Vol. 93, No. 2, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 28, 1980 Pages 392-398

ACTIVATING THE Na-K PUMP WITH MONENSIN INCREASES AMINOISOBUTYRIC ACID UPTAKE BY MOUSE FIBROBLASTS

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Received January 29,1980

SUMMARY

Monensin rapidly tripled the initial rate and extent of α-aminoisobutyric acid accumulation by Swiss 3T3 cells. This ionophore catalyzes the electroneutral exchange of external Na for cellular protons and stimulates the Na-K pump by suppling it with more Na. The stimulation of the Na-K pump and α-aminoisobutyric acid uptake exhibited a similar dependence on monensin concentration. Ouabain prevented monensin from increasing α-aminoisobutyric acid transport. Aminoisobutryic acid transport was more than doubled at low doses of monensin that activated the Na-K pump by elevating cell Na without significantly changing cell K. The rapid activation of α-aminoisobutyric acid transport is probably due to the hyperpolarizing effect of stimulating the electrogenic Na-K pump. The stimulation of the Na-K pump is quiescent fibroblasts by serum or growth factors may be sufficient to activate the Na-dependent amino acid transport systems.

During the last decade numerous investigations have considered the possible relationship between amino acid transport and the control of cell growth and transformation. In general, it has been observed that when cells leave the quiescent (G_1/G_o) phase of the cell cycle, an increase in the transport of certain amino acids occurs (for reviews see Refs. 1 and 2). For example, the transport of AIB[3], (which is a non-metabolizable substrate for the Na-dependent 'A' system (3)), is substantially slower in either confluent 3T3 cells (4), hyperconfluent or serum-deprived, quiescent chick embryo cells (5), quiesient BHK (6) or human diploid fibroblasts (7,8) than in the exponentially growing, non-transformed fibroblasts.

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[3]The abbreviation used is: AIB, α-aminoisobutryic acid.

0006-291X/80/060392-07$01.00/0

Although the control mechanism has not been elucidated, the magnitudes of the transmembrane Na gradient and electrical potential are known to have an important influence on AIB transport (12-15). Moreover, Villereal and Cook (10) recently presented evidence that the higher AIB accumulating capacity of growing cells is due to a serum-associated membrane hyperpolarization. Hence, by adjusting the concentration of external K in the presence of valinomycin the steady-state level of AIB accumulation and apparent membrane potential of growing cells was matched to that of quiescent ones and vice versa (10). Valinomycin is an electrogenic ionophore which is highly specific for K and each complex (Val-K) carries a single positive charge across a lipid barrier and down the gradient of K concentration (17).

Here we have used the Na-selective ionophore, monensin, to investigate the control of amino acid transport by Swiss 3T3 cells. Monensin is an electroneutral ionophore which prefers to exchange cell Na for protons, the complex with the cation bearing no net charge (17). Previously (19), monensin was shown to elicit some of the early, protein synthesis independent alterations in membrane function that occur soon after quiescent cells are re-fed serum or certain purified growth factors. Monensin, like serum and certain peptide growth factors (e.g., thrombin, or vasopressin) increases the 3T3 cell's permeability to Na ions, which stimulates the Na-K pump in the cell membrane (18,19,25). We now show that monensin markedly increases the rate and extent of AIB accumulation by quiescent 3T3 cells. Ouabain, which inhibits the Na-K ATPase, prevented monensin from stimulating AIB transport. These observations help to ascertain the causal relationships between certain early membrane events and further suggest that a primary effect of certain growth factors is to modulate the cell's permeability to Na.

MATERIALS AND METHODS

Cells - Swiss 3T3 cells (20) were obtained from the American Type Culture Collection and propagated in Dulbecco's Modified Eagle's Medium containing 10% calf serum as previously described (21). After seeding

the fibroblasts grew to confluency in 60mm dishes (Falcon) and became arrested in the G_1 phase of the cell cycle. No mycoplasmal contamination was detected in the cultures.

AIB Uptake - Quiescent cultures were washed once with uptake medium at 37°. The uptake medium contained 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 20 mM Hepes buffer adjusted to pH 7.2 with Tris. Then 2.0 ml of uptake medium plus 0.2 mM AIB with or without monensin and/or 1 mM ouabain was added. After a 5 min incubation at 37°, about 0.5 µCi of [^{14}C]-AIB (New England Nuclear) was added and the incubation continued. After 10 min, uptake was stopped by rapidly washing the cells 6-times with 3 to 5 ml of 100 mM $MgCl_2$ (adjusted to pH 7.5 with Tris base) at 4°. The last volume of the final wash was removed by aspiration after a few min of draining the dishes at an angle. Finally the cells were extracted with 1.0 ml of 0.1 N NaOH for 20 min at room temperature and the amount of radioactivity in an 0.8 ml sample of the extract was measured by liquid scintillation counting with 9 ml of Liquiscent (Beckman) and 0.2 ml 1N trichloroacetic acid. Monensin (10 mg/ml) was dissolved in ethanol and added to the uptake medium. The ethanol concentration in the uptake medium was 0.2% or less and had no significant effect on AIB uptake.

^{86}Rb Uptake and Total Cell Na and K. These were measured as previously described (18,19) except 60 instead of 90 mm cultures were used. Cell number was determined with a Coulter Counter and protein by the method of Lowry et al. (22) using bovine serum albumin as a standard.

RESULTS

Figure 1 shows the effects of monensin and ouabain on ^{14}C - AIB uptake by Swiss 3T3 cells. Monensin (15 µM) increased both the initial rate and steady-state level of AIB accumulation by more than 3-fold. The stimulation of AIB uptake was rapid since the maximal effect was observed

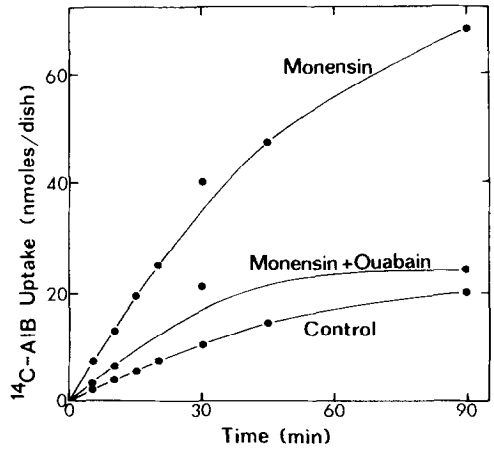

Figure 1. Effects of monensin and ouabain on AIB transport by Swiss 3T3 cells. Monensin and/or ouabain were present as indicated at 15 µM and 1 mM, respectively. Each culture dish contained approximately 2 x 10^6 cells.

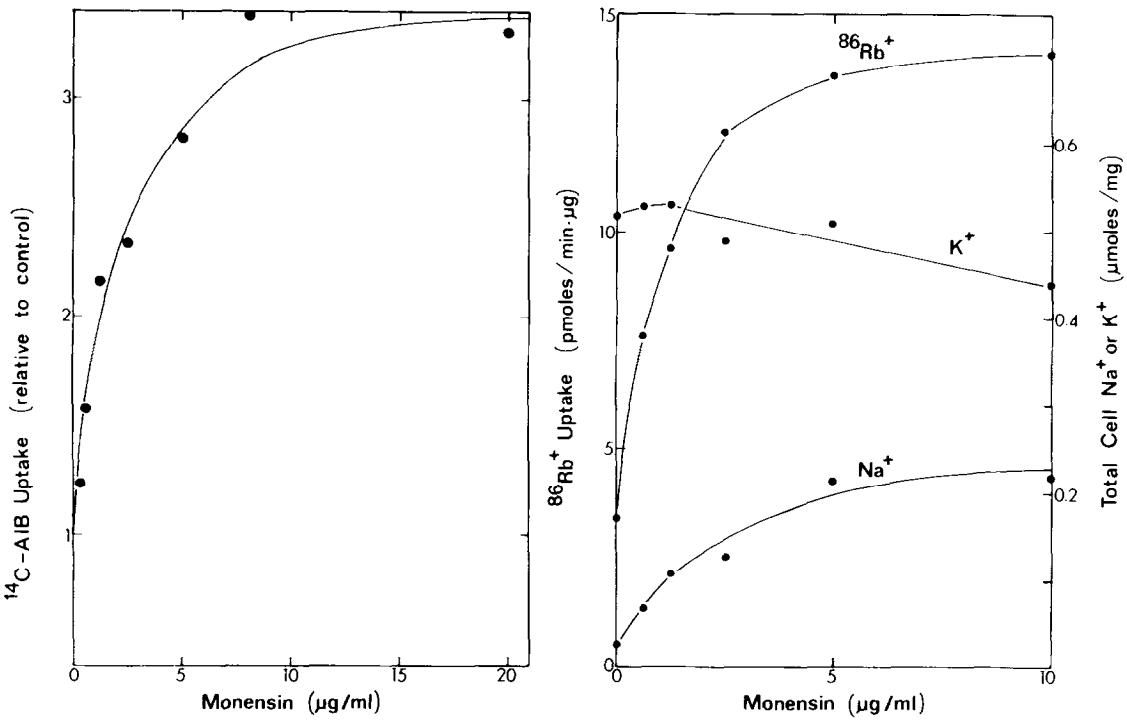

Figure 2. Effect of varying the concentration of monensin on the initial rate of AIB and ^{86}Rb uptake and total cell Na and K. ^{86}Rb uptake and total cell Na and K were measured on the same culture dish, and AIB uptake was assayed on separate cultures of Swiss 3T3 cells (2 x 10^6/dish). There was a 5 min incubation of 37° in the AIB uptake medium (see Materials and Methods') with the indicated concentration of monensin. Then the isotope (^{14}C-AIB or ^{86}Rb) was added and the incubation continued for 10 min.

within 5 min after adding monensin. The apparent concentration ratio (AIB inside/AIB outside) after 90 min incubation with monensin was about 100 compared to 30 in the absence of monensin (assuming 4 μl cell H_2O/mg protein). Ouabain, which had no significant effect on AIB uptake in the absence of monensin, essentially prevented monensin from stimulating AIB uptake. Therefore, the stimulation of AIB uptake probably results from the stimulation of the Na-K pump by monensin.

Maximal stimulation was achieved by 1 μg/ml monensin (Fig. 2). The stimulation of the Na-K pump (^{86}RB uptake) exhibits a similar dependence on the concentration of monensin as the stimulation of AIB uptake and probably results from the elevation of cell Na (righthand panel of Fig. 2 and

ref. 19). Obviously an increase in cell Na would decrease the Na gradient. Hence, the stimulation AIB uptake by monensin is not due to an increase in the Na gradient.

It is noteworthy that the rate of AIB uptake was significantly faster when total cell Na was increased and K was unchanged or perhaps slightly increased (Fig. 2). Thus, the assumed hyperpolarizing effect of monensin does not result from a net loss of Na or K from the cell. Therefore, it is likely that a net difference in some other cellular cation or anion, brought about by the stimulation of the Na-K pump, is responsible for the hyperpolarizing effect of monensin.

DISCUSSION

Monensin is known to catalyze an electroneutral exchange of Na ions for protons (17) and therefore would not directly change the cell membrane potential. However, monensin markedly stimulates the Na-K pump in animal cells (19) which hyperpolarizes the cell membrane (23) since the pump is electrogenic (e.g. the stoichiometry of the pump in red blood cells in 3 Na per 2 K) (24). It appears that the stimulation of AIB uptake by monensin results from the hyperpolarization of the 3T3 cells for the following reasons: (1) the transport of AIB and other substrates of the Na-dependent 'A' system depends in part on the transmembrane electrical potential (10, 13-16); (2) the magnitude of the Na gradient, which is also known to energize the AIB transport (12), is decreased by monensin since it elevates cell Na (Fig. 2 and ref. 19); (3) monensin hyperpolarizes neuroblastoma cells and chick embryo fibroblasts by stimulating the Na-K pump (21); (4) the stimulation of the Na-K pump and AIB uptake exhibited a similar dependence on monensin concentration (Fig. 2) and (5) the stimulation of AIB uptake by monensin was blocked by ouabain which specifically inhibits the Na-K pump (Fig. 1).

Clearly the modulatory effects of the two ionophores, valinomycin (8,10,13) and monensin, on AIB transport occur by different mechanisms.

Valinomycin stimulated AIB uptake in quiescent human fibroblasts incubated in the presence of a reduced level of external Na (50 mM) and normal K (6 mM) (8,10). We have confirmed this observation using 3T3 cells and found that ouabain did not alter the valinomycin effect on AIB uptake (unpublished results). Valinomycin apparently converts the cell membrane into a K ion electrode which allows one to artificially adjust the membrane potential and AIB accumulation by changing the concentration of external K (8). On the other hand, the stimulation of AIB uptake by monensin is prevented by ouabain (Fig. 1) indicating that the monensin effect is mediated by the Na-K pump. Ultimately both ionophores appear to alter amino acid transport by modifying the membrane potential of the cell although by different mechanisms.

Mechanistically, monensin seems to partially mimic the action of serum or certain peptide growth factors or tumor promoters on quiescent fibroblasts. Thus serum (19) or thrombin (18) rapidly increase the activity of NKA in quiescent 3T3 cells apparently by increasing the cell's permeability to Na which supplies the Na-K pump with more of its limiting substrate. Vasopressin and the potent tumor promoter, tetradecanoyl phorbol acetate, which are potent comutogens in 3T3 cells, appear to share a common mechanism of action (25,26), perhaps involving an influence on Na permeability.

ACKNOWLEDGEMENTS

We thank Professor Leon A. Heppel for advice and encouragement, Linda Youngman and Diana Wescott for the preparation of cell cultures, and Kim Muzzy for typing the manuscript.

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