Effect of Cyclic AMP on Placental Active Transport of Amino Acid in Rats

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Tohoku J. exp. Med., 124 (3), 241-249 — In pursuit of the probable relationship
of cAMP to the placental active transport of amino acids as suggested by our
previous human data, experiments were done in this study with dibutyryl cAMP
and theophylline loaded in pregnant rats to examine the changes in level of ^{14}C -
lysine in the maternal liver, placenta and fetus. The results are: (1) Loading of
dibutyryl cAMP had no effect on the uptake of ^{14}C -lysine in the maternal liver,
placenta or fetus, except that the correction of the values with its concentration
in the maternal blood revealed only a slightly significant elevation in the value in
each of them 30 min after the loading. (2) Loading of theophylline significantly
increased the uptake of ^{14}C -lysine in all these organs. (3) The content of cAMP
in the placenta significantly increased with the loading of theophylline. These
results have led the authors to believe that endogenous cAMP is significantly
linked with the placental active transport of amino acids, though there is no
apparent contribution to it by exogenous cAMP. ——— SFD; placenta; cAMP;
amino acids

The placental active transport of substances has long been known to be primarily responsible for feto-maternal transport of amino acids. In other fields, the permeability of amino acids through the cell membrane has been studied in many ways, and as a result, Na^+ , K^+ -ATPase, γ -glutamyl cycle and others have so far been identified as factors influencing amino acid permeation through the cell membrane.

Cyclic AMP (cAMP), aside from its known role as the second messenger of steroid hormones, has been drawing attention for its possible link with the metabolic accelerator of the cell membrane probably facilitating the passage of amino acids and other substances through the cell membrane. The data showing that the uptake of amino acids in the muscle or liver cells distinctly increases with the administration of glucagon or epinephrine, seem to indicate that the activity of cAMP intracellularly invigorated with such agent eventually helped promote the transport of amino acids into the cells.

On the other hand, the presence of cAMP in the placenta was discovered by Sato and Ryan (1971), but its physiological significance has not yet been

elucidated. The present authors previously found a correlation between the placental content of cAMP and the feto-maternal ratio of amino acid concentration (F/M ratio), thereon presuming a possible relationship of cAMP in the placenta to the placental active transport of amino acids (Matsuda et al. 1977). The present study was conducted in an attempt to identify the physiological role of cAMP in the placenta under the in vivo loading of dibutyryl cAMP and cAMP-increasing substance, theophylline, in pregnant rats to examine the changes in uptake of amino acids in the maternal liver, placenta and fetus.

MATERIALS AND METHODS

Animals and agents

The animals used were pregnant rats of the SD strain. Experiments were done in the rats on the 19th day of gestation (the day when vaginal plug was found was decided as the 0 day of gestation). Each test rat was subjected to fasting for 12 hr prior to the start of experiment.

Theophylline (10 mg/ml/100 g) and dibutyryl cAMP (5 mg/ml/100 g) were given in to the abdominal cavity. At the same time, 0.5 ml/300 g of L(U- 14 C)-lysine monohydrochloride (specific activity 330 mCi/mmol), as a marker amino acid, was injected into the femoral vein, and the radioactivities in the maternal liver, placenta and whole fetus were counted on scheduled time. Control rats were given only physiological saline solution (1 ml/100 g) and the same doses of 14 C-lysine.

Determination of radioactivity in the blood and organs

With the rat under ether anesthesia, the blood was sampled from the abdominal aorta. The whole blood (1 ml), to which was added toluene (3 ml), was heated at 50°C for 24 hr to dissolve itself completely. To this solution was added 2 ml of 20% benzoyl peroxide and to 0.1 ml of the resultant solution was added triton (4 ml) and the radioactivity in that was counted using a toluene scintillator. After the blood was taken, the rat was killed, and the liver, placenta and fetuses were taken off. Each of these was washed in saline and, after removal of water with filter paper, was added with 40 ml of 1 N NaOH and heated on a sand bath at 100–200°C to obtain a clear solution. To this solution was further added 0.4 ml of acetic acid, a few drops of ascorbic acid and another 4 ml of triton, then the radioactivity in each of the maternal liver, placenta and fetus was counted also using a toluene scintillator.

Determination of placental cAMP

The placenta was washed in cold physiological saline solution (0°C) to remove blood, and after the removal of water with filter paper, it was frozen with dry ice. The isolation of cAMP was stepped according to the method of White and Zenster (1971) and Ramachandran (1971), and the determination was made using the binding protein method of Walton and Garren (1970) and Gilman (1970).

Quantitation of protein

Proteins were quantitated using Lowry's method (Lowry et al. 1951).

RESULTS

Effects of cAMP and theophylline on the uptake of 14 C-lysine into maternal liver, placenta and fetus

The uptakes of 14 C-lysine per 100 mg of protein in the maternal liver, placenta and fetus are shown in Table 1, and their concentrations of 14 C-lysine per 1 ml of

Fig. 1. Effects of theophylline and dibutyryl cAMP on ^{14}C -lysine uptake in maternal blood, maternal liver, placenta and fetus. ○—○, control; ▲—▲, theophylline; △—△, dibutyryl cAMP.

TABLE 1. Effects of theophylline and dibutyryl cAMP on ^{14}C -lysine uptake into maternal liver, placenta and fetus (dpm/100 mg protein)

	Min	Number of rats	Maternal liver	Placenta	Whole fetus
Control	10	4	3,774±1,029	1,211± 664	1,920±389
	30	7	3,251± 585	1,104± 47	2,013±448
	60	7	2,121± 583	986± 371	1,514±547
	120	4	1,967± 623	1,288± 320	2,017±492
Theophylline	10	4	3,365±1,592	1,060± 226	1,783±440
	30	4	4,184± 776	1,839±1,088	2,857±582
	60	4	1,673± 640	986± 102	1,902±541
	120	4	2,812± 910	1,596± 330	2,548±277
Dibutyryl cAMP	30	6	2,646± 460	1,075± 315	1,368±507
	60	6	1,887± 662	1,077± 396	1,626±398

maternal blood and uptakes of ^{14}C -lysine per 1 g of wet weight of tissue in Fig. 1. As can be seen in the figure, the concentrations of ^{14}C -lysine in the maternal blood, without differences between the rats loaded with theophylline and those with cAMP, decreased gradually as a whole.

In the maternal liver, the cAMP-loaded rats did not show any distinctive difference in the uptake of ^{14}C -lysine from the control animals, their concentrations gradually increasing with time in both. But in the rats loaded with theophylline, the uptakes of ^{14}C -lysine in the liver per 1 g of wet weight and per 100 mg of protein were both significantly higher than the control figures 30 min after loading ($p < 0.05$), the levels were always higher at other times except at 60 min.

In the placenta, the concentrations of ^{14}C -lysine gradually increased without distinction in both the cAMP-loaded and the control group, but in the theophylline-

loaded animals, the uptake of ^{14}C -lysine per 1 g of wet weight was significantly higher than the control 30 min after the loading ($p<0.05$).

The transport of ^{14}C -lysine into the fetus increased gradually without distinction in the cAMP-loaded rats and the control animals, while in the theophylline-loaded rats, the concentration of ^{14}C -lysine in the fetus was ever higher than the control at each measurement, particularly significant at 30 min ($p<0.05$).

^{14}C -Lysine uptake in maternal liver, placenta and fetus corrected with the values of total ^{14}C -lysine concentration in maternal blood

The amounts of ^{14}C -lysine taken in the maternal liver, placenta and fetus per 1 g of tissue and per 100 mg of protein as corrected with the values of total ^{14}C -lysine concentrations in 0.1 ml of maternal blood are shown in Table 2 and Fig. 2, respectively. In the maternal liver, the uptake of ^{14}C -lysine decreased with time

Fig. 2. Rate of ^{14}C -lysine uptake in maternal liver, placenta and fetus (values were corrected with ^{14}C -lysine concentration in maternal blood). ○—○, control; ▲—▲, theophylline; △—△, dibutyl cAMP.

TABLE 2. *Effects of theophylline and dibutyl cAMP on ^{14}C -lysine uptake into maternal liver, placenta and fetus corresponding to maternal blood level of ^{14}C -lysine*

	Min	Number of rats	Maternal liver	Placenta	Whole fetus
Control	10	4	29.7± 7.8	6.1±2.3	6.3±1.9
	30	5	23.2±11.8	6.9±4.4	5.1±1.5
	60	7	10.6± 3.3	2.9±0.4	3.3±0.8
	120	4	4.0± 1.7	2.4±1.2	2.8±2.0
Theophylline	10	4	36.2±13.3	7.0±2.2	7.7±1.4
	30	4	25.4± 9.5	6.0±1.8	7.0±2.1
	60	4	10.7± 2.4	4.2±1.1	5.2±1.8
	120	4	9.4± 3.0	3.6±0.8	3.7±0.5
Dibutyl cAMP	30	5	36.9±21.7	8.9±3.0	10.0±5.8
	60	6	11.9± 4.2	4.5±5.5	4.4±2.1

in each group without distinction as a whole.

In the placenta, the uptakes of ^{14}C -lysine decreased with time in all groups without any significant difference, the only distinction being the peaks shown at 30 min in the groups loaded with theophylline and with cAMP. The transport of ^{14}C -lysine into the fetus generally tended to decrease with time, but in both groups of rats loaded with cAMP and with theophylline, the values were higher than the control figures at each stage of measurement. Particularly, in the theophylline-loaded rats, the values per 100 mg of protein and per 1 g of wet weight were both significantly higher at 60 min ($p < 0.05$). In the cAMP-loaded rats, only the 30 min value per 1 g of wet weight was slightly higher, but not significant ($p < 0.1$).

Placental cAMP levels in theophylline-loaded rats

Table 3 shows the content of cAMP in the placenta in the theophylline-loaded rats 30 min after the loading and the control. The value of 1.239 pmoles/mg wet weight for the theophylline-loaded group was significantly higher compared with 0.456 pmoles/mg wet weight for the control group ($p < 0.025$).

TABLE 3. *Effects of theophylline loading on placental cAMP in rats*
(pmoles/mg wet weight)

	Control (n=6)	Theophylline (n=6)
	0.2348	0.7857
	0.4707	0.6609
	0.9157	1.9100
	0.3606	1.8030
	0.3909	1.5180
	0.3633	0.7544
Mean $\pm$ S.D.	0.4560 $\pm$ 0.2376	1.2386 $\pm$ 0.5694

DISCUSSION

In pursuit of the uptake of amino acids into animal cells, Christensen (1969), Christensen et al. (1973) and Heinz (1974) made studies on Ehrlich cancer cells and others, and reported their basic analyses. On the other hand, since Christensen et al. (1948) and Page et al. (1957) pointed out the active transport as playing a pivotal role in the transport of amino acids through the placenta, analytical investigations into this field have been made by different authors in various ways. Specifically, Enders et al. (1976), classifying neutral amino acids into A, L and ASC types, pursued the problem from the point of their competitive inhibition. Gussek et al. (1975) and Schneider and Dancis (1974), studied uptakes of amino acids by cells in tissue slices, and reported on the influence on them of some agents, such as mixture of amino acids, inhibitors of protein synthesis; and Yoshimura (1965) and Sugawa et al. (1962), in search of some factors possibly influencing the placental transport of amino acids, made studies on estradiol and vitamin B₆, respectively.

As the factors influencing the transport of amino acids through the cell membrane, in addition to Na-K ATPase and γ -glutamyl cycle recently proposed by Meister (1973), cAMP has lately been drawing attention as another influential factor. But so far there has not been any report dealing with its relation to the passage of amino acids through the placenta.

In our previous study on the mechanism for developing SFD from the aspects of amino acid metabolism and the content of cAMP in the placenta, we found a positive correlation between the F/M ratio of amino acid concentrations and the placental content of cAMP, and presumed a link of placental cAMP with the uptake of amino acids into the placenta (Matsuda et al. 1976, 1977). In the present study, in an effort to justify this presumption, we experimented with pregnant rats, loading them with dibutyryl cAMP and theophylline, a cAMP-increasing factor, with ^{14}C -lysine as the marker of amino acid transport.

In the control group with the loading of ^{14}C -lysine, its concentration increased with time in the maternal blood, but decreased in the liver. This is likely to tell that most of the ^{14}C -lysine injected into the femoral vein was first stored in the liver, that is the organ playing a pivotal role in the metabolism of amino acids, and then was released gradually into circulation. In this connection, Dancis et al. (1968) and Rosso (1975), in their experiments with ^{14}C - α -aminoisobutylic acid (AIB) in guinea pigs and rats, noted that the maternal serum level of AIB dropped with time. The inconsistency between their results and ours could be attributed to the differences between the used amino acid (metabolizable lysine by us and non-metabolizable AIB by them), between the test material (whole blood by us and serum by them), and between the concentration of the amino acid administered (our dose was about 1/50 of the serum lysine concentration).

The concentration of ^{14}C -lysine in the maternal liver was significantly higher in the theophylline-loaded group than in the control 30 min after loading, but there occurred no distinction in the group of rats loaded with exogenous cAMP. However, when the values were corrected with the ^{14}C -lysine concentration in the maternal blood, the theophylline-loaded group had no difference from the control, while the cAMP-loaded group showed a slightly higher value than the control at 30 min. Concerning the relation of cAMP to the hepatic uptake of amino acids, Harrison and Christensen (1971), using nonmetabolizable amino acids, reported that the administration of glucagon and theophylline acted on cAMP to help increase the uptake of amino acids. Identical results with similar material were obtained by Scott et al. (1970) through observations of the behaviors of tyrosine aminotransferase and cAMP, and by Tews et al. (1970) through studies with tissue slices. In contrast, Butcher et al. (1972) reported that among the cells of transplanted hepatoma, those actively taking in AIB had a normal content of cAMP, which did not increase with the loading of glucagon. Thus, there is no conclusive view yet elucidating this issue. Our present experiments also failed to find any clear relationship between cAMP and the hepatic uptake of amino acid. For other organs, the contribution of cAMP to the uptake of amino acids was

noted in the cartilage by Drezner et al. (1975). Ring et al. (1974) reported that exogenous cAMP extended no help to increase the uptake of amino acids in *Streptomyces hydrogenans*, but intracellular cAMP positively contributed to that. On the other hand, Kuroki (1973) stated that cAMP contrarily decreased the uptake of amino acids in the cells of the hamster's fetus.

The present experiments could not establish any positive effect of exogenous cAMP increasing the uptake of ^{14}C -lysine in either the placenta or the fetus. But the loading of theophylline evidently caused significant increase in the ^{14}C -lysine uptake in both. In view of the fact that placental cAMP increased with the loading of theophylline, it can be surmised that the increased cAMP in the placenta promotes the passage of amino acid into the placental cells, which then will lead to the transport of increased amino acid into the fetus. Our data, aside from their lack of the time course follow-up, are generally consistent with Rosso's results (Rosso 1975).

The present results have given us the impression that cAMP would have some influence on the passage of amino acids through the placenta. Yet there still remain some doubts about our results, including two major problems: (1) whether theophylline extends or not any influence on the utero-placental blood flow, and (2) whether cAMP acts directly or indirectly on the transport of amino acids. In connection with the latter problem, Nozaki et al. (1975) pointed out a direct effect of cAMP in increasing cardiac output and blood flow in the renal and superior mesenteric arteries, but they did not touch on whether a similar increase in blood flow occurs or not in the placenta.

There remain many other questions of whether cAMP causes or not morphological changes in the cell membrane (Nose and Katsuta 1974, observations on rat's cultured cells), whether the placental uptake of amino acids is influenced or not by cAMP-caused phosphorylation of cell membrane protein, and whether cAMP suppresses or not Na^+ , K^+ -ATPase activity (observed in rat's hepatic cells – Luly et al. 1972; and in toad's bladder – Ferguson 1975). All these stay as puzzle to be unraveled with further extensive investigations.

References

- 1) Butcher, F.R., Scott, D.F., Potter, Van R. & Morris, H.P. (1972) Endocrine control of cyclic-adenosine 3',5'-monophosphate levels in several Morris hepatomas. *Cancer Res.*, **32**, 2135–2140.
- 2) Christensen, H.N. (1969) Some special kinetic problems of transport. In: *Advances in Enzymology*, edited by F.F. Nord, Interscience Publications, New York-London-Sydney-Toronto, Vol. 32, pp. 1–20.
- 3) Christensen, H.N. & Streicher, J.A. (1948) Association between rapid growth and elevated cell concentrations of amino acids in fetal tissues. *J. biol. Chem.*, **175**, 95–100.
- 4) Christensen, H.N., De Cespedes, C., Handlogten, M.E. & Ronquist, G. (1973) Energization of amino acid transport, studied for the Ehrlich ascites tumor cell. *Biochim. biophys. Acta (Amst.)*, **300**, 487–523.
- 5) Dancis, J., Money, W.L., Springer, D. & Levitz, M. (1968) Transport of amino acids by placenta. *Amer. J. Obstet. Gynecol.*, **101**, 820–829.
- 6) Drezner, M.K., Eisenbarth, G.S., Neelon, F.A. & Lebovitz, H.E. (1975) Stimulation

- of cartilage amino acid uptake by growth hormone-dependent factors in serum. Mediation by adenosine 3':5'-monophosphate. *Biochim. biophys. Acta (Amst.)*, **381**, 384-396.
- 7) Enders, R.H., Judd, R.M., Donohue, T.M. & Smith, C.H. (1976) Placental amino acid uptake. III. Transport systems for neutral amino acids. *Amer. J. Physiol.*, **230**, 706-710.
 - 8) Ferguson, D.R. (1975) The effect of diuretics on Na⁺, K⁺-ATPase and c-AMP levels in toad bladder epithelial cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **287**, 111-116.
 - 9) Gilman, A.G. (1970) A protein binding assay for adenosine 3',5'-cyclic monophosphate. *Proc. nat. Acad. Sci. (Wash.)*, **67**, 305-312.
 - 10) Gusseck, D.J., Yuen, P. & Longo, L.D. (1975) Amino acid transport in placental slice. Mechanism of increased accumulation by prolonged incubation. *Biochim. biophys. Acta*, **401**, 278-284.
 - 11) Harrison, L.I. & Christensen, H.N. (1971) Evidence for a hepatic transport system not responsive to glucagon or theophyllin. *Biochem. biophys. Res. Commun.*, **43**, 119-125.
 - 12) Heinz, E. (1974) Coupling and energy transfer in active amino acid transport. *Curr. Top. Memb. Transp.*, **5**, 137-159.
 - 13) Kuroki, T. (1973) Effects of cyclic AMP on the transport of sugars and amino acids. *Kosankinbyo Kenkyu Zasshi*, **25**, 166. (Japanese)
 - 14) Lowry, O.H., Rosenbrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 265-275.
 - 15) Luly, P., Barnabei, O. & Tria, E. (1972) Hormonal control *in vitro* of plasma membrane-bound (Na, K)-ATPase of rat liver. *Biochim. biophys. Acta (Amst.)*, **282**, 447-452.
 - 16) Matsuda, T., Nakano, Y., Takei, H., Ishibashi, S., Ueha, K., Nishikawa, Y. & Yamaguchi, R. (1976) Maternal and fetal amino acid concentrations and placental cyclic AMP levels in SFD and other abnormal pregnancies. *Nippon Sanka-Fujinka Gakkai Zasshi*, **28**, 1465-1472. (Japanese)
 - 17) Matsuda, T., Nakano, Y., Nishikawa, Y. & Yamaguchi, R. (1977) Feto-maternal amino acid patterns and cyclic AMP in human placenta with abnormal pregnancies, particularly with SFD. *Tohoku J. exp. Med.*, **121**, 253-262.
 - 18) Meister, A. (1973) On the enzymology of amino acid transport. *Science*, **180**, 33-39.
 - 19) Nose, K. & Katsuta, H. (1974) Induction of alkaline phosphatase activity by dibutyladenosine 3':5'-cyclic monophosphate in aneuploid rat liver cells. *Exp. Cell Res.*, **87**, 8-14.
 - 20) Nozaki, H., Mori, K. & Okuaki, S. (1975) Responses to exogenous cyclic AMP and dibutyladenosine 3':5'-cyclic AMP on the cardiac output and organ blood flow in dogs. *J. Jap. Coll. Angiol.*, **15**, 329-333. (Japanese)
 - 21) Page, E.W., Glendening, M.B., Margolis, A. & Harper, H.A. (1957) Transfer of D- and L-histidine across the human placenta. *Amer. J. Obstet. Gynecol.*, **73**, 589-597.
 - 22) Ramachandran, J. (1971) A new simple method for separation of adenosine 3',5'-cyclic monophosphate from other nucleotides and its use in the assay of adenylyl cyclase. *Anal. Biochem.*, **43**, 227-239.
 - 23) Ring, K., Tobias, R. & Ehle, H. (1974) Effect of adenosine 3',5'-cyclic monophosphate on active amino acid transport in *Streptomyces hydrogenans*. *Hoppe-Seyler's Z. Physiol. Chem.*, **355**, 1234-1244.
 - 24) Rosso, P. (1975) Changes in the transfer of nutrients across the placenta during normal gestation in the rat. *Amer. J. Obstet. Gynecol.*, **122**, 761-766.
 - 25) Sato, K. & Ryan, K.J. (1971) Adenylyl cyclase in the human placenta. *Biochim. biophys. Acta (Amst.)*, **244**, 618-624.
 - 26) Schneider, H. & Dancis, J. (1974) Amino acid transport in human placental slices. *Amer. J. Obstet. Gynecol.*, **120**, 1092-1098.
 - 27) Scott, D.F., Reynolds, R.D., Pitot, H.C. & Potter, van R. (1970) Co-induction of the hepatic amino acid transport system and tyrosine aminotransferase by theophylline, glucagon and dibutyladenosine 3':5'-cyclic AMP *in vivo*. *Life Sci.*, **9**, 1133-1140.

- 28) Sugawa, T., Yamanaka, S. & Ozaki, M. (1962) Studies on the amino acid and protein metabolism in fetal growth. I. Studies on the transfer of precursor of fetal protein across the placenta and the enzymic degradation of maternal serum protein in the placenta. *J. Jap. Obstet. Gynec. Soc.*, **9**, 257-264.
 - 29) Tews, J.K., Woodcock, N.A. & Harper, A.E. (1970) Stimulation of amino acid transport in rat liver slices by epinephrine, glucagon, and adenosine 3',5'-monophosphate. *J. biol. Chem.*, **245**, 3026-3032.
 - 30) Walton, G.M. & Garren, L.D. (1970) An assay for adenosine 3',5'-cyclic monophosphate based on the association of the nucleotide with a partially purified binding protein. *Biochemistry*, **9**, 4223-4229.
 - 31) White, A.A. & Zenster, T.V. (1971) Separation of cyclic 3',5'-nucleotide monophosphates from other nucleotides on aluminum oxide column. *Anal. Biochem.*, **41**, 372-396.
 - 32) Yoshimura, Y. (1965) The specific effects of the estradiol on the amino acid dynamics in the rabbit uterine and placental tissue. *Adv. Obstet. Gynecol.*, **17**, 7-14. (Japanese)
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