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Positive inotropic and chronotropic effect of alloimmune sera on isolated mouse atria[1]

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Summary. The effects of alloimmune sera on the contractile tension and frequency of spontaneously beating isolated mouse atria were explored. Immune sera enhanced frequency as well as tension; both effects were blocked by the presence of propranolol. In contrast, pretreatment with 6-OH dopamine potentiated the stimulatory action of immune sera.

In previous work it was documented that the sera of chagasic patients containing an antibody (EVI antibody) can interact with the plasma membrane, inducing functional changes in isolated rat atrial preparations[2]. The EVI positive human chagasic sera (EVI(+)S) could influence post-synaptic beta adrenoceptor sites of the plasma membrane acting as a partial beta agonist, increasing tension and frequency. Furthermore, EVI(+)S diminished the reactivity to exogenous norepinephrine through a reversible augmentation of its extraneuronal uptake[2,3]. In addition we have observed that EVI(+)S modified the action of ouabain on cardiac tissue through an activation of the beta adrenoceptors, increasing the influx of calcium[4,5].

In order to investigate whether sera directed againts other antigens could have similar effects on myocardial contractions, a series of experiments using alloimmune sera were done. We show here that BALB/c anti CF1 mouse sera are able to modify the physiological behaviour of isolated CF1 mouse atria. To determine the nature of the action of the alloimmune sera, the effects of propranolol and 6-hydroxydopamine were also studied.

Methods. Animals. Young adult (2-4 months) inbread BALB/c and CF1 mice from our colony were used throughout.

Sera. Immunizations were done with pooled lymphoid cells (from spleen, lymph nodes and thymus) obtained by pressing the organs through a stainless steel mesh. Cells were suspended in phosphate buffered isotonic solution (PBS). All immunizations were carried out between animals of the same sex. BALB/c mice were immunized by 1 i.d. injection of 10^7 CF1 lymphoid cells followed by 1-5 boosters of 3×10^7 CF1 lymphoid cells i.p. at weekly intervals. Animals

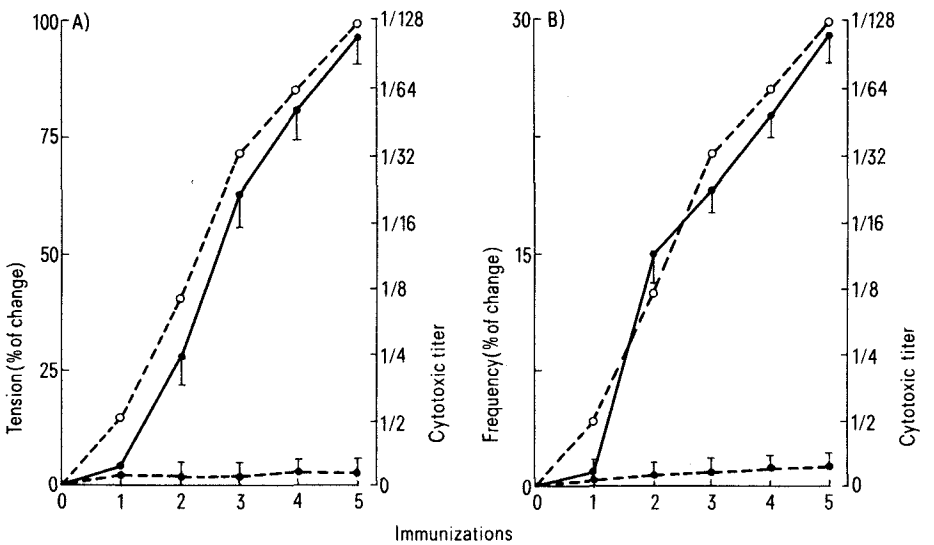

Figure 1. Positive and chronotropic effect of alloimmune sera on spontaneously beating atria. Cytotoxic titers (O - - - O) are shown in A and B in relation with the number of immunization. *A* Tension and *B* frequency in presence (● - - - ●) and absence of propranolol (●——●) are expressed as percent change from initial control ± SEM (n = 8 in each group).

were bled 4–6 days after the last injection. Control BALB/c and CF1 mice received PBS alone.

Antibody titers. Sera were titered for cytotoxic antibodies using a trypan blue exclusion test in a one-step complement-dependent cytotoxic assay. Briefly, CF1 thymus cells were incubated with 1:2 serial dilutions of the test serum; guinea-pig serum was added as a source of complement and 50% lysis was used as the titration point[6].

Atrial preparations. Mice of the CF1 strain were decapitated and their auricles removed and suspended in a modified Krebs-Ringer bicarbonate (KRB) solution gassed with 5% CO_2 in oxygen, maintained at pH 7.4 and 30 °C; the ionic composition of KRB was reported elsewhere[7]. After 1 h of equilibration, initial values for the isometric tension and the frequency of the spontaneously beating atria were measured as previously described[8]. Cumulative dose-response curves for alloimmune sera were constructed for untreated atrial controls as well as for auricles preincubated for 30 min with propranolol (10^{-7} M). Tests were also carried out on preparations from chemically sympathectomized animals injected 24 h prior to sacrifice with 16.5 $mg \cdot kg^{-1}$ of 6-hydroxydopamine (6-OHDA)[9]. In order to asses the efficiency of this denervation, the in vitro influences of tyramine and norepinephrine (NE) were checked. Experimental records were compared with initial controls and expressed as percent change. Differences between mean values were considered significant if $p = 0.05$ or less.

Results. As shown in fugure 1 the addition of 100 µl of BALB/c anti-CF1 serum to isolated CF1 mouse atria beating in KRB increased both tension and frequency; both actions of alloimmune sera were dependent on the number of immunizations and were parallel with the cytotoxic titers. Figure 1 also shows the very significant inhibition of those effects when atria preparations were preincubated during 30 min with (−)-propranolol (10^{-7} M).

As may be seen in figure 2 the degree of stimulation induced by an alloimmune serum with a higher titer of cytotoxicity (1/128) was directly proportional to its concentration. Both effects, on tension and frequency, developed with time and were maximal after 15 min. In contrast, normal sera from BALB/c or CF1 mice had no significant effect on isolated CF1 mice atria. Furthermore, BALB/c anti-CF1 serum had no effect on isolated rat atria (not shown).

Figure 2 also shows the reactivity of atria from CF1 mice subjected to chemical sympathectomy with 6-OHDA as compared with those obtained from untreated animals.

It can be seen that in denervated atria the ino and chronotropic positive effects of alloimmune sera were significantly higher than in normal control atria. Auricles from 6-OH dopamized animals were hypersensitive to 10^{-8} M NE and refractory to 10^{-6} M tyramine.

It should be noted that both tension and frequency of control, 6-OHDA denervated or propranolol treated atria had similar absolute values before the addition of experimental sera (table).

Discussion. The results of this study demonstrate that alloimmune sera can induce positive inotropic and chronotropic effects on isolated beating mice atria.

The isolated atrial preparation was selected as our experimental model because experimental cardiac anaphylaxis has been extensively studied in this tissue[10] and also because the atrium, including the sinoatrial node, is the most responsive part of the heart to anaphylaxis[11].

The fact that the increments of both tension and frequency were inhibited by beta adrenoceptor blockade suggests that the immune serum acted as a beta adrenergic agonist in a way similar to that reported with EVI(+) chagasic human sera[2,12].

Normal BALB/c or CF1 sera were ineffective in this system; this points to the necessity of preimmunization to

Absolute magnitude of tension and frequency of mouse auricles. Effects of (−)-propanolol and 6-hydroxydopamine (6-OHDA)

Conditions	Tension (mN)[a]	Frequency (beats/min)[a]	n
Untreated[b]	1.25±0.13	283±28.1	8
(−)-Propranolol[c] (1×10^{-7} M)	1.15±0.11	264±21.3	8
6-OHDA[b] (16.5 $mg \cdot kg^{-1}$ i.v.)	1.34±0.15	276±22.7	7

[a] Means±SEM. [b] Initial values recorded at 10 min following equilibration (see methods). [c] Values at 30 min after addition.

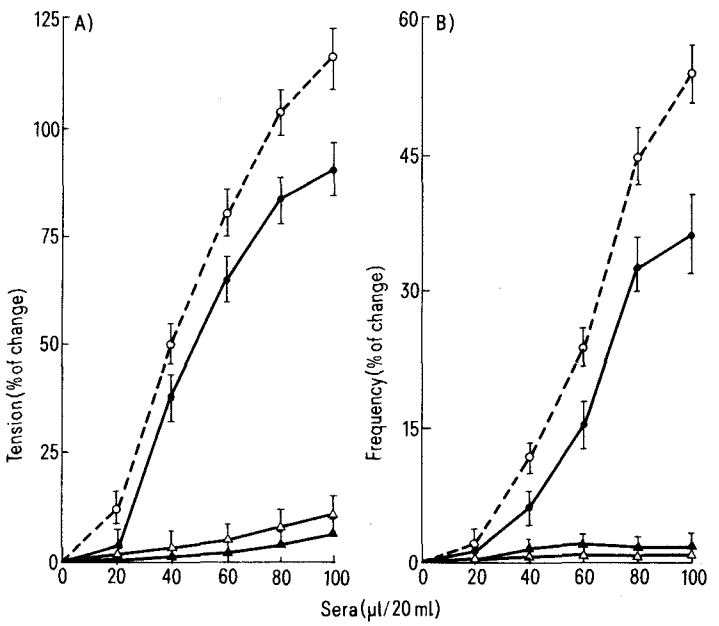

Figure 2. Effect of different concentrations of alloantiserum on *A* tension and *B* frequency of normal (●——●) and 6-hydroxydopaminized (○---○) mouse atria. Note the lack of effect of normal BALB/c (△——△) and CF1 (▲——▲) sera on the same parameters of normal atria.

bring about the response. The degree of stimulatory effects of alloimmune sera depended on serum concentrations and was directly proportional to the cytotoxic titers and the number of immunizations; this suggests that positive ino- and chronotropic effects of sera could depend or be related to the presence of antibodies.

The notion of adrenergic participation in the effects of alloimmune sera is strongly supported by the observation that after blockade of the beta adrenoceptors, all the stimulatory actions of antiserum were blunted.

In order to investigate if the beta adrenergic effect of the alloimmune sera was due to a direct post-synaptic reaction with the beta adrenoceptors or to an indirect action mediated by presynaptic release of endogenous norepinephrine, 6-OH dopaminized animals were used. After denervation, the positive inotropic and chronotropic action of alloimmune sera persisted, suggesting that the alloimmune sera could influence the post-synaptic sites on the plasma membrane.

The mechanism whereby alloimmune sera may trigger a beta adrenergic reaction is not known. Recently it was documented that antibodies raised against beta adrenergic receptors interact with a distinct site from the receptor for hormone interaction, but may still stimulate adenylate cyclase activity[13].

The present preliminary study indicates that alloimmune sera react efficiently with some unknown structure, somehow related to the beta adrenergic mechanism. This structure could be an antigenic determinant. Further studies will be required before any mechanism of action can be proposed.

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Positive inotropic and negative chronotropic effect of pantethine on isolated cardiac muscle of guinea-pigs

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Summary. It was demonstrated that pantethine, a component of co-enzyme A, produces positive inotropic and negative chronotropic actions in isolated cardiac muscle preparations from guinea-pigs.

A growth factor produced by *Lactobacillus bulgaricus* was discovered by Williams[2] and named LBF. LBF, later shown to be a substance that constitutes a part of the structure of co-enzyme A, is composed of pantothenic acid bound with β-mercaptoethylamine. LBF occurs in 2 forms: one being pantetheine and the other pantethine, its stable disulfide. These 2 forms convert into each other in vivo. The potential clinical usefulness of co-enzyme A, a substance essential to living organisms, has not been realized, owing to its in vivo degradation into pantethine and pantothenic acid. Pantethine, a co-enzyme A precursor, which is closer to co-enzyme A in structure than pantothenic acid, gives a high co-enzyme A yield in vivo (fig. 1). The present study was undertaken to determine whether chemically synthesized pantethine[3] has any effect on isolated atria and papillary muscle preparations from guinea-pigs.

Materials and methods. Male guinea-pigs weighing approximately 500 g were used. 1. Papillary muscle preparations obtained from the right ventricle using Gold and Cattell's method[4] were driven by electrical stimulation (rectangular wave, 5 msec duration, 1 Hz). These preparations were suspended in an organ bath containing Locke-Ringer's solution. The bath temperature was maintained at 37 °C and the solution was continuously aerated with a mixture of 95% O_2 and 5% CO_2. The composition of the Locke-Ringer's solution was as follows (mM): NaCl 154.0, KCl 5.63, $CaCl_2$ 2.16, glucose 11.0, $NaHCO_3$ buffer 2.4 (pH 7.4). The drug concentration given in this paper was the final concentration in the organ bath. Isometric tension of papillary muscle preparations was recorded by a strain gauge transducer. 2. Spontaneously beating atria were suspended in the same organ bath and the rate was recorded before and after administration of pantethine.

Results. 1. It was demonstrated that a pantethine concentration of 5×10^{-3} g/ml induced a significant increase in the isometric tension of the papillary muscles of guinea-pigs ($+17.6 \pm 8.8\%$, $p < 0.001$). Figure 2 shows the relationship between the concentration of pantethine and the contractile response of the papillary muscle preparations. No change in contractile force was observed at a concentration of 10^{-5} g/ml, but the contractile force increased dose-dependently at a concentration of 10^{-4} and 5×10^{-3} g/ml. The time-action curve for the positive inotropic effect of pantethine was also studied. Maximum tension was obtained approximately 10 min after administration of pantethine at a concentration of 5×10^{-3} g/ml. 2. The effect of pantethine on the heart rate in spontaneously beating atria of guinea-pigs was studied. Although pantethine concentrations of 10^{-5} g/ml did not produce any change in heart rate, concentration of 5×10^{-3} g/ml produced a significant