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MAJOR HISTOCOMPATIBILITY COMPLEX MODULATION OF β-ADRENOCEPTOR FUNCTION

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Abstract—Reciprocal interaction between β-adrenoceptor specific ligand occupancy and alloantibody binding to specific antigens of cardiac and smooth muscle tissues was observed. Interference of alloimmune antibody fixation to both cardiac and oviductal tract preparations by β_1 or β_2 selective blockers, respectively, was obtained by means of indirect immunofluorescence assays. Reciprocally, alloimmune IgG and monoclonal antibodies directed to class I H-2 antigens, behaving as β-adrenoceptor agonists, modified the contractility of both tissues, increasing intracellular levels of cyclic AMP (cAMP). Additionally, alloantibodies were also capable of inhibiting specific β-adrenoceptor radioligand binding to purified cardiac and smooth muscle membranes. These data suggested a modulation of β-adrenoceptor function by antibodies directed against H-2 class I histocompatibility molecules, probably through molecular interactions between both structures.

The participation of major histocompatibility complex (MHC) genes and products in various ligand–receptor interactions has been studied extensively and documented. It includes the association between MHC haplotypes and glucagon and insulin binding to liver cell membranes [1, 2], the co-immunoprecipitation,but not co-internalization, of insulin receptors by anti-class I monoclonal antibodies (Mab) [3–5], and the interaction between class I antigens and epidermal growth factor (EGF) receptors on human cells [6].

Despite these associations of class I H-2 molecules with growth factor and growth-factor-"like" receptors, we also found interactions with other types of receptors. On the one hand, we have demonstrated that alloiummune antibodies (Ab) are able to induce testosterone synthesis in murine Leydig cells of the appropriate H-2 haplotypes and that these antibodies could precipitate the soluble luteinizing hormone–receptor complex [7]. On the other hand, we also studied associations of class I antigens with sympathetic system receptors, specifically the β-adrenoceptors. Thus we reported that alloimmune sera exert biological effects over the myocardium, increasing its beating tension and frequency through an activation of postsynaptic β-adrenoceptors [7, 8]. Moreover, we demonstrated that the pharmacologic effect of alloimmune sera is exerted by its purified IgG and that the specificity for alloantibody (alloAb) action, in both induction and expression, is directed against murine class I histocompatibility (HC) products [9]. In support of this last point, we found that only those sera obtained from immunizations between H-2 disparate strains are active, exerting their biological actions not only on atria from the immunizing strain but also on atria from other strains carrying the same H-2 haplotype [9]. In addition, by using alloimmune monospecific sera, we confirmed that only those sera directed against class I products have pharmacologic activity; sera directed against class II antigens, though showing high cytotoxic titers compatible with the Ia region haplotype from the assayed strains, have no effect on atria [9].

Wishing to analyze more thoroughly alloAb ligand and β-adrenoceptor interactions, we now show that alloimmune IgG can exert its biological action not only at cardiac β_1-adrenoceptor rich tissue but also over β_2 smooth muscle adrenoceptor preparations, leading in both cases to an increment of intracellular cyclic AMP (cAMP) levels and to consequent contractile activity. We have confirmed that these effects are mediated by class I molecule recognition, as monoclonal antibodies to D and/or K antigens exerted pharmacological activities in both myocardium and smooth muscle preparations from appropriate haplotypes.

We also demonstrated a reciprocal steric interference in both tissues between β-adrenergic ligands and alloAb fixation, that pointed to a close molecular relationship between the above-mentioned structures.

METHODS

Mice. BALB/c (H-2^d) and C_3H (H-2^k) inbred mice (obtained from the Comisión Nacional de Energía Atómica de la República Argentina) were used throughout. All animals were 60- to 90-days old, and H-2 haplotypes were checked by microcytotoxicity tests using monospecific alloimmune sera (origin: National Institutes of Health, Bethesda, MD, U.S.A.) as previously described [8].

Immunizations. Alloimmunizations of BALB/c mice with C_3H lymphoid cells were performed among

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animals of the same sex and following immunizing schedules described elsewhere [7, 8]. Alloimmune BALB/c anti-C_3H sera titers were determined by a trypan blue exclusion two-step complement-dependent microcytotoxicity assay, as described [8], using C_3H spleen or lymph node cell suspensions as targets.

Monoclonal antibodies. Antibodies for mouse MHC class I antigens were supplied by Dr. K. Ozato (Laboratory of Developmental and Molecular Immunity, NICHD, NIH). The monoclocal antibodies against class I *k* haplotype used were: 3.83 (K^kD^k) and 16.1.2 (K^kD^k). Both antibodies gave positive C'-dependent cytotoxic assays over C_3H lymphoid cells but non-significant lysis (titer below 1/10) on BALB/c lymphoid cells. Monoclonal anti-I-E^k (Ia. 7) antibody was from Cedarlane Lab. (CL 8705-A), Canada. This antibody reacts with antigen Ia. 7, which is expressed by the *k*, *d*, *p* and *r* haplotypes.

IgG purification. IgG purification from alloimmune sera was achieved by ion-exchange chromatography in DEAE-cellulose (Sigma Chemical Co.) columns by standard procedures as previously described [8]. IgG samples were concentrated by ultrafiltration (Minicon B 15 concentrator, Amicon Co.) to about 10 mg protein/mL. The purity of the IgG samples was tested by microimmunoelectrophoresis by using goat anti-mouse IgG and anti-mouse total serum proteins (Cappel Lab.). In both cases, only one precipitation line was obtained. The resulting alloimmune IgG had a cytotoxic titer of 1/64 over C_3H lymphoid cells.

Atrial and oviductal tract preparations. After the sacrifice of female C_3H mice, auricles or oviductal tracts (i.e. coiled oviduct plus its surrounding membrane, the mesosalpinx) were quickly removed, attached to a glass holder, and placed in tissue chambers containing a modified Krebs–Ringer–bicarbonate (KRB) solution; the ionic composition of this solution was described previously [10, 11]. Both tissue preparations were gassed with 5% CO_2 in O_2 and maintained at pH 7.4 and 30° or 37° respectively. For smooth muscle preparations, control values for isometric tension (mg) were recorded by using a force transducer coupled to an ink-writing oscillograph as described [11]. For atrial preparations, a constant resting tension of 500 mg was applied, and the activity of the spontaneously beating atria was assessed by recording the maximum rate of isometric force development (dF/dt in g/sec) above externally applied resting tension. Tissues were allowed to stabilize for 60 min. Experimental data obtained with normal or alloimmune IgG were recorded 15 min after each antibody addition, and these values were maintained for 60 min. For each experiment (either a repeat or a different treatment), a separate, not previously manipulated tissue was used.

Cyclic AMP assay. C_3H mice atria or oviductal tracts were excised immediately after sacrifice and were left with their spontaneous activity in 3 mL of KRB gassed with 5% CO_2 in O_2 at 30° or 37°, respectively, for 20 min with or without 10^{-7} M (−)-propranolol and then left for an additional 2 min without or with 5×10^{-7} M normal (IgG_N) or alloimmune BALB/c anti-C_3H (IgG_I) IgG. In all cases, total incubation time was the same under similar experimental conditions. Tissues were then homogenized with 6% ice-cold trichloroacetic acid (TCA) in water and centrifuged at 2500 *g* for 15 min at 4°. Proteins were determined by the method of Lowry *et al.* [12], after dissolving the pellet in 1 mL of NaOH in boiling water. The TCA supernatant fractions were extracted with 4 mL of water-saturated ethyl ether, three times. The ether phase was discarded, and the aqueous phase was heated at 56° to remove the ether and then was evaporated to dryness under a stream of N_2. Cyclic AMP in the residue was dissolved in 300 μL of 0.05 M sodium acetate buffer, pH 6.2. Aliquots of 100 μL were measured for cAMP content by a [^{125}I]-cyclic AMP RIA KIT (New England Nuclear), using a radioimmunoassay procedure. Both contractile activity and cAMP determinations were also carried out on preparations from chemically sympathectomized animals injected, 24 hr prior to being killed, with 16.5 mg/kg of 6-hydroxydopamine (6-OHDA). To assess the completeness of this denervation, the *in vitro* influence of tyramine and norepinephrine was evaluated.

β-Radioligand binding to purified cardiac and oviductal tract membranes. Cardiac and oviductal tract membrane preparations from C_3H mice, obtained as previously described [13], were preincubated for 30 min at 30° alone or with different concentrations of normal (IgG_N), alloimmune BALB/c anti-C_3H (IgG_I) IgG, or monoclonal anti-class I *k* antibodies. Membranes were then assayed for (−)-[^{3}H]dihydroalprenolol ((−)-[^{3}H]DHA) binding. Briefly, 100 μL of membrane suspensions and 1 nM (−)-[^{3}H]DHA (New England Nuclear; sp. act. 99.8 Ci/mmol) were incubated with stirring for 15 min at 37° in a total volume of 150 μL of 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$. At the end of the incubation period, 100-μL aliquots were placed into 2 mL of ice-cold buffer and immediately filtered through Whatman GF/C glass fiber filters. The filters were then washed with 10 mL of cold buffer, dried, added to 10 mL of Triton-toluene based scintillation fluid, and counted. Non-specific binding was determined by filtering aliquots of membranes incubated in the presence of 10^{-6} M (−)-propranolol. Specific binding was always ≥70%.

Indirect immunofluorescence staining. Cryostat sections (6–8 μm) of both heart and oviductal tracts from C_3H mice were fixed for 10 min with acetone, washed three times with phosphate-buffered saline (PBS) and then incubated for 45 min at 37° with BALB/c anti-C_3H IgG (cytotoxic titer 1/64, dilutions indicated in each case) following standard indirect immunofluorescence (IFI) techniques that have been described elsewhere [14]. The following controls were included: (a) a negative control with normal IgG or BALB/c anti-C_3H IgG upon BALB/c slides,and (b) fluorescein-labeled antibody alone. Quantitation was done by direct microscopic observation of the stained sections, by double-blind procedures; a strong green image was assessed as positive staining. It is worth noting that weak tincture was always considered as negative. For studying the effect of β-blockers over antibody fixation, cryostat sections were first incubated with drugs for 15 min at 37° and then incubated with alloimmune IgG as indicated.

Table 1. Biological effects of alloimmune IgG on the mechanical activity of isolated murine muscle tisues

Treatment*	Atria† dF/dt (g/sec)	N‡	Oviductal tract† Tension (mg)	N‡
Control	3.6 ± 0.1	20	381 ± 30	20
Normal IgG	3.4 ± 0.2	6	375 ± 26	8
BALB/c anti-C_3H IgG	5.9 ± 0.1§	8	118 ± 16§	10
Unrelated alloimmune IgG	3.3 ± 0.1	6	355 ± 29	8
3.83	6.2 ± 0.1§	4	180 ± 16§	4
16.1.2	4.5 ± 0.1§	4	234 ± 19§	4
Propranolol, 10^{-7} M, + BALB/c anti-C_3H IgG	3.5 ± 0.2	8	340 ± 32	10
Butoxamine, 10^{-6} M, + BALB/c anti-C_3H IgG	6.0 ± 0.2§	8	370 ± 34	8
Practolol, 10^{-7} M, + BALB/c anti-C_3H IgG	3.2 ± 0.2	8	120 ± 7§	8
para-Oxy-prenolol, 10^{-6} M, + BALB/c anti-C_3H IgG	3.3 ± 0.2	8	115 ± 6§	8

* Normal and alloimmune BALB/c anti-C_3H IgG (10 mg/mL) were tested for their effects on the mechanical activity of C_3H isolated atrial and oviductal tract preparations pretreated or not with solutions of different drugs (type of drug and concentration indicated in each case). Unrelated alloimmune IgG was from C_3H anti BALB/c sera; normal IgG came from BALB/c mice injected with saline. Monoclonal antibodies (Mab) directed to class I *k* products, namely 3.83 and 16.1.2, were also used. In all cases, tissues were exposed to 10 μL/mL of IgG.

† Auricles or oviductal tracts from C_3H female mice were suspended in KRB as described in Methods. Tissues were allowed to stabilize for 60 min. Experimental data obtained with normal or alloimmune IgG were recorded 15 min after addition of each antibody. Experimental procedures were also developed on myocardium and oviductal tract from 6-OHDA C_3H animals, in the presence of Mab 3.83. Values of dF/dt = 6.5 ± 0.2 g/sec ($P \leq 0.05$) and tension = 140 ± 20 mg ($P \leq 0.05$) were obtained.

‡ Number of preparations.

§ $P \leq 0.05$.

Drugs. Freshly prepared solutions of the following drugs were used: (−)-propranolol (Ayerst Lab.), butoxamine (Burroughs Wellcome Co.), practolol (Ayerst Lab.), and para-oxy-prenolol (Ayerst Lab.). Indicated concentrations are the final concentrations in the KRB bath. Except when indicated, all drugs were added 30 min before IgG additions.

Statistical analysis. Paired sample Student's *t*-tests were used to compare initial control and experimental values in the same preparations. When experimental data from different muscle preparations subjected to distinct treatments were compared, non-paired sample Student's *t*-tests were used. For binding data, the statistical considerations of Sagripanti *et al.* [15] were applied. In all cases, results were considered significant when $P \leq 0.05$.

RESULTS

Biological effects of alloimmune IgG on myocardium and smooth muscle preparations. The biological effects of alloimmune IgG on cardiac and smooth muscle preparations are shown in Table 1. It can be seen that alloantibodies increased contractility of electrically conducted auricles of appropriate haplotypes, an effect that was blunted by the non-selective β-antagonist propranolol or by β_1-blockers, i.e. practolol and para-oxy-prenolol, but not by the β_2-blocker butoxamine. On the contrary, in oviductal tract preparations alloimmune IgG induced a β_2-mediated inhibition of the spontaneous smooth muscle motility which was blocked only by non-selective or β_2-antagonists, but not by β_1-blockers. It should be noted that drugs alone had no effect over the contractile activity of either tissue (data not shown). Normal IgG or IgG directed to unrelated haplotypes exerted no effect on either tissue (Table 1). Moreover, Mab directed to class I molecules of the *k* haplotype modified the contractile activity of C_3H ($H\text{-}2^k$) atria and oviductal tract preparations in a manner similar to that of polyclonal alloimmune IgG (Table 1). It is worth noting that these Mab had no effect over BALB/c tissue preparations (data not shown), showing a correlation with data of cytotoxic assays. These data indicate that alloantibodies can induce activation of β-adrenoceptors both in cardiac and smooth muscle tissues, interacting with β_1- or β_2-adrenoceptors, depending on the β-subtype present in the target preparation.

To verify β-adrenergic cardiac and oviductal tract activation by alloantibodies, we analyzed their actions on cyclic AMP levels of muscle preparations. As can be seen in Table 2, a 2-min contact with alloimmune BALB/c anti-C_3H IgG or anti-class I *k* Mab increased the intracellular levels of cAMP nearly 100% above basal values in both tissues. The increment induced by 5×10^{-7} M alloimmune Ab was similar to that observed with 5×10^{-9} M isoproterenol, a β-agonist, and was abrogated completely by 10^{-7} M propranolol, confirming that the stimulatory effects of alloantibodies were mediated by a direct interaction with membrane bound β-receptor cyclase complex.

Table 2. Cyclic AMP production in cardiac and smooth muscle preparations by alloimmune IgG

Tissue		cAMP production* (pmol/ng protein) Without propranolol	With propranolol
Myocardium†	Alone	4.9 ± 0.4	5.3 ± 0.3
	IgG_N	5.0 ± 0.5	5.1 ± 0.4
	IgG_I	10.2 ± 0.6§	5.1 ± 0.5
	3.83	10.0 ± 0.8§	5.5 ± 0.4
	16.1.2	8.7 ± 0.8§	5.2 ± 0.5
	ISO	10.8 ± 0.8§	5.0 ± 0.2
6-OHDA-myocardium‡	IgG_N	4.8 ± 0.4	5.2 ± 0.2
	3.83	11.0 ± 0.9§	4.9 ± 0.1
Oviductal tract†	Alone	0.034 ± 0.003	0.033 ± 0.004
	IgG_N	0.035 ± 0.005	0.034 ± 0.005
	IgG_I	0.066 ± 0.009§	0.033 ± 0.003
	3.83	0.070 ± 0.007§	0.037 ± 0.002
	16.1.2	0.061 ± 0.006§	0.035 ± 0.002
	ISO	0.068 ± 0.007§	0.032 ± 0.002
6-OHDA-oviductal tract‡	IgG_N	0.037 ± 0.004	0.033 ± 0.003
	3.83	0.072 ± 0.006§	0.036 ± 0.003

* Cyclic AMP content of C_3H cardiac and oviductal tract homogenates was measured by a [^{125}I]-cyclic AMP-RIA KIT as described in Methods.

† Murine C_3H myocardium or oviductal tracts were maintained in KRB gassed with 5% CO_2 in O_2 for 20 min with or without 10^{-7} M propranolol and then left for an additional 2 min alone or with 5×10^{-7} M normal (IgG_N), alloimmune BALB/c anti-C_3H (IgG_I) IgG, Mab 3.83 or 16.1.2, or 5×10^{-9} M isoproterenol (ISO).

‡ Murine myocardium or oviductal tracts from animal injected with 6-OHDA 24 hr prior to being killed, as indicated in Methods, were maintained in a KRB bath as indicated above and assays were performed in the presence of IgG_N or Mab 3.83 as indicated.

§ Differs significantly from controls (myocardium or oviductal tract preparations alone), $P \leq 0.05$.

To discard the possibility of an indirect presynaptic action of alloimmune IgG, both the contractile and cAMP biological effects of IgG were measured in tissues from animals whose endogenous stores of catecholamines had been depleted by 6-OHDA. Both mechanical activity (legend Table 1) and cAMP levels (Table 2) were increased by Mab 3.83 on 6-OHDA-treated myocardium and smooth muscle preparations.

Reciprocal interference between alloAb binding and β-adrenoceptor specific ligand occupancy. To analyze more thoroughly the way in which class I products and β-adrenoceptors interact and to study whether or not a resemblance exists among these antigens and the β-adrenoceptor binding site, we investigated if there was a reciprocal interference between the occupancy of β-adrenoceptors and alloAb binding. We first analyzed the action of alloimmune IgG on the binding of the β-radioligand (−)-[^{3}H]DHA to purified murine cardiac and oviductal tract membranes of the correct haplotypes. In Fig. 1, we demonstrate that the specific binding of (−)-[^{3}H]DHA to both cardiac (A) and smooth muscle (B) membranes was inhibited in a concentration-dependent manner by alloimmune IgG, whereas normal IgG did not affect that binding. Monoclonal anti-class I *k* antigens (3.83) gave the same results over C_3H cardiac and smooth muscle purified membranes (Fig. 1, A and B). Neither Mab 3.83 over purified membranes from BALB/c (H-2^d) tissues (data not shown) nor a monoclonal antibody directed against class II *k* molecules on C_3H membranes (Fig. 1) had any inhibitory effect upon radioligand binding, pointing to a specific interaction between β-adrenoceptor and class I products expressed in the target membranes.

To determine if antibody binding to both cardiac and smooth muscle tisues is influenced when β-adrenoceptors are occupied by β-adrenergic ligands, we analyzed the actions of non-selective and selective β-blockers upon alloAb specific antigen recognition in both preparations. AlloAb fixation to cryostat sections of murine C_3H heart and oviductal tracts was analyzed by IFI techniques (Fig. 2). Normal IgG or unrelated C_3H anti BALB/c alloimmune IgG gave a slight green background coloration in cryostat sections of both tissues (data not shown). Alloimmune BALB/c anti-C_3H IgG gave positive images in both myocardium (Fig. 2, top panel) and oviductal tracts (Fig. 2, bottom panel) even though the staining was not homogeneous. When serially diluted, the green sarcolemmal stain was obtained up to a 1/160 dilution in both tissues (Table 3). When cryostat sections were incubated previously with the indicated increasing concentrations of the non-selective β-antagonist propranolol and then used for IFI procedures, an inhibition of antibody fixation in both

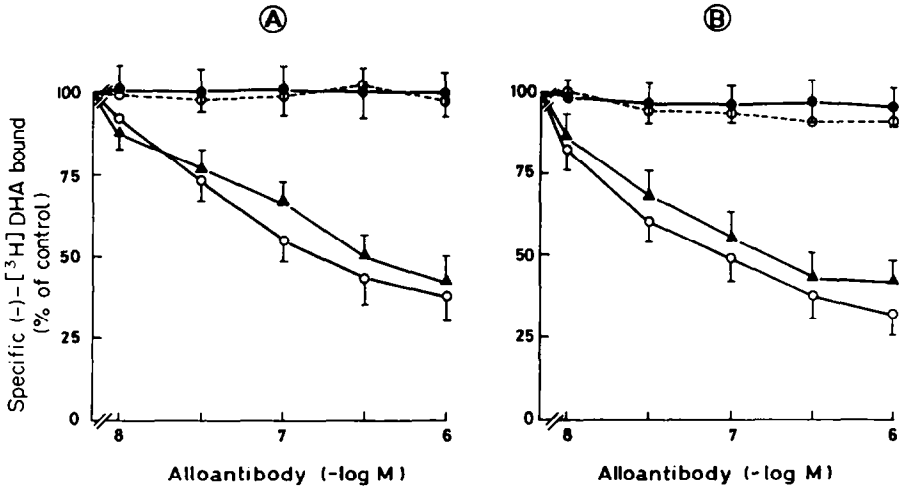

Fig. 1. β-Adrenoceptor radioligand binding inhibition in cardiac and smooth muscle purified membranes by alloimmune IgG. Cardiac (A) and oviductal tract (B) purified membranes from C_3H mice were preincubated for 30 min at 30° alone or with increasing concentrations of normal (●——●), alloimmune BALB/c anti-C_3H IgG (○——○), 3.83 Mab (▲——▲) or I-E^k Mab (○--○). Membranes were assayed for 2 nM (−)-[^{3}H]DHA binding as described in Methods. Control binding of 100% refers to the specific radioactivity bound to muscle membranes preincubated alone; the absolute values for specific binding were: 36.2 ± 2.4 fmol/mg protein and 22.1 ± 1.1 fmol/mg protein for heart and oviductal tract membranes respectively. The means ± SE of six independent experiments are plotted.

tissues was observed: antibody titer diminished in a dose-dependent manner with the drug until no positive image was obtained with 1×10^{-5} M propranolol. To confirm if drug action was specific for alloimmune Ab we analyzed its action over another antigen–antibody complex formation: using red sheep blood cells (RSBC) and an anti-RSBC IgG (origin mice, IFI titer 1/640), we observed that propranolol was not able to interfere with antibody fixation to RSBC preincubated with the drug, even at the higher concentrations (IFI titer with 10^{-4} M propranolol was still 1/640). Furthermore, when using selective β_1- or β_2-blockers at the same concentrations that inhibit alloimmune IgG contractile effects, IFI titers were diminished to 1/40 and 1/20 with practolol and *para*-oxy-prenolol, respectively, in myocardium, but the β_2-blocker butoxamine had no effect. On the contrary, in smooth muscle of oviductal tracts, β_1-drugs had no action on antibody fixation, and butoxamine was able to interfere with antigen recognition to a titer of 1/40. Fixation of cryostat sections with acetone did not seem to affect the structure of the remaining β-adrenoceptors, as was suggested by the (−)-[^{3}H]DHA binding assay over purified muscle membrane pretreated under the same conditions with the solvent. In this case, the number of receptors was reduced (i.e. for cardiac membranes, $K_D = 2.1$ nM, $B_{max} = 71.6$ fmol/mg protein for normal membranes; $K_D = 1.5$ nM, $B_{max} = 35.9$ fmol/mg protein for acetone-treated membranes), indicating the possibility of β-adrenoceptor loss or aggregation. However, the K_D of the receptors still available or functional remained unchanged. These data point to a close molecular relationship between β-adrenoceptors and murine HC antigen that takes place perhaps when they are aggregated in the cell plasma membrane.

DISCUSSION

We have demonstrated previously that alloimmune IgG, while specifically recognizing class I HC antigens of myocardium, is able to activate β-cardiac adrenoceptors, modifying the contractile activity of the tisue [7–9]. In the present study, we show that alloimmune IgG and monoclonal antibodies directed against class I H-2 molecules can exert mechanical activity not only over myocardium but over smooth muscle preparations as well. Similar results were obtained with monoclonal alloantibodies directed to class I products in testosterone-induced synthesis over murine Leydig cells [7]. Thus, alloantibodies increased the contractility of electrically conducted auricles via β_1-adrenoceptor activation as their effects were blunted by β- and β_1-adrenergic blockers only, and, in addition, they induced an inhibition of spontaneous motility of oviductal tract preparations by activating β_2-adrenoceptors of that tissue.

Alloimmune IgG specificity for β_1- and β_2-adrenoceptor reactivity seems to be related to the subtype of β-adrenoceptor that predominates in the responder tissue. It is well known that myocardium is rich in β_1-adrenergic receptors, whereas in smooth muscle β_2-receptors are predominant [16, 17]. This fact could be explained by taking into account alloAb immune specificity for class I HC antigens which are expressed in practically all cells. Thus, recognition of class I membrane antigens may induce activation of β-adrenoceptors present in the responder tissue. A support of this β-adrenergic activation is the increment of cAMP levels in both atrial and oviductal tract preparations. This stimulatory action was maximum with a 2-min contact of immune Ab with murine

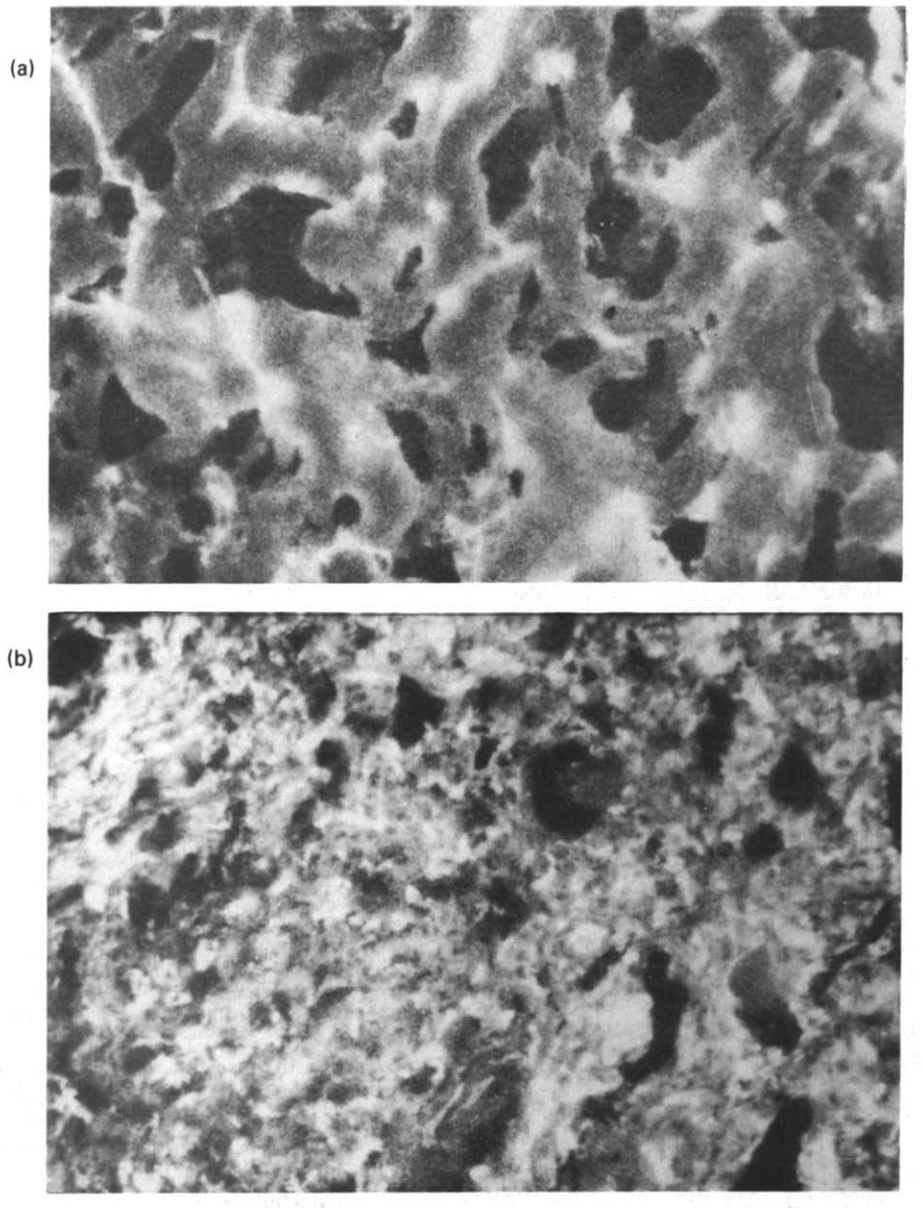

Fig. 2. Cryostat sections of murine C_3H heart and oviductal tracts treated with alloantibodies and fluorescein conjugated antibodies. Cryostat sections of heart and oviductal tracts from C_3H mice were incubated for 45 min at 37° with BALB/c anti-C_3H IgG (diluted 1/10) following standard IFI techniques described in the text. Specific green sarcolemmal coloration from a rabbit antiserum against mice IgG fluorescein conjugate (Miles Yeda Ltd.) (diluted 1/5) was obtained for both cardiac (top panel, 400×) and oviductal tract (bottom panel, 160×) preparations.

hearts and oviductal tracts, and was abrogated by pretreatment of tissues with propranolol.

It is worth noting that after denervation the β-adrenergic mechanical effect and the increment in cAMP levels persisted, confirming a previous report [8] that the alloimmune IgG could influence the postsynaptic sites on the plasma membrane.

Thus, the mechanism whereby alloimmune IgG may trigger mechanical actions is through a direct interaction with membrane-bound β-adrenoceptor cyclase components. Furthermore, polyclonal and monoclonal alloantibodies were able to inhibit in a dose–response manner the binding of (−)-[^{3}H]DHA, as radioligand, to murine purified membranes of myocardium and oviducts expressing the appropriate haplotypes. This action was not obtained when performed with tissues from unrelated haplotypes, confirming previous results [7]. Furthermore, other antibody against other membrane components, like class II products, was ineffective in inhibiting radioligand binding. The lack of interaction between β-adrenoceptors and class II products was reported

Table 3. Interference of alloimmune antibody fixation to cardiac and smooth muscle preparations by β-adrenergic blockers

Pretreatment of cryostat sections*		Antibody IFI titers†	
		Myocardium	Oviductal tract
Control		1/160	1/160
Propranolol	10^{-7} M	1/40	1/80
	10^{-6} M	1/20	1/40
	10^{-5} M	1/5	1/10
Practolol	10^{-7} M	1/40	1/160
Para-Oxy-prenolol	10^{-7} M	1/20	1/160
Butoxamine	10^{-6} M	1/160	1/40

*For the control experiment, cryostat sections of C_3H myocardium and oviductal tract preparations were incubated with alloimmune BALB/c anti-C_3H IgG and then treated with fluorescin-labeled antiserum as described in the text. To test the effects of the β-adrenergic blockers, cryostat sections of C_3H cardiac and oviductal tract preparations were preincubated with different drugs and then used to perform indirect immunofluorescence (IFI) techniques as in controls.

† Last alloimmune IgG dilution that give specific green sarcolemmal stain.

previously on lymphocytes expressing large amounts of these antigens [18]. Since it had been suggested that rodent myocardial membrane expressed small amounts of class I antigens except in the region of the intercalated disk that showed the major staining [19], we tested the alloAb fixation to both cardiac and smooth muscle tissues. As shown in our results, a non-homogeneous staining was seen, specially distributed in muscle membranes. However, alloAb binding to cryostat sections of both tissues could be impaired by β- and β_1-antagonists in cardiac tissues and by β- and β_2-blockers in smoth muscle preparations. The specificity of this blockade was confirmed because propranolol did not alter anti-SRBC fixation to its antigenic determinants on SRBC and because the blockade was observed in both tissues by selective β-adrenoceptor blockers.

These data point to a close molecular relationship between β-adrenoceptors and alloAb class I antigen ligands. Even though the exact mechanism involved in the phenomenon remains unknown, some possible explanations emerge from our results. One possibility is that β-adrenoceptors and class I antigens share antigenic determinants: this seems very improbable because the effect of alloantibodies is extremely specific for class I antigens of the immunizing strain [7, 9] and thus β-adrenoceptors would have as high a degree of polymorphism as HC antigens have [20] and, despite this, it is well known that β-adrenoceptors are phylogenetically conserved structures [21]. However, recent experiments show that insulin receptors in the activated state displace class I antigen β_2-microglobulin heavy chains and that cells depleted of class I antigen expression have reduced insulin receptor activity [22].

The other possibility is that β-adrenoceptor and class I antigen molecular associations are a consequence of membrane receptor aggregation. This could justify the reciprocal steric interference between alloAb and β-specific ligands. Aggregation of receptors may occur precisely during cryostat section fixation with acetone, as shown by binding data obtained with tissue membrane pretreated with the solvent. This membrane redistribution phenomenon would explain β-ligand impaired alloAb fixation in IFI assays. Other authors also observed a decrease in anti-HLA A_1 cytotoxic titers on appropriate HLA A_1 lymphocytes by means of drugs that bind to lymphocyte β-adrenergic receptors [23]. It was also demonstrated that binding of EGF inhibits the binding of anti-HLA antibody to human cells [6].

In addition, Schreiber *et al.* [6] have shown that alloAb inhibition of EGF interaction with its receptor could not be managed by Fab fractions, but it could be restored by using a cross-linking second antibody, indicating that antigen aggregation is necessary for alloAb action. Furthermore, Simonsen and Olsson [24] had proposed the existence of compound receptors, that attain their final active structure by rearrangement and assembly in the membrane of different subunits, with class I HC molecules, suggesting that biologically active insulin receptor may include the MHC class I heavy chain as a subunit. In our case, the biologically active form of β-adrenoceptor probably could be achieved in cellular membranes by physical interactions with class I alloantigens, leading in this way to aggregation and subsequent coupling to the other components of the adenylate cyclase system. This last step is essential for the biological activity of β-adrenoceptors, and it is also well known that the formation of microaggregates that include β-adrenoceptors after agonist binding is necessary for hormonal signal transmission within the cell [25].

Even though the exact mechanism involved in class I antigens and hormonal receptor interactions remains to be elucidated, these associations are not experimental artifacts: they have been demonstrated in various animal models and by different scientific groups from distinct parts of the world. Here, we are not only showing the existence of that sort of interaction between HC antigens and β-adrenoceptors, but we are also pointing to biological consequences of that MHC-mediated receptor modulation.

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