THE ABILITY OF EXOGENOUS HEME TO RESTORE GLOBIN SYNTHESIS IN RETICULOCYTES WITH IMPAIRED HEME FORMATION

P. Poňka, J. Neuwirt, M. Šperl and Z. Březík

Department of Pathological Physiology, Faculty of General Medicine, Charles University, U nemocnice 5, Praha 2, Czechoslovakia

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SUMMARY. The addition of isonicotinic acid hydrazide (INH) or cycloheximide decreases the incorporation of ^{75}Se-methionine and ^{14}C-labeled aminoacids into globin. 10^{-4}M hemin added to the incubation mixture removes the inhibition of globin synthesis caused by INH but is without effect on the synthesis of globin inhibited by cycloheximide.

In recent years evidences have been collected that the synthesis of specific proteins in animal tissues is quantitatively regulated not only at the transcriptional but also at the translational level (1). The addition of hemin to rabbit reticulocytes in which no new RNA is synthesized, stimulates the rate of globin synthesis (2, 3). This finding which supports the idea of quantitative regulation of globin synthesis in reticulocytes at the level of translation was confirmed by many authors (4, 5, 6).

In the previous paper we have reported that isonicotinic acid hydrazide (INH) is a potent inhibitor of heme synthesis in rabbit reticulocytes (7). In this work the effect of heme on the rate of globin synthesis in reticulocytes incubated with INH or cycloheximide was studied. INH is a primary inhibitor of heme synthesis and it inhibits the synthesis of globin component of hemoglobin secondarily and reversibly. Reti-

culocytes with inhibited heme synthesis serve as a model for the studies of the regulation of globin synthesis in intact cells which may be different from regulation of globin synthesis in a cell-free system.

MATERIALS AND METHODS

Reticulocyte rich blood was obtained from bled rabbits. The incubation mixture was prepared as described in previous paper (7, 8). The incubation conditions, termination of incubation and the separation of cells were the same as described previously (7, 8).

^{75}Se-methionine (specific activity 5 mC/1 mg) was obtained from the Radiochemical Centre, Amersham, and added in amount of 1-3 uC per 30 ml of the total incubation mixture. The amount of 2-^{14}C-glycine used and its specific activity was the same as in previous experiments (8). 1.50 uC of ^{14}C-leucine (spec. activity 35 mC/mM) per 50 ml of total incubation mixture were added.

^{14}C-heme and ^{14}C-globin were isolated from reticulocytes and their specific activities measured using methods described in previous paper (8). The procedure with ^{75}Se-globin was the same as with ^{14}C-globin up to the drying of globin (8). The dried globin was weighed and its radioactivity measured in the scintillation counter (Tesla NRQ 612).

The times of preincubation of cells with inhibitors and of their incubations with the label are presented in appropriate tables and figures.

RESULTS

Table 1 shows that INH inhibits the incorporation of glycine-2-^{14}C into heme in the period of two-hour incubation and this inhibition is dependent on the concentration. At the

TABLE 1

The effect of various concentrations of INH on the incorporation of glycine-2-^{14}C into heme and globin and of leucine-^{14}C into globin. Results are expressed as percentages of incorporation into corresponding controls. The reticulocytes were preincubated 30 minutes before addition of the label.

Concentration of INH	Time of incubation	Incorporation of (expressed as % of control) glycine-2-^{14}C into heme	glycine-2-^{14}C into globin	leucine-^{14}C into globin
2×10^{-3}M	15	75.7	104.4	86.7
	30	82.4	-	106.6
	60	63.5	99.6	78.3
	120	41.6	83.8	81.0
4×10^{-3}M	15	24.4	59.0	56.9
	30	26.3	43.0	57.2
	60	21.6	42.6	44.3
	120	21.5	33.1	35.6
8×10^{-3}M	15	10.1	32.5	35.6
	30	9.5	26.1	33.0
	60	6.1	24.0	20.6
	120	5.4	21.2	20.7

same time the incorporation of glycine and leucine into globin decreases. We do not know whether greater suppresion of incorporation of labeled precursors into heme than that of globin during incubation with INH is due to the more pronounced suppresion of heme synthesis. The difference between the incorporation into globin and heme may be also caused by another factor since in the present experiments the specific activity of only partially purified globin (8) was measured. It is known that the purification of globin under certain conditions may decrease its specific activity (9). The solving of

these questions is beyond the scope of the present communication and is under investigation.

From Fig. 1 it is evident that 10^{-4}M hemin added to reticulocytes from bled rabbits exerts a small stimulation of globin synthesis of globin synthesis measured by the incorporation of ^{75}Se-methionine. Synthesis of globin in the presence of INH decreases similarly as in the previous experiment. The addition of hemin to reticulocytes incubated with INH pre-

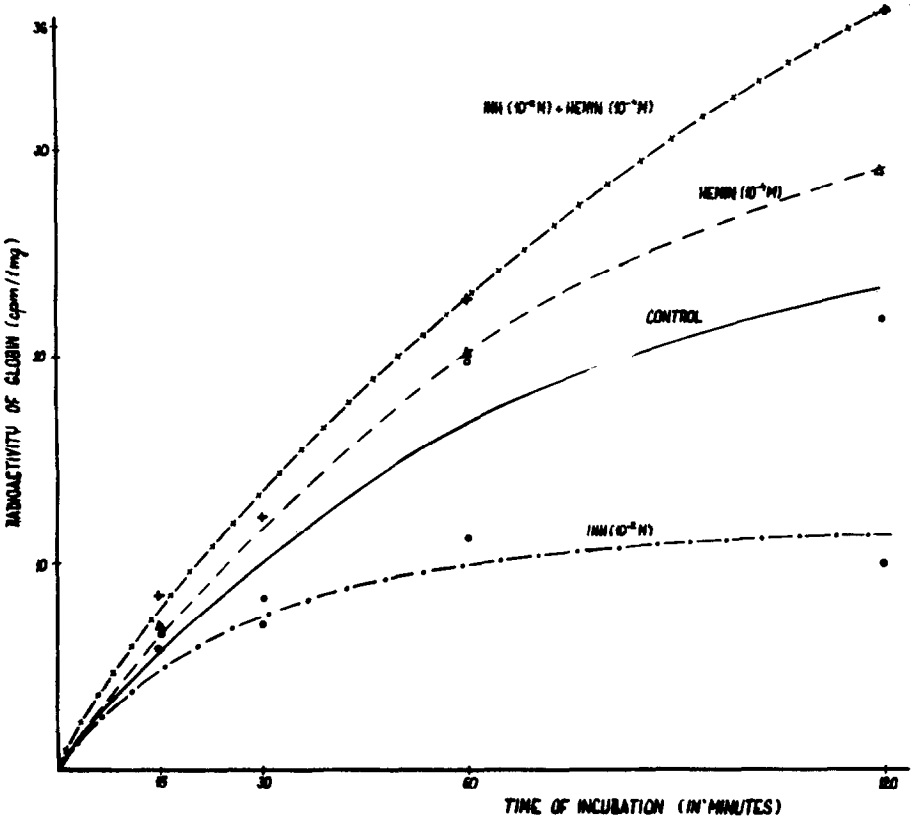

Figure 1. The effect of hemin on the incorporation of ^{75}Se-methionine into globin of control reticulocytes and of reticulocytes incubated with an inhibitor of heme synthesis. Cells were 10 min. incubated with or without INH and hemin was added (in final conc. 10^{-4}M) to the incubation mixture.10 min. after addition of hemin ^{75}Se-methionine was added to cells of all groups.

vents the inhibition of globin synthesis; the incorporation of ^{75}Se-methionine into globin was in the group with INH and heme even higher than in the control group.

In the further experiment we studied whether exogenous hemin abolishes the inhibition of globin synthesis caused by cycloheximide which primarily inhibits the synthesis of globin. Table 2 shows that hemin has no effect on the inhibition

TABLE 2

The effect of hemin on 60-minute incorporation of ^{75}Se-methionine into globin of reticulocytes incubated with the inhibitor of globin synthesis. Cells were preincubated 30 minutes before addition of hemin and then 15 minutes before addition of the label. Each value represents the mean of results from three incubation flasks

	cpm/mg of globin	% of incorporation into appropriate control
Control	70.3	100.0
Hemin (10^{-4}M)	71.5	101.8
Cycloheximid (2×10^{-4}M)	13.7	100.0
Cycloheximid (2×10^{-4}M) + hemin (10^{-4}M)	12.1	88.5

of incorporation of ^{75}Se-methionine into globin caused by cycloheximide.

^{75}Se-methionine incorporates into hemoglobin of erythroblasts and reticulocytes (10) and was used in these experiments because of the uncomplicated experimental procedure. This amino acid is, however, not the natural component of proteins and the kinetics of its incorporation into proteins need not be the actual measure of proteosynthesis. In an experiment recorded in Table 3 evidences are provided that the results with both 2-^{14}C-glycine and ^{14}C-leucine are similar

TABLE 3

60-minute incorporation of glycine-2-^{14}C into heme and globin and of leucine-^{14}C into globin of control reticulocytes and of reticulocytes incubated with INH or hemin. Cells were preincubated with or without INH 40 minutes before addition of the label. Cells in the last group were preincubated for 30 minutes with INH only, then hemin was added and after 10 minutes isotope was added (in the same time as to all samples). Each value represents the mean of four samples.

	Incorporation of				
	glycine-2-^{14}C into			leucine-^{14}C into	
	heme (cpm/mg)	globin (cpm/mg)	% of appropriate control	globin (cpm/mg)	% of appropriate control
Control	1598	69	100.0	171	100.0
Hemin (10^{-4}M)	1090	97	140.6	296	173.1
INH (10^{-2}M)	132	25	100.0	44	100.0
INH (10^{-2}M) + hemin (10^{-4}M) (hemin added in the same time as INH)	132	116	464.0	376	854.5
INH (10^{-2}M) + hemin (10^{-4}M) (hemin added 30 minutes after INH)	133	133	532.0	403	915.9

as with ^{75}Se-methionine. From Table 3 it is also evident that 10^{-4}M hemin stimulates globin synthesis inhibited by INH beyond control values regardless if hemin is added to reticulocytes in the same time as INH or after 30-minute preincubation of cells with the inhibitor of heme synthesis. Differences in the percentage of stimulation of leucine and glycine incorporation by heme may be related to different membrane transport of both substrates. This possibility is currently studied.

DISCUSSION

The present experiments lead to the conclusion that INH primarily inhibits the synthesis of heme and secondarily reduces globin synthesis. Exogenous hemin added to reticulocytes incubated with INH enters these cells (7) and probably replaces insufficiently formed endogenous hemin which is essential for the normal synthesis of globin (3, 11).

In some reticulocytes the synthesis of endogenous heme may be maximal (Table 2) and added hemin is without effect on the synthesis of globin. The various degree of response of control cells to the stimulatory effect of hemin may be related to different availability of iron either during the maturation of erythroid cells (12) or in the incubation mixture (3, 6).

It is difficult to explain why both hemin and INH incubated reticulocytes incorporate more labeled precursors into globin than only hemin treated reticulocytes (Fig. 1, Table 3). It was recently described that the amount of iron taken up by reticulocytes incubated with INH and heme is greater than that taken up by cells incubated with heme (7). The question of some direct functional importance of iron for the regula-

tion of globin synthesis has therefore arisen. Iron incorporates into polysomes (13) but it is believed that it does not effect directly the stabilization of polysomes (14).

Recent finding of Maxwell and Rabinowitz (15) that only fresh reticulocyte cell-free system responses to the influence of heme and that during short incubation of such system an inhibitor is formed which prevents the stimulatory effect of heme stimulated us to the experiment depicted in Table 3. It follows from this table that integrated reticulocytes incubated 30 minutes in the absence of heme synthesis did not lose their response to the effect of hemin. Further experiments will elucidate whether preserved response of preincubated whole cells is due to the lack of formation or due to the destruction of such inhibitor. In each case it is evident that regulatory mechanisms of globin synthesis are different in cell-free system and in integrated reticulocytes.

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