Biochem. J. (1974) **144**, 607–609
Printed in Great Britain

Induction of Cystathionase in Human Foetal Liver

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(*Received* 30 *September* 1974)

Incubation of liver explants from second-trimester human foetuses with dexamethasone, glucagon or dibutyryl cyclic AMP (plus theophylline) increased the activity of liver cystathionase from unmeasurable or trace values to adult values. Simultaneous incubation with cycloheximide or actinomycin D inhibited this effect.

In adult human liver methionine is converted into cyst(e)ine via the trans-sulphuration pathway (Rose & Wixom, 1955). The last enzyme in this pathway, cystathionase (EC4.4.1.1), is not measurable in human foetal liver (Sturman *et al.*, 1970; Gaull *et al.*, 1972), and immunochemical studies have demonstrated that the human foetus does not synthesize more than trace amounts of cystathionase (Pascal *et al.*, 1972). We have therefore examined whether this enzyme can be induced in human foetal liver in organ culture by agents such as dibutyryl cyclic AMP (6-*N*,2′-*O*-dibutyryladenosine 3′:5′-cyclic AMP), glucagon or dexamethasone.

Materials and methods

Liver tissue was obtained at legal therapeutic abortions by hysterotomy. A complete description of the organ culture system used has been reported previously (Räihä *et al.*, 1971). The culture medium was Eagle's minimum essential medium with Hanks balanced-salt solution containing twice the normal concentrations of glucose, glutamine and bicarbonate, pH 7.6, and with 100 i.u. of penicillin and 100 μg of streptomycin/ml (Schwartz, 1972). Explants less than 1 mm^3 were prepared from foetal liver tissue and placed on wire grids at the liquid–gas interphase. During the incubation the explants were gassed with O_2+CO_2 (95:5). The explants were preincubated for 24h before the agents were added to the culture medium. The activity of cystathionase was assayed by the method of Gaull *et al.* (1969). The amount of cysteine formed was quantified by the method of Gaitonde (1967), but the amount of acid ninhydrin reagent was doubled in order to increase the intensity of the colour reaction. The protein concentrations were determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. The enzyme activity was expressed as nmol of cysteine/60 min per mg of protein.

Results and discussion

In fresh specimens of foetal liver tissue, no cystathionase activity was detected, which is in agreement with previous reports (Sturman *et al.*, 1970; Gaull *et al.*, 1972). For reference, a post-mortem sample from a full-term male infant (age 50h) was obtained and analysed for cystathionase activity, which was 70 nmol of cysteine/60 min per mg of protein. This result agrees well with previous observations on the activity of cystathionase in liver samples from newborn humans (Gaull *et al.*, 1972).

Without added agents, no activity of cystathionase was detected in liver explants from the smallest foetuses (crown–rump length 35–50 mm) incubated for 24 or 48 h. In late first-trimester foetuses and in all second-trimester foetuses studied (crown–rump length 90–170 mm), trace amounts of cystathionase activity were observed at least after a 48 h incubation (Table 1). Although liver cystathionase activity in adult human subjects increases after the administration of large doses of vitamin B_6 (Gaull *et al.*, 1969), addition of pyridoxal 5′-phosphate (from 1 to 100 μg/ml) to the culture medium failed to elicit any increase in the activity of foetal liver cystathionase during a 48 h period of incubation.

Theophylline, an inhibitor of phosphodiesterase which breaks down cyclic AMP, potentiated the effect of dibutyryl cyclic AMP on the activity of foetal liver cystathionase. This was shown in a series of experiments performed on liver explants from a mid-trimester human foetus (crown–rump length 170 mm). When the explants were incubated with dibutyryl cyclic AMP (0.2 mM) alone, the activity of cystathionase was 43 ± 12 nmol of cysteine/60 min per mg of protein (mean $\pm$ S.E.M., $n = 8$), which was not different from the enzyme activity found in control explants (31 ± 9 nmol/60 min per mg of protein, $n=7$, $P^*>0.05$). A 10-fold concentration of dibutyryl cyclic AMP (2.0 mM) increased the cystathionase activity to 95 ± 13 nmol/60 min per mg of protein ($n = 5$); this effect was significant ($P<0.01$). However, incubation of the explants with a combination of dibutyryl cyclic AMP (0.2 mM) and theophylline (0.5 mM) increased the activity of cystathionase to 192 ± 20 nmol/60 min per mg of protein ($n = 4$); this was

*Significance of difference was calculated by the Student's t test; $P < 0.05$ is not significant.

Table 1. *Effect of dexamethasone, glucagon or dibutyryl cyclic AMP plus theophylline on cystathionase activity in human foetal liver explants*

The explants were preincubated for 24h before the experiment; duration of the experiments was 24h. Each value represents the mean±S.E.M. (number of determinations). For each determination (in duplicate) 25–30 explants were required. – means no measurable activity of cystathionase.

Crown–rump length (mm)	Activity of cystathionase (nmol of cysteine/60min per mg of protein)					
	Fresh	Preincubated (24h)	Dexamethasone (60μg/ml)	Glucagon (100μg/ml)	Dibutyryl cyclic AMP+theophylline (0.2mM+0.5mM)	None
35	–	– (3)	– (7)	– (6)	– (8)	– (5)
40	–	– (4)	– (6)	– (7)	– (7)	– (6)
50	–	– (5)	– (5)	– (4)	– (5)	– (6)
75	–	– (4)	198± 9 (4)*	164± 3 (4)	92±18 (4)	– (5)
90	–	– (4)		128±18 (5)	168±25 (5)	28± 7 (5)
95	–	– (4)			153±20 (5)	40±12 (5)
115	–	– (4)	114±21 (4)	102±18 (4)†	120±14 (4)	– (4)
120	–	21± 6 (6)	188± 8 (6)			41±15 (6)
135	–	– (4)	111±15 (4)	140±21 (4)	191±26 (4)	21± 7 (4)
145	–	18± 7 (5)			195± 2 (6)	25± 8 (6)
170	–	19±10 (5)	112± 2 (4)	168± 7 (4)	192±20 (4)	29± 5 (5)

* Concentration of dexamethasone in this experiment 20μg/ml.
† Concentration of glucagon in this experiment 150μg/ml.

Table 2. *Effect of cycloheximide or actinomycin D on the dibutyryl cyclic AMP-plus-theophylline-, dexamethasone- or glucagon-stimulated activity of cystathionase in human foetal liver explants*

After a 24h preincubation cycloheximide (10μg/ml) or actinomycin D (30μg/ml) was added to the incubation medium simultaneously with either dibutyryl cyclic AMP plus theophylline, dexamethasone or glucagon. The duration of the experiments was 24h. Each value represents the mean±S.E.M. (number of determinations).

Crown–rump length (mm)	Agent	Activity of cystathionase (nmol of cysteine/60min per mg of protein)
170	Dibutyryl cyclic AMP (0.2mM) plus theophylline (0.5mM)	192±20 (4)
	Dibutyryl cyclic AMP (0.2mM) plus theophylline (0.5mM) and cycloheximide (10μg/ml)	41±22 (5)
	None	29±5 (5)
110	Dexamethasone (60μg/ml)	200±11 (4)
	Dexamethasone (60μg/ml) and cycloheximide (10μg/ml)	58±10 (4)
	Dexamethasone (60μg/ml) and actinomycin D (30μg/ml)	60± 7 (4)
	None	No measurable activity
170	Glucagon (100μg/ml)	168± 7 (4)
	Glucagon (100μg/ml) and cycloheximide (10μg/ml)	59±14 (6)
	None	19±10 (5)

significantly more than the effect produced by either of the concentrations of dibutyryl cyclic AMP alone ($P<0.001$ and <0.01 respectively). Theophylline (0.5 and 5.0mM) was ineffective alone. 5′-AMP, the degradation product of cyclic AMP, had no effect on cystathionase activity in human foetal liver explants, which suggests that dibutyryl cyclic AMP itself caused the observed increase in the activity of this enzyme.

The effects of dibutyryl cyclic AMP plus theophylline, glucagon or dexamethasone on the activity of cystationase in human foetal liver explants during a 24h incubation period are presented in Table 1. None of the agents had an effect on cystathionase activity in liver explants from the smallest first-trimester foetuses. In liver explants from late first-trimester foetuses and from all second-trimester foetuses, incubation with dibutyryl cyclic AMP (0.2mM) plus theophylline (0.5mM), glucagon (100 or 150μg/ml) or dexamethasone (20 or 60μg/ml) increased liver cystathionase activity to values similar to those observed previously in liver samples from healthy adult humans (Sturman *et al.*, 1970; Gaul *et al.*, 1972). There was no significant difference between the effects of these agents. When treated with either of these agents, the activity of liver cystathion-

ase increased progressively throughout the 24h incubation period.

Table 2 shows the effect of cycloheximide or actinomycin D on the dibutyryl cyclic AMP-, glucagon- or dexamethasone-stimulated activity of cystathionase in human foetal liver explants. Simultaneous incubation with cycloheximide (10μg/ml) or actinomycin D (30μg/ml) abolished the effects of these agents. These data suggest that protein synthesis is required for the increased enzyme activity.

The present results show that cystathionase activity can be induced in mid-trimester human foetal liver *in vitro*. The failure to stimulate the activity of this enzyme in liver explants from the smallest foetuses suggests that a certain developmental stage must be reached before foetal liver tissue has this competence to respond to externally administered pharmacological agents.

This study was supported by a grant from Sigrid Juselius Foundation.

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