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Effect of Hormonal Steroids on the Uptake of Labeled Amino Acids by HeLa Cells[1]

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The uptake of glycine-1-^{14}C and AIB[3]-1-^{14}C by HeLa cells into the 60% alcohol-soluble fraction and the effect of steroid (10 μg/ml of medium) on it were studied. After exposure of the cells to cortisol for 48 hr during culture, glycine uptake by the cells in 3, 5, 10 and 60 min of incubation in lactalbumin medium was depressed to 55 to 84% of the control. Larger depressing effect of cortisol on glycine uptake in 10 min of incubation was observed when the cells were incubated in Eagle's medium. Deoxycorticosterone also decreased glycine uptake to 55.6% of the control in 10 min of incubation in Eagle's medium. Cortisol decreased AIB uptake by 33 and 41%, and deoxycorticosterone by 9% (not significant) and 21% in 10 and 30 min of incubation, respectively. 17β-Estradiol and estriol did not significantly affect glycine uptake.

There is a considerable evidence that supports the direct effect of hormones on transport of amino acids across cell membrane or concentration ratios between intra- and extra-cellular amino acids at steady state in various tissues and cells. It has been found that growth hormone accelerates amino acid transport into rat tissues *in vivo*[4] and *in vitro*,[5] and there are many reports[6] on experiments which have demonstrated the aceclerating action of insulin on amino acid transport into rat diaphragm *in vitro*. Estradiol injected into rabbit increased amino acid concentration in the immature uterus.[4,7] It has been shown that the uterus of estradiol-primed rats takes up amino acids much more than that of control *in vitro*.[8] Thyrotropic hormone increases AIB transport into thyroid *in vitro*.[9] Amino acid concentration increased in the liver of rats,[4] and in muscle and plasma of mice[10] after cortisol injection. Another experiment, however, showed that free glycine concentration decreased in the skin and muscle of rats after a week of daily administration of cortisone.[11] These glucocorticoids injected into rats caused depression of amino acid accumulation in diphragm[12] and, on the other hand, acceleration of amino acid release from thymus[13] *in vitro*. Mohri reported previously that 48 hr or 72 hr of cortisol treatment depressed the steady state distribution ratios (ratio of intra- to extra-cellular concentration) of individual free amino acids in the cells of HeLa and other two culture cell lines.[14]

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2) Location: *1-33 YaYoi-Cho, Chiba.*
3) α-Aminoisobutyric acid.
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Established tissue culture cells are very valuable material for studies on amino acid accumulation and transport in mammalian cells and on direct hormonal effect on cellular functions, because a system which consists of one type of cells can be obtained, they give minimum errors in estimating cellular space, and can be exposed quantitatively to any water-soluble substances even for a long time without any influence from other cell systems.

In the present work, rate of amino acid uptake by HeLa cells into alcohol-soluble fraction and the effect of treatment with cortisol, deoxycorticosterone, 17β-estradiol, and estriol on this uptake were investigated.

Material and Method

1. **Steroids and Treatment**——All the steroids used were obtained from the Teikoku Hormone Mfg. Co., Ltd., Tokyo, in free forms. Each steroid dissolved in ethanol was added to culture medium at a concentration of 10 μg/ml (alcohol concentration was less than 0.1% in final medium) 48 hr before measuring the amino acid uptake.

2. **Labeled Amino Acids**——Glycine-1-^{14}C (5.0 mCi/mmole) was purchased from the Daiichi Pure Chemicals Co., Ltd., Tokyo, and AIB-1-^{14}C (4.0 mCi/mmole) from the New England Nuclear Corp., Mass., U.S.A.

3. **Media and Cell Culture**——The lactalbumin medium for cultivation and for some of measurements of amino acid uptake was composed of Hanks balanced salt solution[15] and 0.4% (w/v) of lactalbumin hydrolysate (the Nutritional Biochemical Corp., Ohio, U.S.A.), supplemented with 10% (for cultivation) or 5% (v/v) (for measurement of amino acid uptake) of inactivated bovine serum.

Eagle's basal medium[16] was substituted for lactalbumin medium in most of the measurements of amino acid uptake, supplemented with 5% (v/v) of bovine serum. All the components were sterilized and no antibiotics were added to the medium. Cells were grown without agitation in monolayers on a glass surface inside flattened 250 ml culture bottles. Each bottle contained 15 ml of cell suspension inoculated with 1×10^5 cells/ml, and kept at 38° until a confluent layer of cells was obtained after several renewals of the medium.

4. **Measurement of Amino Acid Uptake**——Bottles of the cells, some of which had been exposed to steroid, were pre-incubated at 38° in a test medium, but without radioactivity, for 40 min, and then, after discarding the used medium, incubated for terms as indicated in the text in a test medium with 0.02—0.1 μCi/ml of glycine-^{14}C or AIB-^{14}C. Two pairs of bottles of cells per group were rapidly chilled in an ice bath at a given time to stop the uptake of amino acid, and then rinsed gently twice, each time with 10 ml per bottle of Hanks balanced salt solution containing 0.5 mM of corresponding carrier amino acid. The cells were peeled off the glass surface by rubber-policeman into 60% (v/v) ethanol, and deep-frozen in centrifuging tubes. It was found that specific radioactivity of amino acid in the medium remained essentially constant throughout the incubation time examined here.

5. **Radioactivity and DNA Analysis**——After thawing and centrifuging, aliquots of the alcohol-soluble fractions of the cells were dried on planchets and their radioactivity was counted in a gas-flow counter. In some experiments, radioactivity of the alcohol-insoluble fractions was determined by the gas-flow counter. The correction of counts was made for self absorption when necessary. DNA was determined with perchloric acid extract of the alcohol-insoluble gractions by a slightly modified Dische method.[17]

Result

1. Effect of Cortisol on Glycine-^{14}C Uptake in the Lactalbumin Medium

The effect of cortisol treatment during cultivation and incubation on glycine uptake into the alcohol-soluble fractions was examined after 1, 3, 5, 10, and 60 min of incubation of the cells in the lactalbumin medium (Fig. 1). Radioactivity in the first minute of incubation was so low that it was not reliable. The values after 60 min might contain some activities of metabolites of glycine-^{14}C, because it was found by paper chromatographic analyses in pilot experiments that glycine-1-^{14}C taken up by HeLa cells from the lactalbumin medium was converted in about 15% into radioactive metabolites in 60 min of incubation. Throughout

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this period, except in 1 min of incubation, cortisol decreased glycine uptake to 55—84% of each control. The depressions at 3, 5, and 10 min were significantly large.

2. Time Courses of Glycine-^{14}C Uptake in Different Media

The uptake of glycine-^{14}C by non-treated HeLa cells was determined after 1, 3, 10, and 30 min of incubation with Eagle's and lactalbumin medium, and also after 60 min with the later (Fig. 2).

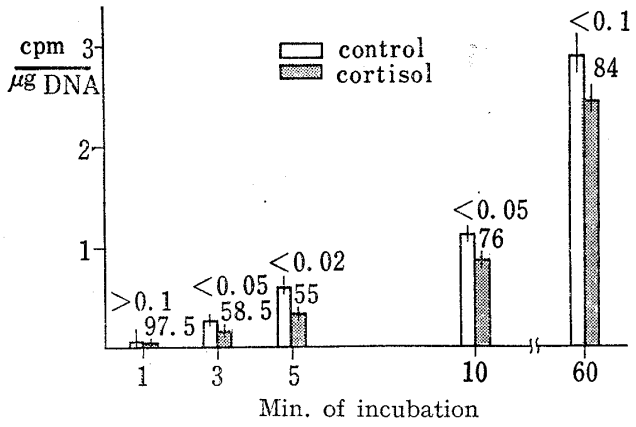

Fig. 1. Effect of Cortisol on Glycine-^{14}C Uptake into Alcohol-Soluble Fractions during Incubation in Lactalbumin Medium

Each column shows mean of uptake of radioactivity and ±S.E. of the mean determined with four fraction samples obtained from eight culture bottles of cells. Numbers shown above the columns indicated probability in t test and % uptake by treated cells compared to controls.

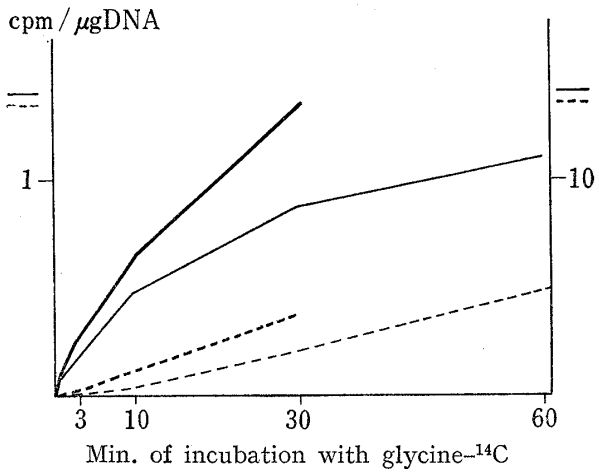

Fig. 2. Time Course of Glycine-^{14}C Uptake during Incubation in Lactalbumin and Eagle's Medium

The solid lines show count in alcohol-soluble fractions, and the broken lines that in alcohol-insoluble fractions. Radioactivity uptake was determined in 1, 3, 10, and 30 min of incubation in both media, and also in 60 min in lactalbumin medium. Each point shows average of uptake determined with four bottles of cells.
: lactalbumin medium, : Eagle's medium

Radioactivity of glycine added to Eagle's medium was about four times that added to lactalbumin medium. Nevertheless, the uptake of radioactivity into the alcohol-soluble and-insoluble fraction of HeLa cells incubated in Eagle's medium was always more than 10 times that of the cells incubated in lactalbumin medium. In both media, rate of radioactivity uptake into the soluble fractions decreased with incubation time, whereas that into the insoluble fractions remained constant even after 10 min of incubation. Ascending paper chromatography (with solvent systems of butanol-acetic acid and phenol-ammonia) of the soluble fractions obtained from the cells incubated with glycine-^{14}C in Eagle's medium showed that one radioactive spot corresponding to glycine could account for more than 90% of the total radioactivity in 10 min of labeling, but less than 40% in 30 min.

3. Comparison of Cortisol Effect on Glycine-^{14}C Uptake between the Incubation Media

The decreasing effect of cortisol on glycine uptake was demonstrated again by replacing lactalbumin medium with Eagle's medium in incubation with glycine-^{14}C (Table I). The depression was as much as 34% of the control in Eagle's medium in 10 min of incubation compared with 26% in lactalbumin medium.

4. Effect of Deoxycorticosterone on Glycine-^{14}C Uptake

HeLa cells were exposed to deoxycorticosterone for 48 hr during cultivation, and then glycine uptake by the cells was measured in Eagle's medium (Table II). Radioactivity uptake was depressed by the steroid to 55 and 58% of each control in 10 and 30 min of incubation, respectively. Radioactivity in the alcohol-soluble fractions is probably contaminated

Table I. Comparison of Cortisol Effect on Glycine-^{14}C Uptake into the Alcohol-Soluble Fractions between Incubation Media

Media	Control	Cortisol	p
Eagle	5.21±0.35	3.46±0.20	<0.01
Lactalbumin	1.42±0.07	1.20±0.05	<0.05

Each value indicates mean of uptake of radioactivity in cpm/μg DNA and±S.E. of the mean. The uptake was determined with four bottles of cells per group incubated for 10 min with glycine-^{14}C after 48 hr of cultivation with or without cortisol (10μg/ml).

with that of glycine metabolites in 30 min of labeling in Eagle's medium as mentioned earlier.

Table II. Effect of Deoxycorticosterone on Glycine-^{14}C Uptake into the Alcohol-Soluble Fractions in Eagle's Medium

	Min of incubation	
	10	30
Control	6.12±0.40	12.04±0.78
Deoxycorticosterone	3.41±0.17	6.99±0.59
p	<0.01	<0.01

Expression of the values is same as in Table I. The uptake was determined with four bottles of cells per group after 48 hr of cultivation with or without deoxycorticosterone (10 μg/ml).

5. Effect of Cortisol and Deoxycorticosterone on AIB-^{14}C Uptake

Cortisol and deoxycorticosterone were tested for their effect on AIB-^{14}C uptake by HeLa cells during incubation in Eagle's medium after 48 hr of treatment (Table III). Cortisol decreased the uptake by 33 and 41% of each control in 10 and 30%min of incubzation, repectively. The decreasing effect of deoxycorticosterone on the uptake was not evident in 18 min of incubation, but significantly large (by 21% of control) in 30 min.

Table III. Effect of Cortisol and Deoxycorticosterone on AIB-^{14}C Uptake in Eagle's Medium

	Min of incubation	Control	Treated	p
Cortisol	10	2.30±0.17	1.54±0.06	<0.01
	30	3.43±0.19	2.02±0.24	<0.01
Deoxycorticosterone	10	2.16±0.09	1.97±0.09	>0.1
	30	3.16±0.14	2.65±0.1	<0.05

Expression of the values is same as in Table I. The uptake was determined with four bottles of cells per group after 48 hr of cultivation with or without steroids shown.

6. Effect of 17β-Estradiol and Estriol on Glycine-^{14}C Uptake

The effect of treatment with one of these estrogens for 48 hr on glycine uptake during incubation was examined with Eagle's medium (Table IV). The results show that these two estrogens have a slight depressing effect on glycine uptake by HeLa cells, although the effect was not significantly large, so far as examined. The depression by estriol always appeared to be larger than that by estradiol.

Table IV. Absence of Effect of 17β-Estradiol and Estriol on Glycine-^{14}C Uptake into the Alcohol-Soluble Fractions in Eagle's Medium

	Min of incubation	Control	Treated	
17β-Estradiol	10	1.21±0.05	1.10±0.05	n.s.[a]
	30	2.19±0.13	1.95±0.12	n.s.
Estroil	10	1.07±0.06	0.93±0.05	n.s.
	30	2.42±0.16	2.01±0.18	n.s.

Expression of the values is same as in Table I. The uptake was determined with four bottles of cells per group after 48 hr of cultivation with or without steroids shown.
a) No significance between two averages of control and treatment.

Discussion

Lactalbumin hydrolysate–Hanks medium and Eagle's mediun, both supplemented with human or bovine seum, are usually used in the cultivation of HeLa cells. Lactalbumin medium was used in the present work both as growth and test medium, and it was compared with Eagle's medium in measuring amino acid uptake by HeLa cells. Eagle's medium was proved to be preferable to lactalbumin medium for efficient uptake of labled glycine (Table I). The difference in glycine-^{14}C uptake between these two media is probably due to the difference of glycine concentrations in the media, *i.e.*, only a small concentration of glycine originating from the serum is expected to be in Eagle's medium, while the concentration may be as much as 0.4 mM in lactalbumin medium.[18] Saturation mechanism can prevent proportional uptake of amino acid by the cells in such a wide range of concentration of the amino acid in the medium. It is also possible that alanine (about 1.3 mM) and other competitive amino acids inhibit glycine uptake in lactalbumin medium.[19]

Both cortisol and deoxycorticosterone treatment decreased glycine and AIB uptake by HeLa cells in a short period of incubation. The effect of cortisol on amino acid uptake by HeLa cells is considered to be consistent with the observation in isolated diaphragm obtained from cortison-treated rats.[12] It was demonstrated by Kostyo[20] that cortisol and deoxycorticosterone depressed AIB uptake of isolated diaphragm obtained from normal rats during 4 hr of incubation in concentrations of 10^{-7} to 10^{-5}M. From the apparent effect of these steroids on the short-term uptake of amino acids by HeLa cells demonstrated in the present work, it would be reasonable to admit that the depression of amino acid uptake by corticoids is due to direct impairment of the entry of amino acids across the cell membrane.

HeLa cells were originally derived from human uterus, but two estrogens, estriol and 17β-estradiol, neither stimulated nor significantly depressed glycine uptake of the cells (Table IV). It was shown by Endo, *et al.*[21] that cortisol inhibited HeLa cell growth in 0.01 μg/ml and more, and estradiol in 10 μg/ml. Estriol, on the other hand, stimulated the cell growth in concentrations from 0.01 to 10 μg/ml in four days of treatment. Therefore, effect of the steroids on HeLa cell growth and on amino acid uptake was not found to be completely parallel. The effect of cortisol and deoxycorticosterone on amino acid uptake observed in the present experiments may not be assumed so far to be a physiological action of the steroids in view of their excessive concentration in the medium as compared to their circulating concentration in normal human being. Restriction of the activity to these adrenocortical steroids, however, would suggest the specificity of their chemical structures in interaction with transport mechanism for amino acids of cells.

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