Table 3 Inhibition of ^{3}H-leucine sequestration by other amino acids and calcium

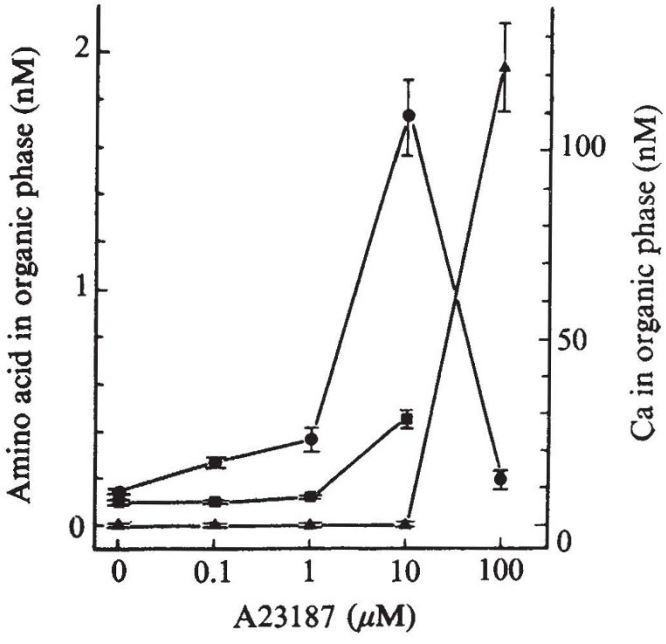
Composition of the initial water phase	Amount of ^{3}H-leucine in the organic phase after extraction c.p.m.	μM (mean)	% Relative inhibition (mean)
^{3}H-leucine (50 μM)+A23187 (5 μM)	7,102±53	0.890	—
^{3}H-leucine (50 μM)+A23187 (5 μM)			
+L-leucine (50 μM)	5,678±322	0.710	100
+L-glycine (50 μM)	5,723±293	0.715	97
+L-lysine (50 μM)	5,832±122	0.725	89
+L-arginine (50 μM)	6,025±585	0.750	79
+L-aspartic acid (50 μM)	6,125±535	0.765	69
+L-glutamic acid (50 μM)	6,805±545	0.850	21
+β alanine (50 μM)	7,343±42	0.915	0
+ε amino caproic acid (50 μM)	7,458±178	0.930	0
+$CaCl_2$ (50 μM)	6,730±320	0.840	26

^{3}H-leucine (1 μCi) and the additions as indicated were extracted with 1 ml toluene–butanol mixture as described in Table 1. Relative inhibition of ^{3}H-sequestration by the added compounds is calculated from the c.p.m., taking the inhibition caused by 50 μM L-leucine as 100%. Mean and range of the results from two separate extractions are given.

A23187 to sequester leucine, but it could be explained by assuming that in an aqueous medium the highly hydrophobic A23187 can acquire two different micellar forms, depending on concentration. At lower concentrations of A23187 a form with a relatively high affinity to leucine is prevalent, whereas at higher concentrations the ionophore exists mainly in another form, which can bind calcium but not leucine. The final answer to this question requires further study.

We also found that leucine is not the only amino acid with which A23187 can interact. Whereas the partitioning of ^{3}H-uridine and ^{3}H-thymidine remained practically unaffected at 0.1 to 10 μM concentrations of the ionophore (data not shown) ^{35}S-methionine was effectively transferred to the organic phase by 10 μM A23187 (Fig. 1). Furthermore, several other α amino acids were able to inhibit organic sequestration of ^{3}H-leucine. They showed increasing relative affinity for the ionophore in the following order: Glu ≪ Asp < Arg < Lys < Gly < Leu (Table 3). Other experiments have shown that cysteine and glutamine can also inhibit sequestration of ^{3}H-leucine. Non-α amino acids tested, β alanine and ε amino caproic acid, did not show any affinity for A23187 in this system (Table 3). Again, compared with cold leucine, $CaCl_2$ was less effective in inhibiting ^{3}H-leucine sequestration (Table 3).

Fig. 1 Dose dependence of sequestration of ^{3}H-leucine, ^{35}S-methionine and ^{45}Ca into organic phase by A23187. Radioisotope (1 μCi) and indicated concentrations of the ionophore in 1 ml PBS were mixed with 1 ml toluene–butanol (1:1 v/v) mixture and shaken vigorously for 1 min at room temperature. Initial concentrations in the water phase were: ^{3}H-leucine, 18.5 nM; ^{35}S-methionine, 8.0 nM; $^{45}CaCl_2$, 800 nM. For other experimental details see Table 1. Mean ± s.d. of two to four experiments are given. ●, Leucine; ■, methionine; ▲, calcium.



The relevance of these findings to the effects of A23187 on cellular physiology is difficult to assess. Although we detected EGTA-resistant induction of an enhanced rate of leucine uptake within a few hours in lymphocyte cultures incubated with the ionophore, our attempts to show direct facilitation of leucine uptake in the presence of A23187 have so far been unsuccessful. Nevertheless, the findings reported in this paper indicate that the ionophore A23187 can cause reversible organic sequestration of amino acids at concentrations as low as, or lower, than those needed to form lipid-soluble complexes with calcium, and that equimolar concentration of calcium can only slightly inhibit this complex formation. This means that apart from the well documented action of A23187 as a potent divalent cation ionophore it may have effects on cellular metabolism resulting from its affinity for amino acids. One other implication of these findings is that when A23187 is used in complex experimental conditions, caution is necessary before concluding that all the phenomena detected are simply caused by increased permeability of membranes to divalent cations.

T.H. was supported by EMBO and by the Sigrid Juselius Foundation, Helsinki, Finland. A23187 was a gift from Dr O. K. Behrens (Eli Lilly).

T. Hovi
S. C. Williams
A. C. Allison

Clinical Research Centre,
Watford Road,
Harrow HA1 3UJ, Middlesex, UK

Received January 15; accepted May 2, 1975.

1 Reed, P. W., *Fedn. Proc.*, **31**, 432 (1972).
2 Reed, P. W., and Lardy, H. A., *J. biol. Chem.*, **247**, 6970 (1972).
3 Foreman, J. C., Mongar, J. L., and Gomperts, B. D., *Nature*, **245**, 249 (1973).
4 Steinhard, R. A., and Epel, D., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1915 (1974).
5 Maino, V. C., Green, N. M., and Crumpton, M. J., *Nature*, **251**, 324 (1974).
6 Entman, M. L., Gillette, P. C., Wallick, E. T., Pressmann, B. C., and Schwartz, A., *Biochem. biophys. Res. Commun.*, **48**, 847 (1972).
7 Pressmann, B. C., and de Guzman, N. T., *Ann. N.Y. Acad. Sci.*, **227**, 380 (1974).
8 Pressmann, B. C., *Fedn. Proc.*, **32**, 1968 (1973).
9 Van den Berg, K. J., and Betel, I., *Expl Cell. Res.*, **66**, 257 (1971).
10 Pfeiffer, D. R., Reed, P. W., and Lardy, H. A., *Biochemistry*, **13**, 4007 (1974).

Corrigendum

In the article "The function of phytochrome in plants growing in the natural environment" by M. G. Holmes and H. Smith (*Nature*, **254**, 512; 1975) the two arable weeds used were *Tripleurospermum martimum inodorum* (scentless mayweed) and *Chenopodium album* (fat-hen).