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Right atrial dilatation increases inositol-(1,4,5)trisphosphate accumulation

Implications for the control of atrial natriuretic peptide release

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Stretching the right atrium of isolated perfused [^{3}H]inositol-labelled rat hearts was shown to stimulate the phosphatidylinositol turnover pathway as demonstrated by the accumulation of [^{3}H]inositol-(1,4,5)trisphosphate and its degradation products. Stimulation was detectable after 1 min with larger increases observed after 10 or 20 min. These findings demonstrate that the myocardium can respond to dilatation by an activation of the phosphatidylinositol turnover pathway. Such a mechanism has implications for the release of atrial natriuretic peptide following right atrial distention.

Atrial stretch; Inositol phosphate; Inositol-(1,4,5)trisphosphate; Atrial natriuretic peptide; (Rat heart)

1. INTRODUCTION

As it is currently understood the phosphatidylinositol (PtIns) turnover pathway involves the hydrolysis of a plasma membrane phospholipid, phosphatidylinositol-(4,5)bisphosphate following binding of a hormone or neurotransmitter to an appropriate receptor. This hydrolysis yields two second messengers, *sn*-1,2-diacylglycerol (DAG) and inositol-(1,4,5)trisphosphate (Ins(1,4,5)P_3). DAG stimulates the membrane-bound phospholipid-dependent, Ca^{2+}-dependent protein kinase C and Ins(1,4,5)P_3 releases calcium from endoplasmic reticulum stores in a number of different tissues [1]. The PtIns turnover pathway has been well studied in many tissues but relatively little detailed information is available for myocardial tissue. In isolated ventricular myocytes [2], ventricle strips [3], perfused ventricles [4] and intact perfused hearts [5], 2–4-fold stimulations by noradrenaline (α_1-receptors) and acetylcholine (muscarinic receptors) have been observed. In the present study isolated perfused rat hearts have been used to study the PtIns turnover response to right atrial distention, a stimulus known to cause release of atrial natriuretic peptide.

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2. MATERIALS AND METHODS

2.1. *Preparation of [^{3}H]inositol-labelled hearts*

The methods used for the preparation of [^{3}H]inositol-labelled perfused hearts were essentially as described previously [5,6]. The rats used in the current study weighed 300–350 g and phospholipids were labelled by perfusing for 2 h with [^{3}H]inositol (Amersham, Bucks, England) (10 μCi/ml). Following the labelling period, the perfusion was continued for 10 min with 5×10^{-3} M non-radioactive inositol and 10^{-2} M LiCl. In hearts to be subjected to right atrial stretch, a latex balloon (size 5, obtained from Hugo Sacks, Elektronic 7801 Masch-Hugotetten, FRG) was inserted into the right atrium via the inferior vena cava prior to the labelling. The balloon was attached to a 1 ml syringe via a catheter and inflated to a volume of 75 μl. The stretch signal was terminated by chilling the hearts in ice-cold saline. Right atria were dissected, weighed and inositol phosphates extracted using a chloroform/methanol extraction method as described elsewhere [7].

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2.2. *Separation and identification of inositol phosphates*

Water-soluble [^{3}H]inositol-labelled compounds were separated using anion-exchange high-performance liquid chromatography as described elsewhere [6] or Dowex column chromatography [7]. The column used was a Whatman partisil SAX column packed by Waters (Milford, MA, USA) for use in a radial compression system on a Waters model 441 liquid chromatograph. [^{3}H]Inositol-labelled compounds were eluted using a linear gradient of 0–2 M ammonium formate buffered to pH 3.7 with phosphoric acid. The flow rate was 1 ml/min. Ins-1P, Ins(1,4)P_2 and Ins(1,4,5)P_3 were identified using ^{3}H-labelled standards (from Amersham, Bucks, England). ^{3}P-labelled glycerophosphoinositol, glycerophosphoinositol-4-phosphate and glycerophosphoinositol-(4,5)bisphosphate were prepared as described by Downes et al. [8]. Standard Ins(1,3,4,5)P_4 was prepared from [^{3}H]Ins(1,4,5)P_3 using a crude preparation of Ins(1,4,5)P_3 kinase from rat brain [6,9].

2.3. *Assay of atrial natriuretic peptide (ANP)*

ANP was measured using a radioimmunoassay as described elsewhere [10].

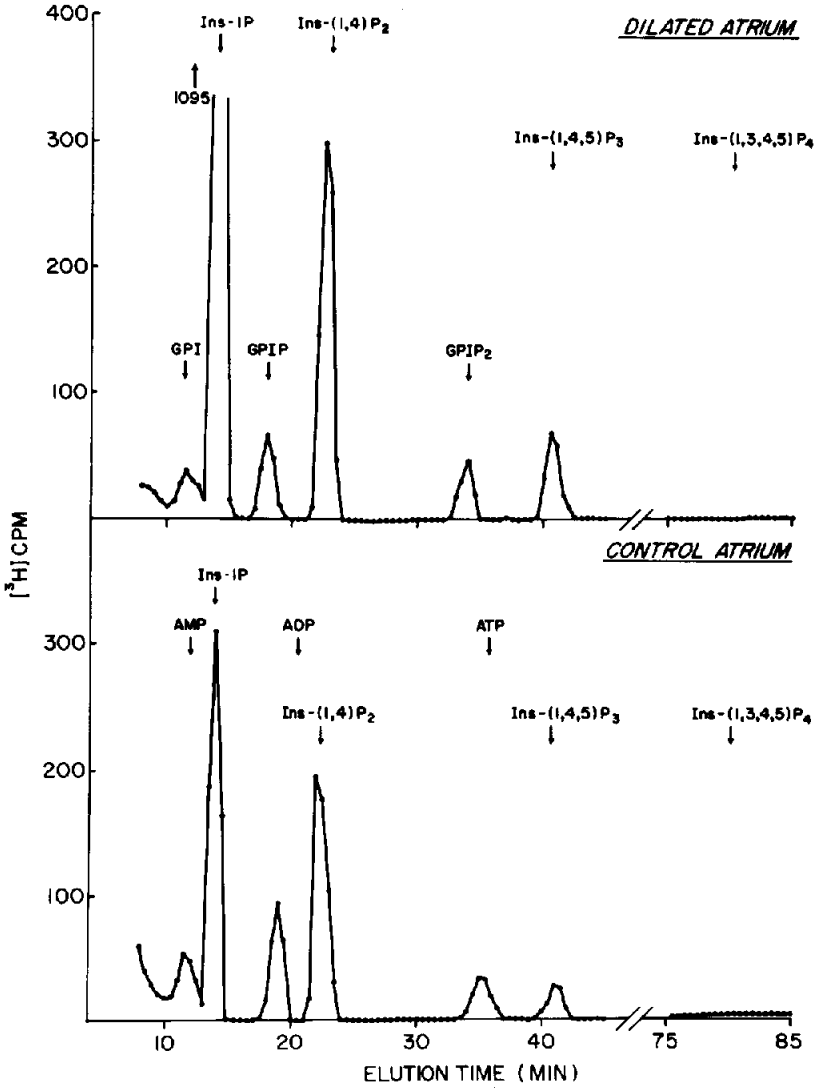

Fig.1. Effect of 10 min right atrial dilatation on the profile of inositol phosphates in right atria. HPLC analysis was performed as explained in section 2. GPI, glycerophosphoinositol; GPIP, glycerophosphoinositol-4-phosphate; $GPIP_2$, glycerophosphoinositol-(4,5)bisphosphate. The experiment was performed 4 times with similar results.

3. RESULTS

3.1. *Effect of 10 min right atrial dilatation on inositol phosphate accumulation*

[^{3}H]Inositol-labelled rat hearts were subjected to 10 min dilatation of the right atrium. Water-soluble, [^{3}H]inositol-labelled products were extracted and examined using anion-exchange high-performance liquid chromatography. An increase in Ins(1,4,5)P_3 was observed in right atria following 10 min dilatation, together with increases in its degradation products, Ins(1,4)P_2 and Ins-1P (fig.1). In four different experiments the average cpm in Ins(1,4,5)P_3 in dilated atria was 7850 ± 1700 (cpm/g tissue; mean ± SE) compared with 4523 ± 1200 in control preparations ($P < 0.01$, Perutz F-test). Other peaks on the chromatograms were identified as glycerophosphoinositol (GPI), glycerophosphoinositol-4-phosphate (GPIP) and glycerophosphoinositol-(4,5)bisphosphate (GPIP_2). These compounds were not increased by right atrial stretch. Profiles obtained in right atria were similar to those reported previously in ventricular tissue and differed from those reported in other tissues [6]. In none of the profiles observed in atria was there any peak at the position of Ins(1,3,4,5)P_4 or any of its dephosphorylation products. Similar profiles have been reported by others in muscle cells in culture [11]. Thus atria, like ventricles, appear to lack the products of the Ins(1,4,5)P_3 kinase pathway, at least under the conditions of our perfused heart preparations. However, others have reported finding Ins(1,4,5)P_3 kinase activity in rat heart preparations [4].

3.2. *Time dependence of PtIns responses to right atrial dilatation*

Effects of right atrial dilatation on inositol phosphate accumulation in the right atrium itself were detectable after 1 min balloon stretching, with larger increases being observed after 10 or 20 min (fig.2). Increases in $InsP_1$, $InsP_2$ and $InsP_3$ were observed. These experiments were performed

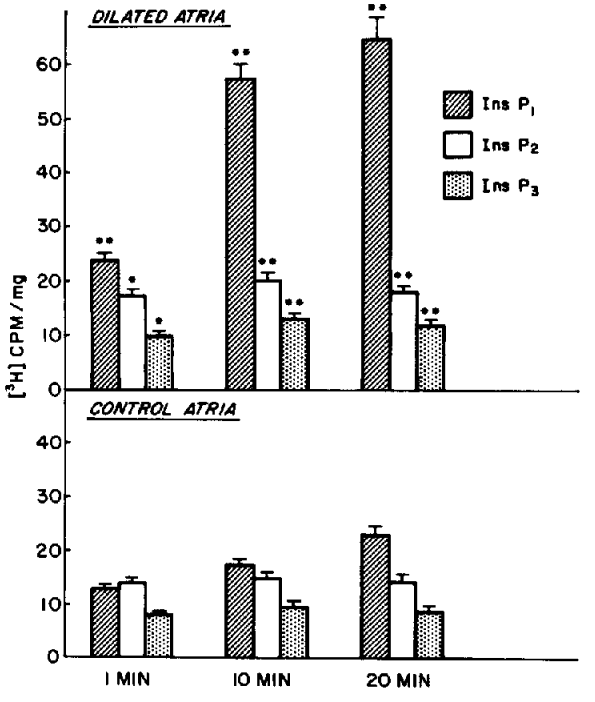

Fig.2. Accumulation of inositol phosphates in the right atria of isolated, perfused rat hearts subjected to right atrial dilatation for 1, 10 and 20 min. Values shown are mean ± SE of 6–8 experiments (** $P < 0.01$ and * $P < 0.05$, Perutz F-test [19]).

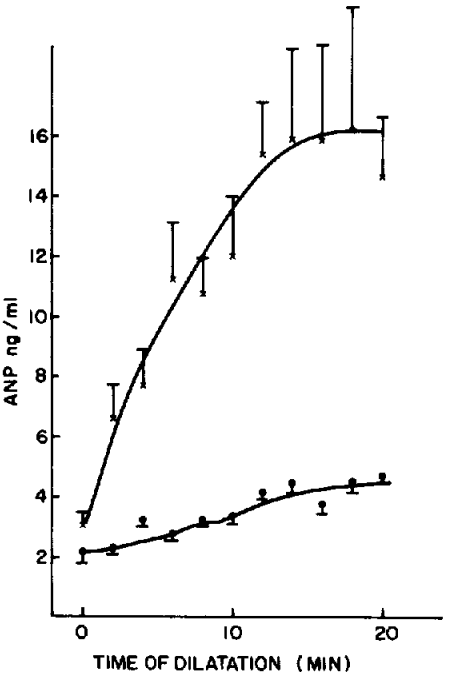

Fig.3. Effect of right atrial dilatation on the release of atrial natriuretic peptide (ANP) into the perfusate. Dilatation was performed as described in the legend to fig.1. Values shown are mean ± SE of 4 hearts from the 20 min dilated group shown in fig.2. (●) Control; (×) right atrial dilatation.

using Dowex column chromatography to separate the inositol phosphates. Preliminary experiments had demonstrated that the $InsP_2$ and $InsP_3$ fractions contained, in addition, glycerophosphoinositol-4-phosphate and glycerophosphoinositol-(4,5)bisphosphate, respectively. As these products did not change during dilatation, results obtained using column chromatography should underestimate changes in inositol phosphates. Addition of 1 μM concentrations of propranolol, prazosin and atropine to block respectively β-adrenoceptors, α_1-adrenoceptors and muscarinic acetylcholine receptors did not alter the response indicating that it was not secondary to release of noradrenaline or acetylcholine.

3.3. *Release of ANP*

Dilatation of the right atrium also caused an increase in the release of ANP into the perfusate. ANP release from hearts with right atriaily dilated for 20 min is shown in fig.3. Release of ANP continued steadily for 12 min and plateaued thereafter.

4. DISCUSSION

Experiments in vivo and in vitro have demonstrated release of ANP following distention of the right atrium but the mechanism whereby such a stimulus is transmitted has remained unknown. Atrial stretch has been reported to increase calcium influx by causing plasma membrane depolarization [12] and the increased cytosolic calcium might stimulate release of ANP. Other studies have shown that noradrenaline acting at α_1-adrenoceptors can stimulate release of ANP, apparently by activation of the PtIns turnover pathway [13]. Direct stimulation of this pathway using phorbol esters and calcium ionophores also releases ANP from atria in vitro [14]. Thus, to date, the best described stimulants of ANP release are atrial stretch or activation of the PtIns turnover pathway. In this study we have addressed the question of a possible relationship between atrial stretch and activation of the PtIns turnover pathway and have shown that stretching the right atrium causes an activation of this pathway. Stimulation was not secondary to release of neurotransmitters. Furthermore, PtIns turnover in heart is not stimulated by increases in cytosolic calcium [2,5], making it unlikely that the response to dilatation is mediated by calcium. While mediation by some other change caused by dilatation cannot be ruled out, it remains possible that 'stretch receptors' can directly activate the PtIns-(4,5)P_2-specific phospholipase C.

$Ins(1,4,5)P_2$ has been reported to be active [15], inactive [16] and weak [17] in releasing calcium from cardiac sarcoplasmic reticulum. Therefore its role in the heart is unclear. The other second messenger released in concert with $Ins(1,4,5)P_3$, DAG, via its activation of protein kinase C, can activate calcium channels in the sarcolemma [18]. Such a mechanism may be important in raising intracellular calcium concentrations which in turn may be important in stimulating release of ANP and in increasing myocardial contractility. Clearly, the data do not prove that ANP release following right atrial dilatation is mediated by the PtIns turnover pathway. However, stimulation of PtIns turnover, either by receptor activation or by phorbol esters and calcium ionophores can release ANP. Therefore, it is reasonable to suggest that stimulation of PtIns turnover at least contributes to the stimulated release of ANP following dilatation of the right atrium.

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