BIOCHEMICAL LETTERS

ATP and the binding of [³H]inositol 1,4,5-trisphosphate to its receptor

There are now several reports of the specific reversible binding of inositol 1,4,5-trisphosphate (InsP₃) to permeabilized cells or particulate preparations of several tissues [1,2,3,4]. In some [1,2,3], though not all [4], of these preparations, equilibrium dissociation constants (*K_D*) for InsP₃ binding have been reported to be in the low nanomolar range, whereas effective concentrations to release Ca²⁺ from intracellular stores, often from the same preparation, are one to two orders of magnitude greater. This could suggest that the binding site and sites associated with Ca²⁺ release are different or that the conditions of the assays substantially alter the binding affinity of InsP₃.

In this context we have reported that ATP, which is generally present in millimolar concentrations in Ca²⁺-release assays, inhibited the specific binding of [³H]Ins-(1,4,5)P₃ to a particulate site prepared from rat cerebellum [*I*C₅₀ (concentration producing 50% inhibition) 51 μM] [5]. Since these sites have been characterized for stereo- and positional specificity and possess qualitative properties in this respect very similar to those associated with Ca²⁺ release [6], we considered that the difference in apparent affinity between the systems could relate to the presence of ATP in the Ca²⁺ assays. Inhibitory effects of ATP on InsP₃ binding have been confirmed in other systems [1, 3], but a recent letter to this journal [7] suggested that this inhibitory effect was not related to ATP itself but to some impurity in certain preparations. In particular, it was reported that ATP from Pharmacia (type 27-1006-0) is inactive on InsP₃ binding to pituitary microsomes.

Using our cerebellar membrane preparation, which has been carefully evaluated for stereo- and positional specificity [5], we have failed to reproduce these results. For example, in our hands ATP (Sigma A2383) and ATP (Pharmacia, type 27-1006-0) produce superimposable inhibition curves against 15 nM-[³H]Ins(1,4,5)P₃ with *I*C₅₀ values of 7.9 (±0.67) × 10⁻⁴ M and 7.6 (±0.80) × 10⁻⁴ M

respectively. Furthermore, ATP preparations from several other commercial sources were identical in this respect. AMP and ADP possessed *I*C₅₀ values in excess of 5 × 10⁻³ M.

It is not clear why these data differ from those in pituitary microsomes, or indeed if the inhibition by ATP is competitive; however, it still remains possible that the discrepancy between the effectiveness of Ins(1,4,5)P₃ in releasing Ca²⁺ and its affinity for receptor binding relates to the presence of greater-than-millimolar concentrations of ATP in many of the former systems. In this context it should be noted that a very recent report [8] has shown that, in the absence of ATP, Ins(1,4,5)P₃ can release Ca²⁺ from intracellular stores of permeabilized rat basophilic leukaemic cells in a highly co-operative manner in the 4–40 nM concentration range.

Alan L. WILLCOCKS and Stefan R. NAHORSKI

Department of Pharmacology and Therapeutics, University of Leicester, P.O. Box 138, Medical Sciences Building, University Road, Leicester LE1 9HN, U.K.

- Guillemette, G., Balla, T., Baukal, A. J. & Catt, K. J. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8195–8199
- Spat, A., Bradford, P. G., McKinney, J. S., Rubin, R. P. & Putney, J. W. (1986) *Nature (London)* **319**, 514–516
- Guillemette, G., Balla, T., Baukal, A. J. & Catt, K. J. (1988) *J. Biol. Chem.* **263**, 4541–4548
- Worley, P. F., Baraban, J. M., Colvin, J. S. & Snyder, S. H. (1987) *Nature (London)* **325**, 159–161
- Willcocks, A. L., Cooke, A. M., Potter, B. V. L. & Nahorski, S. R. (1987) *Biochem. Biophys. Res. Commun.* **146**, 1071–1078
- Strupish, J., Cooke, A. M., Potter, B. V. L., Gigg, R. & Nahorski, S. R. (1988) *Biochem. J.* **253**, 901–905
- Eberhardt, I., Kiesel, L. & Spat, A. (1988) *Biochem. J.* **250**, 311
- Meyer, T., Holowka, D. & Stryer, L. (1988) *Science* **240**, 653–656

Received 28 July 1988