Volume 217, number 1, 85–88 FEB 04788 June 1987

Effect of inositol 1,4,5-trisphosphate and GTP on calcium release from pituitary microsomes

L. Kiesel, G.L. Lukács*, Inge Eberhardt, B. Runnebaum and A. Spät*

Department of Gynecology and Obstetrics, University of Heidelberg, D-6900 Heidelberg, FRG and **Department of Physiology, Semmelweis University Medical School, PO Box 259, H-1444 Budapest, Hungary*

Received 10 April 1987

Microsomal vesicles from bovine anterior pituitary accumulate Ca^{2+} and maintain a steady-state ambient Ca^{2+} level of 200 nM. IP_3 and GTP both induce calcium release from the microsomal vesicles. The effect of IP_3 is inhibited by polyethylene glycol (PEG), and the effect of GTP is absolutely dependent on PEG. Half-maximal effect of IP_3 (without PEG) is 0.26 μm, the maximal calcium release attaining 7% of the A23187-releasable pool. The same values for GTP (in the presence of PEG) are 80 μM and 10%, respectively. GTP potentiates the effect of IP_3. This potentiation is not mediated by protein phosphorylation.

GTP; Inositol trisphosphate; Ca^{2+} release; Microsome; Polyethylene glycol; Protein phosphorylation

1. INTRODUCTION

Several hormones trigger the biological response of the cell by inducing the formation of IP_3 and diacylglycerol (reviews [1,2]). Interaction of IP_3 with specific binding sites in the endoplasmic reticulum [3,4] elicits the release of Ca^{2+} from this store (review [5]). Dawson's [6] pioneering observation revealed that Ca^{2+} efflux from the endoplasmic reticulum may be controlled by both IP_3 and GTP. The precise mode of action of these two agonists and their interrelationship have not yet been sufficiently elucidated. Here we report on our observations on the actions of IP_3 and GTP in the microsomes obtained from an endocrine tissue, the anterior pituitary.

Correspondence address: A. Spät, Dept of Physiology, Semmelweis University Medical School, PO Box 259, H-1444 Budapest, Hungary

Abbreviations: EC_{50}, concentration required for half-maximal effect; IP_3, inositol 1,4,5-trisphosphate; PEG, polyethylene glycol

2. MATERIALS AND METHODS

IP_3 was a gift from Dr R.F. Irvine (AFRC, Cambridge, England). All other chemicals were purchased from Sigma or Serva. Fresh bovine pituitaries were collected in liquid nitrogen and stored at −80°C until homogenization. The separated anterior pituitary was homogenized first in a Polytron homogenizer and then with a glass-teflon Potter homogenizer at 4°C. The microsomal fraction was prepared as described [7] with the modification that the pellet obtained after the first 35000 × *g* centrifugation was resuspended in 250 mM sucrose, containing 5 mM Hepes, 10 mM KCl, 1 mM dithiothreitol, 0.15 mM EGTA (pH 7.0) and recentrifuged at 35000 × *g* for 30 min.

For calcium transport measurements microsomes (~1 mg/ml) were incubated at 30°C in the following medium: 100 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 10 mM Hepes, 1.5 mM ATP, 4 mM creatine phosphate, 20 μg/ml creatine kinase, 0.5 mM dithiothreitol and 1 μg/ml oligomycin, pH 7.0. The free Ca^{2+} concentration was monitored with a Ca^{2+}-selective electrode [8], calibrated with calcium buffers [9]. Ca^{2+} release

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

was quantitated on the basis of the electrode response to known amounts of $CaCl_2$ added after microsomes reached the steady-state Ca^{2+} concentration.

Protein phosphorylation with [γ-^{32}P]GTP or [γ-^{32}P]ATP (New England Nuclear) was carried out under conditions identical to those used for calcium transport measurements, in the presence of 5% PEG, however, with or without an ATP-generating system (v.s.). Phosphorylation was initiated by the addition of 740 kBq (= 1.7 nmol) labelled ligand per 85 μl microsomal suspension. Incubation lasted for 30 or 120 s at 30°C. The proteins were separated by means of electrophoresis on SDS-polyacrylamide slab gels [10]. Autoradiograms were prepared with intensifying screens at −80°C.

3. RESULTS AND DISCUSSION

Pituitary microsomes in the presence of ATP accumulate Ca^{2+} and buffer the ambient Ca^{2+} concentration at about 200 nM (fig.1). The Ca^{2+} accumulation by mitochondria is negligible, since it is not influenced either by the mitochondrial uncoupler drug carbonyl cyanide *m*-chlorophenylhydrazone (2 μM) or by ruthenium red (2 μM), an inhibitor of mitochondrial Ca^{2+} uptake (not shown).

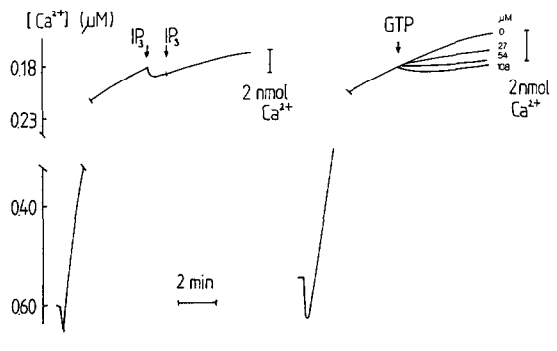

Fig.1. Effect of IP_3 (1.3 μM, left) and GTP (at concentrations indicated, right) on ambient calcium concentration of pituitary microsomes. The microsomes were prepared and incubated as described in section 2. Ca^{2+} concentration was measured with an ion-selective electrode as also described. IP_3 was added twice, and GTP once at the times indicated by arrows. Calibration signals are shown on the right end of both curves. Representative curves are shown.

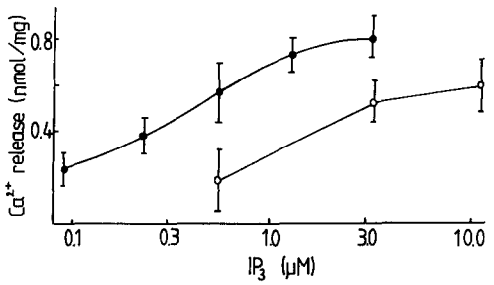

Fig.2. Effect of increasing concentrations of IP_3 on Ca^{2+} release from pituitary microsomes in the presence (○) (n = 3) and absence (●) (n = 4) of 5% PEG. Means ± SE are shown.

IP_3 is also formed in response to Ca^{2+}-mobilizing stimuli in pituitary cells (e.g. [11]). Its Ca^{2+}-releasing effect in the pituitary has been demonstrated in permeabilized tumor cells only [12,13]. Now we have localized the site of action of IP_3 in the microsomal fraction prepared from intact pituitary. IP_3, added at or near steady-state Ca^{2+} concentration, induces the release of Ca^{2+}, the effect being complete within 15–20 s. The observed Ca^{2+} release is not due to Ca^{2+}(-like) contamination of IP_3 (as we observed with a commercial preparation) since the medium Ca^{2+} concentration is not increased by IP_3 in the absence of microsomes and since a second pulse of maximally effective IP_3 fails to induce further Ca^{2+} efflux (fig.1, left). As shown in fig.2, half-maximal and maximal release is attained by 0.26 and about 3 μM IP_3, respectively. PEG (5%) increases the EC_{50} of IP_3 to more than 1 μM (fig.2). The extent of maximal release is about 0.8 nmol/mg protein, corresponding to about 7% of the A23187-releasable Ca^{2+} pool.

GTP, in the presence of 5% PEG, induces the release of Ca^{2+} but its action lasts longer than that of IP_3 (fig.1, right). The EC_{50} of GTP is about 80 μM and the maximal extent of Ca^{2+} release (within 1 min) is about 1 nmol/mg protein (fig.3). This amount represents about 10% of the A23187-releasable Ca^{2+} pool.

In contrast to the effect of IP_3, which was inhibited by PEG (fig.2), no effect of GTP was found in the absence of PEG (not shown). While the PEG dependence of the GTP effect was observed in several cell types, the lack of enhancement of the IP_3 effect by PEG was only recently

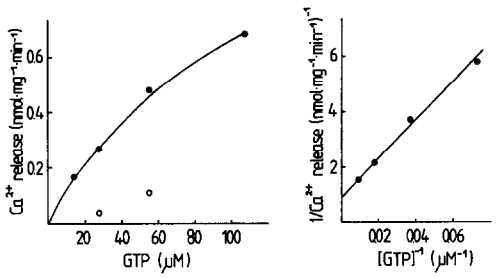

Fig.3. Effect of GTP in the presence (○) or absence (●) of 164 μM Gpp(NH)p on calcium release from incubated pituitary microsomes. The microsomes were incubated in the presence of 5% PEG, as described in section 2. The right-hand panel is a double-reciprocal plot of the data shown in the left panel. Results from one of two similar experiments are shown.

described [14,15]. The discrepancy in the effect of PEG on the IP_3- and GTP-induced Ca^{2+} release together with the different temperature and intravesicular Ca^{2+} sensitivity [14–18] support the concept that IP_3 and GTP exert their action by separate mechanisms.

The Ca^{2+}-releasing effect of GTP has been regarded as an indication of GTP-induced protein phosphorylation [19] as well as that of a microsomal GTPase activity [20]. In accordance with both concepts, the non-hydrolyzable GTP analogue, guanylyl imidodiphosphate (Gpp(NH)p) does not induce Ca^{2+} release per se and strongly antagonizes the effect of GTP (fig.3, left). We examined protein phosphorylation and observed that the same protein bands were labelled by [^{32}P]GTP and [^{32}P]ATP and the labelling by [^{32}P]GTP was completely overcome by the addition of an ATP-generating system (not shown). Thus, protein phosphorylation is not involved in the Ca^{2+}-releasing action of GTP in pituitary microsomes. The absence of GTP-specific phosphorylation was also described in neuroblastoma × glioma hybrid microsomes [15].

Finally, we examined the interaction of IP_3 and GTP. GTP potentiates the Ca^{2+}-releasing effect of IP_3 [6] and IP_3 also potentiates the effect of subsequently added GTP (Lukács, Hajnóczky, Hunyady and Spät, in preparation) in rat liver microsomes. In contrast to liver, in a pituitary microsomal preparation, incubated without PEG, GTP (54 μM) reduced the EC_{50} of IP_3 by about 50% (not shown). On the other hand, IP_3 (up to 20 μM) failed to potentiate the effect of GTP (54 μM, not shown). Since, in our studies, the potentiating effect of IP_3 in the liver and the lack of such an effect in the pituitary were observed under comparable conditions, the difference may be attributed to tissue variation. While the requirements for the potentiating effect of IP_3 remain to be elucidated, the potentiating effect of GTP shows that the two separate mechanisms may control the cellular calcium metabolism in a complex, cooperative manner.

ACKNOWLEDGEMENTS

We are indebted to Dr R.F. Irvine (Cambridge, England) for the generous gift of IP_3. We appreciate Dr S. Rose-John's help in the protein phosphorylation studies and the valuable comments of Dr E. Ligeti.

REFERENCES

[1] Berridge, M.J. and Irvine, R.F. (1984) Nature 312, 315–321.
[2] Williamson, J.R. (1986) Hypertension 8, II-140–II-146.
[3] Spät, A., Fabiato, A. and Rubin, R.P. (1986) Biochem. J. 233, 929–932.
[4] Spät, A., Bradford, P., McKinney, J.S., Rubin, R.P. and Putney, J.W. jr (1986) Nature 319, 514–516.
[5] Berridge, M.J. (1986) J. Cell Sci. suppl. 4, 137–153.
[6] Dawson, A.P. (1985) FEBS Lett. 185, 147–150.
[7] Dawson, A.P. and Irvine, R.F. (1984) Biochem. Biophys. Res. Commun. 120, 858–864.
[8] Lukács, G.L. and Fonyó, A. (1986) Biochim. Biophys. Acta 858, 125–134.
[9] Tsien, R.Y. and Rink, T.J. (1980) Biochim. Biophys. Acta 599, 623–638.
[10] Laemmli, U.K. (1970) Nature 227, 680–685.
[11] Kiesel, L., Bertges, K., Rabe, T. and Runnebaum, B. (1986) Biochem. Biophys. Res. Commun. 134, 861–867.
[12] Gershengorn, M.C., Geras, E., Purrello, V.P. and Rebecchi, M.J. (1984) J. Biol. Chem. 259, 10675–10681.
[13] Biden, T.J., Wollheim, C.B. and Schlegel, W. (1986) J. Biol. Chem. 261, 7223–7229.
[14] Chueh, S.-H. and Gill, D.L. (1986) J. Biol. Chem. 261, 13883–13886.

[15] Jean, T. and Klee, C.B. (1986) J. Biol. Chem. 261, 16414–16420.
[16] Wolf, B.A., Florholmen, J., Colca, J.R. and McDaniel, M.L. (1987) Biochem. J., 137–141.
[17] Henne, V. and Söling, H.-D. (1986) FEBS Lett. 202, 267–273.
[18] Gill, D.L., Ueda, T., Chueh, S.-H. and Noel, M.W. (1986) Nature 320, 461–464.
[19] Dawson, A.P., Comerford, J.G. and Fulton, D.V. (1986) Biochem. J. 234, 311–315.
[20] Nicchitta, C.V., Joseph, S.K. and Williamson, J.R. (1986) FEBS Lett. 209, 243–248.