Volume 249, number 2, 302-306 FEB 07215 June 1989

Stimulation of phosphoinositide hydrolysis via class I antigen-specific recognition in murine cardiac tissue

Graciela A. Cremaschi and Leonor Sterin-Borda

Centro de Estudios Farmacológicos y de Principios Naturales, CONICET, Serrano 665, (1414) Buenos Aires, Argentina

Received 31 March 1989

Induction of polyphosphoinositide hydrolysis in cardiac tissue by specific recognition of class I histocompatibility antigens was assayed. C3H (H-2^k) mice auricles were labelled with *myo*-[^{3}H]inositol precursor and inositol phosphate production in the presence or absence of anti-class I k products was measured. Anti-class I, but not anti-class II products specifically increased phosphoinositide turnover. This increment was partially blocked by muscarinic cholinergic and α-adrenergic blockers and even more so by the phospholipase C inhibitor NCDC. Alloantibodies specifically directed against class I antigens could then exert stimulation of phospholipase C-mediated phosphoinositide hydrolysis through the interaction with muscarinic cholinergic and/or α-adrenergic receptors. The induction of intracellular second messengers by class I antigens and hormone-receptor interactions is discussed.

Histocompatibility antigen; Alloantibody; Phosphoinositide turnover; Phospholipase C; Adrenergic receptor, α-; Muscarinic cholinergic receptor

1. INTRODUCTION

Hormones, neurotransmitters and a wide variety of growth-promoting factors exert their physiological responses through intracellular signals induced by their interactions with specific cell surface receptors. In addition to receptors that regulate cyclic AMP (cAMP) formation, there is another type of cell surface receptor which stimulates phospholipase C-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DG) [1]. Muscarinic cholinergic and α_1-adrenergic receptors of the heart are coupled to stimulated polyphosphoinositide turnover [2,3] and it has been suggested that some of the physiological and metabolic effects of adrenergic and cholinergic actions upon myocardium are secondary to receptor-mediated hydrolysis of phosphoinositides [1,3]. Furthermore, it has been demonstrated that the products of phospholipase C hydrolysis, namely DG and PI_3, may serve as intracellular second messengers. On the other hand, it was recently reported that major histocompatibility (HC) antigens (Ag), especially class I molecules, play an important role in some ligand-receptor interactions [4,5], including insulin [6–9], epidermal growth factor, luteinizing hormone [11] and β-adrenergic receptors [11–14]. In the last case our group demonstrated that alloimmune anti-class I serum activation of cardiac β_1-adrenoceptors leads to intracellular signaling by increasing cAMP production [14]. Our purpose was the investigation of whether alloantibody action was restricted to cAMP production or if other intracellular signals, i.e. phospholipase C product formation, could also be stimulated. Here we show that alloimmune antibody, while specifically recognizing class I molecules of cardiac tissue, activates phosphoinositide turnover through muscarinic cholinergic and/or α-adrenergic receptor interactions.

Correspondence address: L. Sterin-Borda, Serrano 665, (1414) Buenos Aires, Argentina

Published by Elsevier Science Publishers B.V. (Biomedical Division)

2. MATERIALS AND METHODS

2.1. *Materials*

myo-[2-^{3}H]Inositol ([^{3}H]MI) precursor (S = 15 Ci/mmol) was from New England Nuclear. Bio-Rad AG 1-X8 (100–200 mesh, formate form) anion-exchange resin was used. Methoxamine (Met) was from Burrough's Wellcome; phentolamine (Phent) from Ciba; carbachol, atropine and 2-nitro-4-carboxyphenyl-*N,N*-diphenylcarbamate (NCDC) from Sigma. Other chemicals used were of analytical grade. For chromatographic procedures the following standards from Sigma were used: *myo*-inositol 2-monophosphate, D-*myo*-inositol bisphosphate, D-*myo*-inositol trisphosphate, DL-*myo*-inositol 1-monophosphate, DL-*myo*-inositol 2-monophosphate and DL-*myo*-inositol 1,2-cyclic monophosphate. Alloimmune mouse antiserum against H-2^k HC Ag was obtained by immunizing BALB/c (H-2^d) mice with C3H (H-2^k) lymphoid cells as described [12,14]. Subsequent purification of alloimmune IgG was achieved by DEAE-cellulose ion-exchange chromatography and the degree of purification was determined by immunoelectrophoresis as in [12]. Monoclonal anti-class I k alloantigens, 3.83 (K^k, D^k), were kindly supplied by Dr K. Ozato (Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD). Monoclonal anti-class II alloantigens of k haplotypes were I-E^k (Ia.7) from Cedarlane, specific for Ia Ag coded by the E subregion of the k haplotype.

2.2. *Incubation*

Adult C3H mice were killed by decapitation and atrial appendages (nearly 10 mg wet wt) were rapidly excised after allowing hearts to beat in modified Krebs-Ringer-bicarbonate (KRB) solution, gassed with 5% CO_2 in O_2, in order to expel all remaining blood. The ionic composition of KRB was reported in [15]. Auricles were transferred to continuously oxygenated vessels containing 0.5 ml buffer with 1 μCi [^{3}H]MI, and 10 mM LiCl was added for inositol monophosphate accumulation, according to Berridge et al. [16]. Atrial preparations were incubated at 37°C in a shaking water bath for 120 min. Alloantibody or agonists were added 30 min before the end of the incubation period. When blockers were used, they were added 30 min before addition of alloantibody.

2.3. *Total labelled inositol phosphate (IP) measurement*

After incubation, water-soluble IPs were extracted following Berridge et al. [16]. Briefly, auricles were quickly washed with KRB and homogenized in 0.3 ml KRB with 10 mM LiCl and 2 ml chloroform/methanol (1:2, v/v), for stopping incubation reactions. Chloroform (0.62 ml) and water (1 ml to separate phases) were then added. Samples were centrifuged at 2000 × g for 15 min and the upper aqueous phase (~ 1.8 ml) was applied to a 0.7 ml column of Bio-Rad AG-X8 suspended in 0.1 M formic acid and previously washed with 10 mM Tris-formate (pH 7.4). The resin was then washed with 20 vols of 5 mM *myo*-inositol followed by 6 vols water and IPs were eluted with 1 M ammonium formate in 0.1 M formic acid. Fractions of 1 ml were recovered and the radioactivity determined by scintillation counting. Results were expressed as the percentage of control peak areas which were determined by triangulation.

2.4. *Chromatography of radiolabelled compounds*

In order to determine the absence of [^{3}H]MI in the IP eluted peak, chromatography on silica gel 60 F_{254} sheets (Merck) was performed using propan-2-ol/6 N NH_4OH (14:5) as developing solvent [17]. Spots were located by spraying with freshly prepared 0.1% $FeCl_3$ in ethanol, followed, after air-drying, with 1% sulfosalicylic acid in ethanol, as described by Hokin-Neaverson and Sadeghian [17]. To determine radioactivity, a histogram was constructed from cutting up of the sheet gel, placing each sample in Triton-toluene-based scintillation fluid and counting.

3. RESULTS

In order to analyze alloantibody actions on phospholipase C-induced intracellular second messengers in cardiac tissue, C3H auricles were incubated with radiolabelled MI precursor in the presence of 10 mM LiCl and water-soluble IPs formed in the presence and absence of alloimmune BALB/c anti-C3H IgG were subjected to anion-exchange chromatography. We gathered fractions corresponding to 5 mM *myo*-inositol washing (first peak) that contains remaining [^{3}H]MI, and 1 M ammonium formate elution (second peak) that contains total IPs, namely IP_3, inositol bisphosphates (IP_2), with particular accumulation of inositol monophosphates (IP) as described before [16,18] (see fig.1A). Alloimmune BALB anti-C3H IgG was capable of producing a 2.0-fold increase in the second peak without modifications of the first one (fig.1A,B). This pointed to the fact that alloantibody increased cardiac PI turnover, since it could augment IP formation without affecting incorporation of [^{3}H]MI into the tissue. It should be noted that normal IgG (obtained from BALB/c mice injected with saline) gave results similar to those of controls (not shown).

In order to ensure that the 2nd peak did not contain contaminating [^{3}H]MI, we analyzed control and alloimmune antibody produced peaks by chromatography using MI, [^{3}H]MI, IP_3, IP_2 and distinct IP [inositol 1-monophosphate (I-1-P), inositol 2-monophosphate (I-2-P) and inositol 1,2-cyclic monophosphate (cIP)] as standards. As shown in fig.2, the products in the first peak remain at the origin and those of the 2nd peak gave a spot with the same R_f value as that of cIP. When the radioactivity in the chromatograms was evaluated, it was observed (see histogram bars; fig.2) that [^{3}H]MI remained at the origin and that the radioactivity run with the spot of the 2nd peak only when IPs were used as carrier.

In order to investigate the participation of hor-

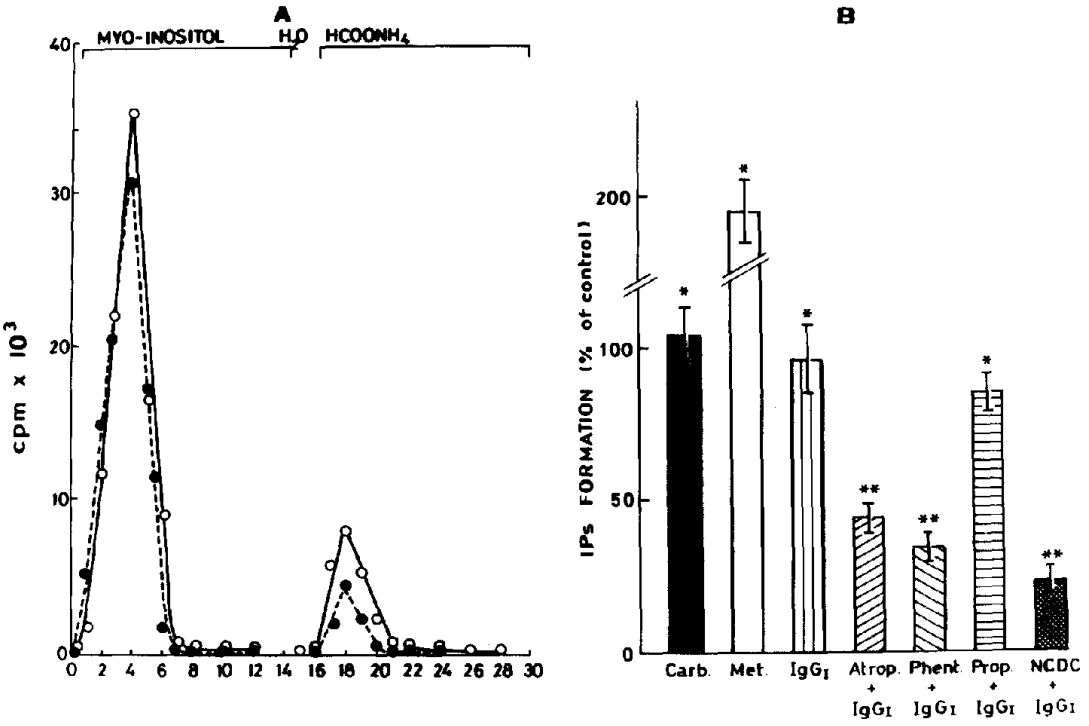

Fig.1. Alloantibody effect on formation of IPs. Action of different hormone receptor blockers. C3H mouse atria were incubated for 60 min with [^{3}H]MI and for an additional 30 min in the absence or presence of different hormone receptor blockers [10^{-6} M atropine (Atrop.), 10^{-6} M phentolamine (Phent.), 5×10^{-7} M propranolol (Prop.)] or a phospholipase C inhibitor (5×10^{-6} M NCDC). Tissues were then left for a further 30 min in the presence or absence of BALB/c anti-C3H IgG or in the presence of hormone receptor agonists [5×10^{-6} M carbachol (Carb.) or 10^{-4} M methoxamine (Met.)]. (A) Anion-exchange chromatography of [^{3}H]MI and its derived radiolabelled products, obtained in the presence (○—○) or absence (●—●) of alloantibody. Peaks eluting with 5 mM *myo*-inositol (first peak) and with 1 M ammonium formate (second peak) are displayed. (B) Formation of IPs was analyzed in the presence of the indicated drugs. Values represent the amount of radioactivity, for each experimental condition, as percentage of control. Each set of data is the mean of 3 independent experiments ± SE. * $p<0.001$ vs control and ** $p<0.001$ vs IgG_1 alone, by one-way analysis of variance and the Dunnett test.

mone receptors in the phenomenon, the actions of the alloantibodies were assayed in the presence of muscarinic cholinergic and α-adrenergic blockers. Since we had previously observed alloantibody-induced β-adrenergic receptor activation in cardiac tissue, we also analyzed the effect of the β-blocker propranolol, in order to exclude any indirect unknown action of β-adrenergic activation upon stimulation of phosphoinositide hydrolysis. As seen in fig.1B, 5×10^{-7} M propranolol did not affect alloantibody action upon stimulation of PI turnover, but is was significantly diminished by 10^{-6} M atropine and 10^{-6} M phentolamine, pointing to the fact that muscarinic cholinergic and α-adrenergic receptor interactions with alloantibody are responsible for the increase in PI turnover. Furthermore, 5×10^{-6} M NCDC, a phospholipase C inhibitor, also abrogated the effect of alloimmune IgG on PI turnover, indicating that phospholipase C-mediated hydrolysis of PIP_2 is involved in the phenomenon. Alloantibody action was also compared with that of α-adrenergic and muscarinic cholinergic agonists. In fig.1B, it is shown that alloimmune IgG mimics the effect of 5×10^{-6} M carbachol, but was less than that of 10^{-4} M methoxamine. High doses of α-agonist were used, since these were previously reported [18] to be effective in inducing contractile effects on a murine cardiac isolated preparation.

To demonstrate the marked specificity of alloimmune antibody in exerting these effects on phosphoinositide turnover, its action was assayed on an

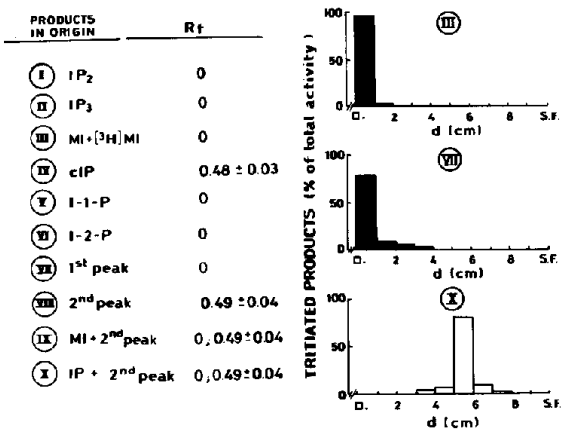

Fig.2. Silica gel sheet ascending chromatographic analysis of peaks eluted on anion-exchange chromatography. Propan-2-ol/6 N NH_4OH (14:5) was used as developing solvent for ascending chromatography performed as described in section 2. All compounds were applied in 10 μg amounts. [^{3}H]MI was diluted 100-times and 2 μl were applied using unlabelled *myo*-inositol (MI) as carrier. For 1st and 2nd peaks, aliquots of 200 μl were separated prior to scintillation counting of eluted fractions, and those with the greatest amount of radioactivity were chosen for chromatographic analysis. Where indicated the 2nd peak products were applied using MI or a pool of inositol monophosphate (IP) as carriers. The following standards were used: inositol bisphosphate (IP_2), inositol trisphosphate (IP_3), inositol 1,2-cyclic monophosphate (cIP), inositol 1-monophosphate (I-1-P) and inositol 2-monophosphate (I-2-P). The histograms show serial 1 cm strip counting of the radioactivity from radiolabelled standard MI, 1st and 2nd peaks. S.F., solvent front; O, origin.

Table 1

Class I AG participation in alloantibody-induced stimulation of PI turnover

Alloantibody	Atria	IP formation (% of control)
BALB/c anti-C_3H IgG	C_3H	92 ± 9[a]
	BALB/c	1.1 ± 5
Mo 3.83	C_3H	91 ± 8[a]
Mo Ia.7	C_3H	2.0 ± 3

[a] Differs significantly from control, with $p < 0.001$, by one-way analysis of variance and the Dunnett test

Murine atria (C_3H or BALB/c where indicated) were incubated in KRB with 1 μCi [^{3}H]MI and 10 mM LiCl for a total of 120 min as described. In the last 30 min of incubation, different alloantibodies were added: polyclonal BALB/c anti-C_3H IgG, monoclonal anti-class I k alloantigens (Mo 3.83) or monoclonal anti-class II products of the k haplotype (Mo Ia.7). Water-soluble radiolabelled products were extracted and separated by anion-exchange chromatography as described in the text. Percentage of IP formation with respect to controls was determined for each experimental condition. Results are means of 3 independent experiments ± SE

unrelated cardiac BALB/c preparation, and monoclonal antibodies specific to class I or II products were also used. As shown in table 1, BALB/c anti-C3H IgG has no effect upon formation of IPs in BALB/c cardiac tissue. Furthermore, only monoclonal 3.83 directed to class I alloantigens showed the same action as that of polyclonal alloantibody, with anti-class II product antibody having no effect on IP formation.

4. DISCUSSION

Molecules of major histocompatibility complex class I interact with several hormone receptors and it has been proposed that this sort of association is required for triggering cell activation [11,14]. We had previously reported that cAMP formation induced by β-adrenoceptor–class I molecule interactions took place in cardiac tissue [14]. Therefore, these associations could produce transmembrane signalling necessary for the biological effects of hormones. Here, we report that class I Ag specific recognition by alloantibody is able to stimulate formation of IPs via polyphosphoinositide hydrolysis, as the effects of alloimmune IgG were blunted by the phospholipase C inhibitor, NCDC. Hence, other intracellular second messengers are capable of being activated by class I product-hormone receptor interactions. In this case, the receptors that participate appear to be cardiac muscarinic cholinergic and α-adrenergic receptors, since the effect of alloantibody upon IP formation was significantly diminished by atropine and phentolamine, respectively. Others have previously demonstrated that some of the physiological and metabolic effects of α-adrenergic and cholinergic stimulation on myocardium are secondary to receptor-mediated hydrolysis of PI [18,20,21]. The reason why the action of alloantibody upon the formation of IPs could implicate that both types of receptor are involved, without distinguishing between the two, is uncertain, but it has been proposed that α-adrenergic and cholinergic receptors are functionally associated on isolated murine atria [19].

Nevertheless, the present data support the idea that class I molecules and hormone receptors interact in the cell membrane, implicating perhaps the corresponding regulatory nucleotide-binding proteins. The physiological importance of this interaction is in need of further clarification.

Acknowledgements: This work was supported by grant 3904801/85 from CONICET. We thank Mrs Elvita Vannucchi for excellent technical assistance and Mrs Beatriz Costigliolo for secretarial assistance.

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