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Heparin inhibits the inositol 1,4,5-trisphosphate-induced Ca^{2+} release from rat liver microsomes

Peter J. Cullen, John G. Comerford and Alan P. Dawson

School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, England

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Inositol 1,4,5-trisphosphate (Ins (1,4,5)P_3)-stimulated Ca^{2+} release is inhibited by low concentrations of heparin (IC_{50} = 4.5 μg/ml). GTP-stimulated Ca^{2+} release is unaffected at a heparin concentration of 16 μg/ml. Addition of heparin after Ins (1,4,5)P_3 causes the rapid re-uptake of Ins (1,4,5)P_3-releasable Ca^{2+}.

Ca^{2+}; Inositol 1,4,5-trisphosphate; GTP; Heparin; Microsome; Polyphosphoinositide

1. INTRODUCTION

Upon stimulation of plasma membrane-bound receptors, the phospholipase C-catalysed hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdIns (4,5)P_2) results in the production of Ins (1,4,5)P_3 and diacylglycerol (reviews [1,2]). Ins (1,4,5)P_3 has been shown to be effective at mobilising Ca^{2+} from rat liver microsomal fractions (microsomes) [3], this release being greatly increased if GTP together with 5% poly(ethylene glycol) is present in the medium [4-6]. It has been proposed, from work in our laboratory, that GTP brings about its effect by causing the fusion of the microsomal vesicles into larger structures [4-6]. As well as promoting Ins (1,4,5)P_3-dependent Ca^{2+} release, GTP itself causes Ca^{2+} release from microsomes. However, there is mounting evidence that the Ins (1,4,5)P_3-stimulated efflux and GTP-stimulated Ca^{2+} release from endoplasmic reticulum occur by different mechanisms [7-11].

It has previously been shown that the sulphated polysaccharide, heparin, can compete with Ins (1,4,5)P_3 for its specific binding site [12]. We have investigated the effect of heparin on the Ins (1,4,5)P_3- and GTP-stimulated Ca^{2+} release from rat liver microsomes. It was found that heparin at micromolar concentrations strongly inhibited the Ins (1,4,5)P_3-promoted Ca^{2+} release whilst no effect was observed on the Ca^{2+} release promoted by GTP.

Correspondence address: P. Cullen, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, England

Abbreviations: PEG, poly(ethylene glycol), M_r 8000; DTT, dithiothreitol

2. MATERIALS AND METHODS

Ca^{2+}-sensitive electrode membranes containing the neutral ionophore ETH1001 were prepared as described by Clapper and Lee [13]. Ins (1,4,5)P_3 was a kind gift from Dr R.F. Irvine (AFRC, Babraham, Cambridge, England). Heparin (sodium salt, grade 1), phosphocreatine, and creatine kinase were purchased from Sigma (England). ATP, GTP and DTT were from BCL (Lewes, Sussex).

The microsomal fraction sedimenting at 35 000 × *g* for 20 min was prepared from the livers of fed 250 g male rats as described by Dawson and Irvine [3]. Protein was determined by the method of Lowry et al. [15], using bovine serum albumin as standard.

Ca^{2+} uptake and release were routinely measured with a Ca^{2+}-sensitive electrode as described [6] with the exception that oligomycin and ruthenium red were omitted from the assay medium [16]. The assay medium consisted of 150 mM sucrose, 50 mM KCl, 10 mM Hepes-KOH, pH 7.0, 5% (w/v) PEG, 1 mM DTT, 2 mM $MgCl_2$, 5 mM ATP, 36 μg/ml creatine kinase and 5 mM phosphocreatine in a volume of 2.45 ml. Experiments were carried out at 30°C.

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3. RESULTS AND DISCUSSION

The lack of effect that heparin has on the GTP-induced Ca^{2+} release is shown in fig.1a (incubation medium as described in section 2). This shows that addition of 16 μg/ml of heparin has no effect on the rate or extent of Ca^{2+} release when added during the phase of Ca^{2+} release following GTP addition. It was also found that if heparin was present in the incubation mixture before the addition of microsomes, the time course of Ca^{2+} uptake and that of GTP-stimulated Ca^{2+} efflux were identical to those observed in the absence of heparin (not shown). Also, we have found that heparin has no effect on the GTP-dependent vesicle fusion (not shown), as determined by the fluorescence energy transfer procedure of Comerford and Dawson [16].

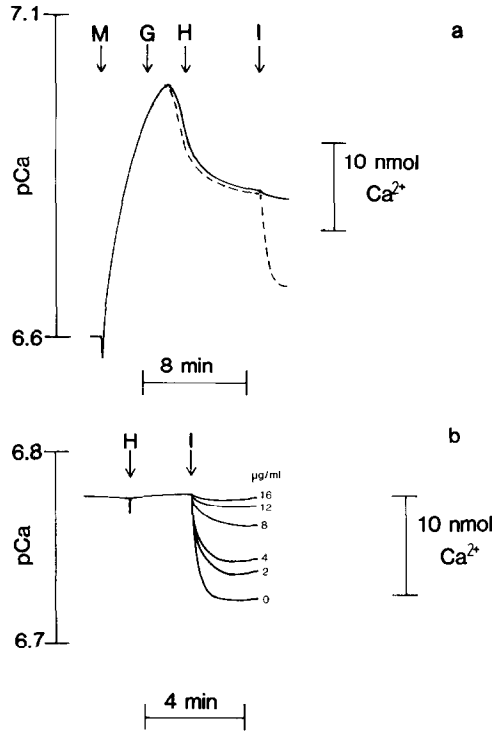

Fig.1. Effect of heparin on the GTP-stimulated and Ins (1,4,5)P_3-stimulated Ca^{2+} release. (a). Heparin (H) (16 μg/ml) was added during GTP-stimulated release. Arrows indicate the addition of microsomes (3.5 mg protein), M; GTP (20 μM), G; Ins (1,4,5)P_3 (1 μM), I. Dashed line indicates a typical trace in the absence of heparin. The trace is slightly displaced downwards to ease clarification. (b) Heparin (at the concentrations shown) was added when the new steady state of Ca^{2+} loading had been reached after the addition of GTP. Ins (1,4,5)P_3 (1 μM) was added as shown by the arrow. The trace shows only the Ins (1,4,5)P_3-stimulated Ca^{2+} release, however the whole trace is similar to that shown in (a), except that heparin was added after GTP-dependent Ca^{2+} release was complete.

Fig.1a,b shows the heparin inhibition of the Ins (1,4,5)P_3-induced Ca^{2+} release. In fig.1b, heparin, at the indicated concentration, was added after a new steady state of free Ca^{2+} ($pCa \sim 6.75$) had been attained after GTP-stimulated Ca^{2+} release. Ins (1,4,5)P_3 was added 2 min after heparin. It should be noted that occasionally the addition of higher concentrations of heparin (i.e. 16 μg/ml) resulted in some small amounts of Ca^{2+} re-uptake prior to Ins (1,4,5)P_3 addition. This may be due to antagonism, by heparin, of some endogenous bound Ins (1,4,5)P_3, or may indicate a blockade of open Ca^{2+} channels. The data of fig.1 clearly show that heparin acts as a specific antagonist against the Ins (1,4,5)P_3-stimulated Ca^{2+} efflux, but has no effect on the GTP-stimulated Ca^{2+} efflux. This reinforces the idea that in rat liver microsomes the Ins (1,4,5)P_3-stimulated release and GTP-stimulated Ca^{2+} efflux occur by different mechanisms.

Fig.2 is a typical trace showing the effect of adding heparin after the Ins (1,4,5)P_3-induced Ca^{2+} release has been completed. Heparin addition clearly results in the rapid re-uptake of Ca^{2+} previously released by Ins (1,4,5)P_3. This could be

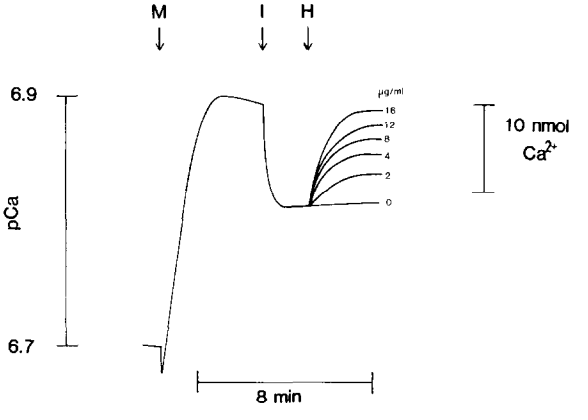

Fig.2. Effect of adding heparin after the Ins (1,4,5)P_3-stimulated Ca^{2+} release has ended. Microsomes were prepared as described in section 2. GTP (20 μM) was present in the incubation mixture prior to the addition of microsomes. Arrows mark the addition of microsomes (3.0 mg protein), M; Ins (1,4,5)P_3 (1 μM), I; heparin (at concentrations shown), H.

due to displacement of bound Ins (1,4,5)P_3 by heparin, or to direct blockage of the Ins (1,4,5)P_3-sensitive Ca^{2+} channels (see below). The dose-response curves for both the inhibition of the Ins (1,4,5)P_3-induced Ca^{2+} release and the heparin-stimulated Ca^{2+} re-uptake are shown in fig.3. In both cases, a half-maximal effect is obtained at approx. 5 μg/ml of heparin.

Spät and co-workers [14] have shown that the rate of dissociation of [^{32}P]Ins (1,4,5)P_3 from rat liver microsomal binding sites occurs monoexponentially, with a half-time of approx. 5.9–4.7 min at 4°C. Our results, however, show that the effect of heparin in causing Ca^{2+} re-uptake is extremely rapid. The concentration dependence of inhibition of Ca^{2+} release and stimulated Ca^{2+} re-uptake described here is very similar to that of inhibition of Ins (1,4,5)P_3 binding found by Worley et al. [12], strongly suggesting that heparin inhibition is due to displacement of Ins (1,4,5)P_3. Clearly, the time course of Ca^{2+} re-uptake shown in fig.2 suggests an off-rate for Ins (1,4,5)P_3 with a time constant of the order of seconds (within the time response of the electrode) rather than minutes. Therefore, either the off-rate is very temperature dependent, or heparin is binding to a binding site distinct from the Ins (1,4,5)P_3 site, leading to an increased Ins (1,4,5)P_3 off-rate.

The structure of heparin, being a sulphated polysaccharide, suggests that it might be acting as a structural analogue of Ins (1,4,5)P_3, and could therefore be binding to the Ins (1,4,5)P_3-binding site. If this is the case it suggests that the off-rate for Ins (1,4,5)P_3 leaving the receptor at physiological temperature is very much faster than would be predicted from the low-temperature data of Spät et al. [14], but would be very much in line with the time response of the termination of the Ca^{2+} signal on removal of the agonists [17].

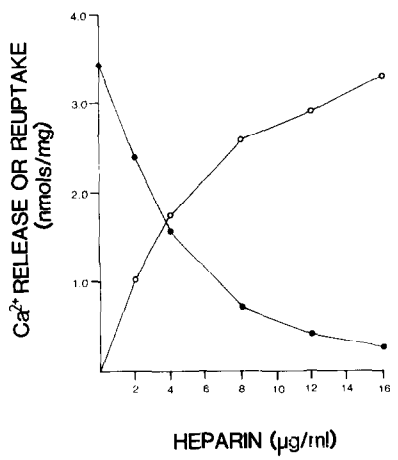

Fig.3. Effect of heparin concentration on Ca^{2+} release and re-uptake. The effect of various heparin concentrations on the Ins (1,4,5)P_3-stimulated Ca^{2+} release (●), and on the Ca^{2+} re-uptake after the completion of the Ins (1,4,5)P_3-stimulated Ca^{2+} release (○) were measured by the techniques shown in figs 1 and 2. Each point is the mean of three separate experiments carried out on different samples of microsomes.

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