Vol. 97, No. 2, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 28, 1980 Pages 642-648

THE EFFECT OF REVERSE TRANSFORMATION AGENTS ON α-AMINOISOBUTYRIC ACID UPTAKE IN TRANSFORMED AND NON-TRANSFORMED CELLS[1]

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Received October 14,1980

SUMMARY: It has been reported that dibutyryl cyclic AMP plus testololactone, agents which restore normal morphological properties and contact inhibition of movement to transformed cells, stimulate the net uptake of α-aminoisobutyric acid. In those experiments, cells were pre-incubated with the agents for 90 min; using similar conditions we have confirmed this observation and have shown that the effect is accounted for by a 2.8-fold increase in the V_{MAX} of influx with no change in K_M. We have also shown that these "reverse transformation agents" cause an increase in the net uptake of α-aminoisobutyric acid in non-transformed cells as well as in transformed cells.

In 1971, two different laboratories reported that the treatment of transformed cells with cAMP[2] or certain of its derivatives causes them to regain the morphological characteristics of normal cells: e.g., the treated cells have fewer knobs (blebs) on their surface, become more spindle-shaped, adhere more tightly to the substratum, and exhibit contact-inhibition of movement (1,2). These changes, which have been attributed to effects of cAMP on the microtubule-microfilament system (3), have been called "reverse transformation". The effect of dibutyryl cAMP on transformed Chinese hamster ovary (CHO) cells is potentiated by testosterone or testololactone (1,3).

Recently, Puck reported a 2.3-fold increase in the steady-state internal concentration of the amino acid analogue, α-aminoisobutyric

1. A preliminary report of this work was presented at the 71st Annual Meeting of the American Society of Biological Chemists, in New Orleans, La., in abstract (14).

2. Abbreviations: cAMP, cyclic adenosinemonophosphate; AIB, α-aminoisobutyric acid; MeAIB, N-methylaminoisobutyric acid; CHO, Chinese hamster ovary; RTA, reverse transformation agents.

0006-291X/80/220642-07$01.00/0

acid (AIB), when transformed CHO cells were incubated with the radioactive substrate in the presence of dibutyryl cAMP plus testololactone, following a 90-min pre-incubation with those agents (3). This increase in steady state level could be due to either a stimulation of influx or an inhibition of efflux; the experiments reported here were designed to resolve this question, as well as to determine which kinetic parameters of transport are altered by 90-min pre-treatment of cells with reverse transformation agents (RTA).

MATERIALS AND METHODS

Cells and Growth Media. Experiments were performed with cells of a transformed mouse fibroblast line, SVT2, and a non-transformed line, BALB/C 3T3, both gifts of Dr. Ruth Sager. The cells were maintained in Falcon flasks at 37°C, 5% CO_2 in the α modification of minimal essential medium (α-MEM) with Earl's salts (Flow catalogue #10-311).

Preincubation Media. The basic medium (TRANS-EBSS) was patterned after Earl's balanced salt solution, 300 mOsm/kg, as previously described (4). For RTA experiments, 8 mM dibutyryl cAMP and 240 μM testololactone were added to this medium.

Transport Measurements. Logarithmic phase cells were collected by brief exposure to 0.25% trypsin, washed twice, and plated on glass coverslips in α-MEM, 10% dialysed fetal calf serum, with antibiotics, (penicillin G, streptomycin, kanamycin), at 5 x 10^4 cells per coverslip, after the method of Roth (5). After 36 hr appropriate batches of coverslips were preincubated for 90 min in either TRANS-EBSS or TRANS-EBSS with RTA. Initial rates of uptake were measured at 37°C in 0.1, 0.5, 1.0, 2.0, 5.0, and 10.0 mM [^{3}H]AIB (20 μCi/ml) in TRANS-EBSS. For each run, triplicate coverslips with attached cells were first rinsed in TRANS-EBSS and then immersed in the appropriate [^{3}H]AIB solution for either 20, 40, 60 or 80 sec. The transport measurement was stopped by serial washes in 4°C TRANS-EBSS and the coverslips were placed directly in liquid scintillation vials containing 1 ml 12% perchloric acid. Extracellular water was determined using separate coverslips and [^{3}H] inulin. After incubating at room temperature for at least one min, 9 ml of an aqueous counting cocktail (Formula 963, New England Nuclear) were added and the vials were mixed and counted 10 min in a Beckman LS 250 or 335 counter. Protein per coverslip was determined by the method of Lowry et al (6).

Calculations. Data values for each time point are the average of triplicate measurements. In experiments measuring initial rates, uptake was linear over the indicated time interval. Initial rates of uptake were calculated from the slopes of these lines and were used to determine kinetic parameters by a computer fit using the Marquardt algorithm (7), a general nonlinear curve-fitting procedure (IBM SHARE No. 3094).

Materials. Testololactone (1,2-dehydrotestololactone) was the generous gift of Squibb Pharmaceuticals (SQ 9538). Dibutyryl cAMP was purchased from Sigma Chemical Corporation, and used without ether extraction of butyrate.

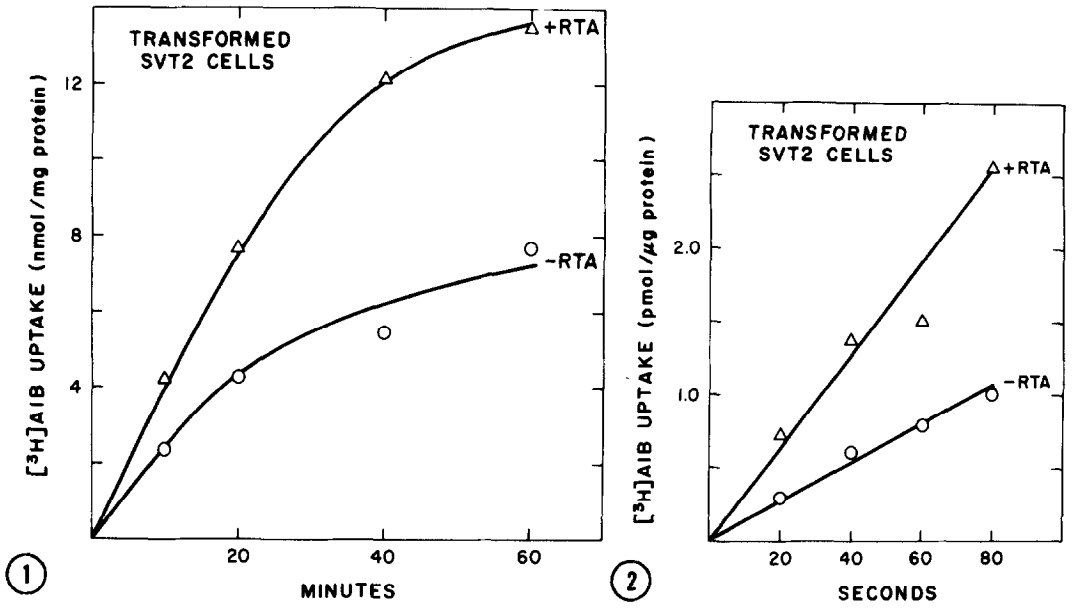

Fig. 1. Long term time course of uptake of 0.5 mM [^{3}H]AIB into transformed SVT2 cells. Cells were incubated in the presence or absence of RTA as described in "Materials and Methods".

Fig. 2. Short term time course of uptake of 0.5 mM [^{3}H]AIB into transformed SVT2 cells. Cells were incubated in the presence or absence of RTA as described in "Materials and Methods".

RESULTS

First, we asked if another transformed cell line besides CHO-K1 exhibits an increased steady-state level of AIB after pre-incubation with the RTA, dibutyryl cAMP and testololactone. The uptake of 0.5 mM AIB was measured over a one-hour time period using SVT2 cells. Pre-incubation of the cells for 90 min did enhance the accumulation of AIB by about 2-fold (Fig. 1). A control experiment indicated that RTA had no effect on AIB uptake when present only in the assay mixture, without prior pre-incubation of the cells with these agents.

The increase in accumulation of AIB could be due to an increased influx or a decreased efflux of AIB. To determine if the enhancement was due to an increased influx, we measured the initial uptake rate of AIB over an 80-second time period. Fig. 2 illustrates the uptake of 0.5

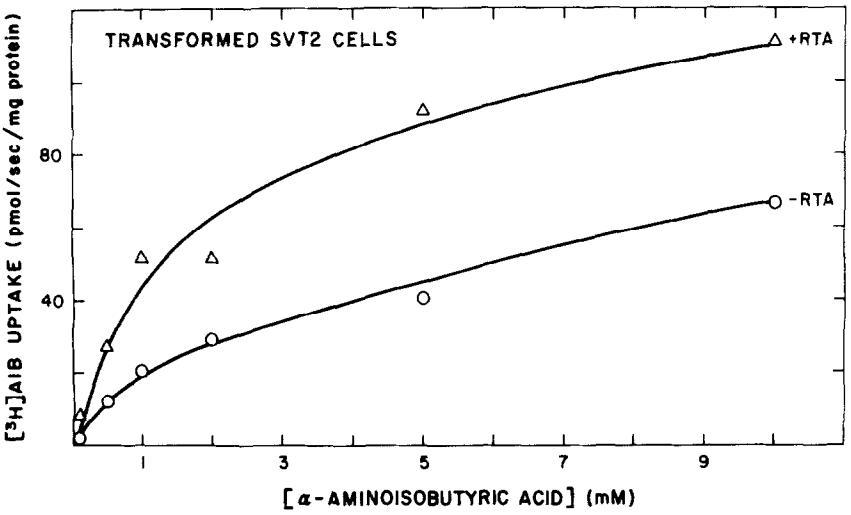

Fig. 3. Uptake rates of [^{3}H]AIB into transformed SVT2 cells. Cells were incubated in the presence or absence of RTA as described in "Materials and Methods". The curves represent the least squares computer fit of the data over the concentration range 0.1 to 10.0 mM according to the following equation:

$$v = \frac{V_{MAX}[S]}{K_M + [S]} + K_D[S]$$

Kinetic parameters are presented in Table 1.

mM AIB with and without pre-incubation of the cells with RTA. The initial uptake rates were 28.6 and 12.0 pmol/sec/mg protein, respectively, with and without pre-treatment. This represents a 2.3-fold increase in uptake rate, consistent with the 2-fold enhancement in steady-state levels seen in Fig. 1.

The increase in initial uptake rate could be due to an increase in the affinity of the transport protein for AIB (decrease in K_M) or an increase in the number of transport proteins or their turnover rate (increase in V_{MAX}). In order to determine the kinetic parameters, the initial rate of uptake was measured over a wide concentration range (0.1 to 10 mM) with and without pre-incubation of the cells with RTA (Fig. 3). The kinetic parameters are given in Table 1. In both cases, uptake was found to be due to a single transport system plus diffusion. As

TABLE I

Effect of RTA on the Kinetic Parameters of AIB uptake in SVT2 cells[a]

Condition	V_{MAX}	K_M	K_D
	(pmol/sec/mg)	(mM)	(pmol/sec/mg/mM)
INDIVIDUAL FITS			
Without pre-treatment	25.58 ± 5.86	0.67 ± 0.34	4.14 ± 0.58
With pre-treatment	78.77 ± 25.36	0.94 ± 0.59	4.12 ± 2.29
JOINT FITS[b]			
Common V_{MAX}			
Without pre-treatment		8.95 ± 1.71	
With pre-treatment	125.79 ± 32.51	1.92 ± 0.79	0.22 ± 2.13
Common K_M			
Without pre-treatment	28.93 ± 7.96		
With pre-treatment	80.40 ± 11.21	0.95 ± 0.30	3.92 ± 0.88

[a] Data were fitted by computer to one transport system and a diffusion term (see "Materials and Methods").

[b] In the joint fits, one kinetic parameter (either the K_M or the V_{MAX}) and the diffusion term were held in common.

indicated by the individual fits, pre-incubation of the cells with RTA had little effect on the diffusion constant (K_D) or on the K_M. When the data were jointly fitted, a very good fit was obtained when experimental conditions were assumed to have altered only the V_{MAX} (bottom, Table 1). We conclude that the enhancement in initial uptake rate after pre-incubation with RTA is due to a 2.8-fold increase in V_{MAX} (from 28.93 to 80.40 pmol/sec/mg protein) with no effect on K_M (0.95 mM) or on the diffusion constant (3.92 pmol/sec/mg protein/mM).

Finally, we were interested in determining whether a non-transformed cell, pretreated with RTA, would also exhibit a stimulation of transport. Cells of the non-transformed, genetically-similiar BALB/C 3T3 cell line were pre-incubated with RTA using identical conditions to those above. As shown in Fig. 4, these cells also exhibited a significant increase (1.4-fold) in the steady-state level of AIB after incubation.

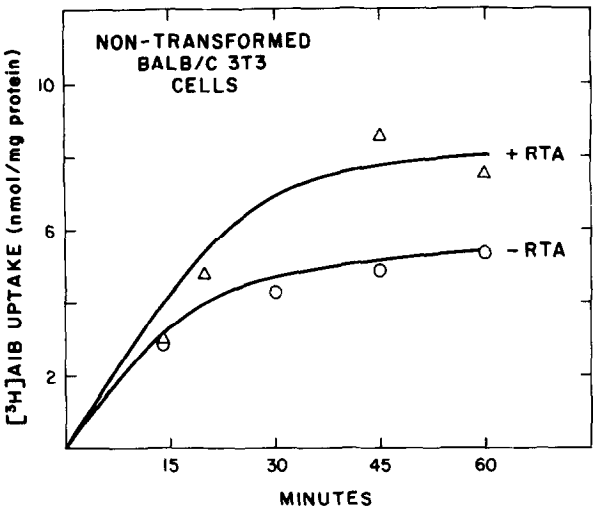

Fig. 4. Long term time course of uptake of 0.5 mM [^{3}H]AIB into non-transformed BALB/C 3T3 cells. Cells were incubated in the presence or absence of RTA as described in "Materials and Methods".

DISCUSSION

Studies on the effects of cAMP or dibutyryl cAMP (RTA) on transport have yielded what appear to be conflicting results. Some studies on amino acid transport for example, report an increase in uptake (3,8,9, and present paper) and others a decrease (9,10,11,12). This variability, however, can undoubtedly be attributed to differences in cell type, in the parameters measured (e.g., initial rates of uptake versus steady-state levels), in experimental conditions (e.g., short-term versus long-term pre-incubation with RTA), and in the transport systems affected.

Previous papers describing effects on AIB transport of short-term pre-incubation with cAMP have reported either steady-state levels of AIB (3), or uptake rates that were not linear during the measurement period (9). In the present studies we have carried out a kinetic analysis of unidirectional AIB influx over a series of AIB concentrations, and have shown that the stimulating effect of dibutyryl cAMP plus testololactone is accounted for by a 2.8-fold increase in the V_{MAX} of influx.

Since non-transformed cells generally exhibit a reduced transport activity when compared to transformed cells (13), the restoration of the normal phenotype by RTA would be expected to reduce the uptake of small nutrient molecules. Thus, the stimulation of amino acid transport seen in some experiments (including our own) is incompatible with reverse transformation, and is presumably a second, unrelated effect of RTA. Consistent with this view is the finding that RTA stimulate the uptake of AIB in non-transformed as well as in transformed cells (8, and the present paper).

Acknowledgements

This work was supported by Grant PCM76-02818 from the National Science Foundation, and a National Institutes of Health Postdoctoral Fellowship 5F32GM06197-02 to A.H. Dantzig.

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