specific activity of sucrase; maltase was increased only by simultaneous injection. This increased activity was most probably caused by a cessation in growth of the small intestine (see Discussion) because the total activity in cycloheximide and actinomycin D injected rats was always lower than in those receiving cycloheximide only (not shown).

DISCUSSION

The present results have thus shown that cycloheximide, a posttranslational inhibitor of protein synthesis, causes in increase of sucrase and maltase activities in the jejunum of suckling rats. This effect differed from that of actinomycin D, a RNA synthesis inhibitor. The latter did not influence substantially the sucrase and maltase of control suckling rats and inhibited the cortisone-evoked increase of these enzymes.

Generally, the effect of cycloheximide described in this paper belongs to a group of various experiments recently reviewed by Tomkins *et al.*[7] where a paradoxical effect of inhibitors of protein synthesis was observed.

With the exception of experiments performed on suckling mice and chicken embryo (see below), it has been found in various laboratories using adult rats and mice as well as post-hatching chickens that cycloheximide inhibits various functions of the small intestine such as the incorporation of amino acids into the mucosal protein[16–19], DNA synthesis[16,17], RNA synthesis[16], and mitosis[17,20]. These changes usually lasted only for several hours. Cell migration could be also inhibited by repeated injections of cycloheximide[19]. Furthermore, a transient shortening of microvilli[21] and a decrease in alkaline phosphatase[20,21] and leucyl naphthylamidase activities[21] were observed after application of cycloheximide. Imondi *et al.*[19] found a decrease of thymidine phosphorylase, adenosine deaminase and purine nucleoside phosphorylase. Although the doses were sometimes higher than those which we used in our study, no substantial necrosis of enterocytes was observed. Finally, Norman *et al.*[22] prevented by cycloheximide injection the increase of microvilli alkaline phosphatase mediated by vitamin D in rachitic chickens.

On the other hand, in immature animals it was reported that cycloheximide caused an elevation of several enzymatic activities. Moog[23] showed that cycloheximide increased the specific activity of alkaline pnosphatase in the duodenum of suckling mice (doses ranged from 0.47 to 12 mg/100 g body wt); in older mice (3–5 weeks of age) its effect was irregular. Furthermore, in 17-day-old chicken embryo this inhibitor (10 μg/embryo) caused an increase in specific activity (100%) of sucrase in the small intestine after 72 h[24].

In our experiments we have expressed the results in two ways, namely, as specific activity (per mg of protein) and as total activity per jejunum. We believe that this is important, especially where growing animals are studied. Sometimes a halt in growth of the organ or its diminishment associated with no change in total amount of enzyme may give increase in values of specific activity which may lead to erroneous conclusions. This approach enabled us to differentiate the effect of cycloheximide on sucrase and maltase. Although they both reacted to cortisone, cycloheximide and actinomycin D strikingly similarly, they displayed some differences (see data in Tables I and II, Fig. 3). Thus, although these two hydrolases are localized in the same cell particle (microvilli) and probably share some similar regulatory control mechanism, they have additionally different mechanisms of control. Furthermore, whereas sucrase activity can be attributed to one enzyme, maltase activity is actually a product of the activities of several isozymes whose relative contribution to the total activity is unknown[25]. A similar conclusion was drawn in experiments where the actinomycin D effect was studied on sucrase and maltase in adult rats[26].

In the present study the involvement of adrenals in the mechanism of the

cycloheximide-evoked increase was most apparent. The relationship between the adrenals and cycloheximide could already be seen in the toxicity of this drug. All suckling intact animals survived the used doses (20–60 μg/100 g body wt). In adrenalectomized animals injected with 30 μg/100 g body wt there was approximately a 33% mortality but simultaneous injection of cortisone acetate protected completely these animals. This is in good agreement with previous observations on adult rats[13,14]. Dependence of the cycloheximide effect on the endocrinal system was shown previously in adult rats[14,15]. Cycloheximide increases the RNA content in adrenals and intact hypophysis was essential for this effect[14]. The cycloheximide-stimulated incorporation of amino acids *in vitro* into the liver microsomes of adult rats was abolished in adrenalectomized rats[15]. Interestingly, this process was independent of the pituitary function because it occurred in hypophysectomized animals with intact adrenals[15].

The first possible interpretation of the cycloheximide-evoked increase of both hydrolases in our experiments was to assume that adrenal secretion of endogenous corticosteroid was stimulated and thus we have observed only a steroid effect. Although this may be one mechanism of the whole process, our experiments do not preclude other explanations. Whereas actinomycin D in intact rats inhibited the cortisone-evoked increase, the cycloheximide-evoked increase was not affected by application of actinomycin D at different periods (preceding, simultaneously or after the cycloheximide injection). This was done in order to inhibit the effect of possible secreted steroids. Furthermore, cycloheximide evoked the increase of sucrase in adrenalectomized animals. Without the adrenals the increase was small but definitive as compared to controls (Table I); there was a 400% increase of both specific and total activity of sucrase.

The question of the mechanism of the cycloheximide effect remains open and could be approached further by (1) determining the effect of cycloheximide on secretion and degradation of corticosteroids, and (2) following the rate of sucrase formation as well as its degradation by immunochemical methods.

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