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Research Article

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Enhancement of Murine Cardiac Chronotropy by the Molecular Transfer of the Human β_2 Adrenergic Receptor cDNA

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Abstract

Cardiac pacemaking offers a unique opportunity for direct gene transfer into the heart. An experimental system was developed to assay the effects of transferring the human β_2 adrenergic receptor (β_2AR) under in vitro, ex vivo, and finally in vivo conditions. Constructs encoding either β_2AR or LacZ were used in chronotropy studies with isolated myocytes, and transplanted as well as endogenous murine hearts. Murine embryonic cardiac myocytes were transiently transfected with plasmid constructs. The total percentage of myocytes spontaneously contracting was greater in β_2AR transfected cells, as compared with control cells (67 vs. 42±5%). In addition, the percentage of myocytes with chronotropic rates > 60 beats per minute (bpm) was higher in the β_2AR population, as compared with control cells (37 vs. 15±5%). The average contractile rate was greater in the β_2AR transfected myocytes at baseline (71±14 vs. 50±10 bpm; $P < 0.001$) as well as with the addition of 10^{-3} M isoproterenol (98±26 vs. 75±18 bpm; $P < 0.05$). Based on these results, a murine neonatal cardiac transplantation model was used to study the ex vivo effects of targeted expression of β_2AR. The constructs were transfected into the right atrium of transplanted hearts. Injection of the β_2AR construct increased the heart rate by ~ 40% (224±37 vs. 161±42 bpm; $P < 0.005$). Finally, the constructs were tested in vivo with injection into the right atrium of the endogenous heart. These results were similar to the ex vivo data with injection of the β_2AR constructs increasing the endogenous heart rates by ~ 40%, as compared with control injected hearts (550±42 vs. 390±37 bpm; $P < 0.05$). These studies demonstrate that local targeting of gene expression may be a feasible modality to regulate the cardiac pacemaking activity. (*J. Clin. Invest.* 1998. 101:337–343.)
Key words: heart rate • pacemaker • gene therapy • heart transplantation • cardiac myocytes

Introduction

The physiologic depolarization of the heart originates in the sinus node located in the high right atrium. This depolarization spreads from the sinus node through the surrounding atrial tissue and then into the atrial-ventricular node before proceeding into the ventricular conduction system. The rate of sinus node depolarization results from the spontaneous depolarization of myocytes within the node (for review see reference 1). These spontaneous cellular depolarizations are automatic, and are, in turn, subject to both sympathetic and parasympathetic regulation. Myocytes from other areas of the heart also depolarize spontaneously, but at physiologic frequencies significantly lower than those of sinus nodal myocytes. Thus, impulses originating from sinus node depolarization suppress the spontaneous activity of myocytes in other areas. Increased activation of the sinus node elevates heart rate, whereas depressed activation of sinus node may lead to cardiac activation by impulses originating from other areas of the heart.

Extensive previous research has demonstrated that β adrenergic receptors (βAR)[1] regulate cardiac myocyte inotropic and chronotropic responses through a G protein–linked signaling pathway (for reviews see references 2–4). These signaling pathways involve both $G_{\alpha s}$-direct and cAMP-mediated interactions with ion channels involved in myocyte depolarization. Stimulation of βAR increases heart rate as well as cardiac inotropic force. Conversely, blockade of βAR decreases heart rate and cardiac contractility. The βAR-regulated response is also seen in cultured cardiac myocytes which exhibit an increased spontaneous depolarization rate as well as an augmented contractile force.

The period of automatic depolarization of the heart is shortened by stimulation of βAR in part through an increase in the flux of diastolic depolarization current (I_f) in cardiac myocytes (5). Moreover, the sinus node has a higher density of βAR compared with the surrounding atrium (6, 7), which in turn has a higher βAR density than the rest of the heart (8). This density of βAR and its regulation of the I_f current suggest that increases in the density of βAR in the vicinity of the sinus node may lead to an increase in heart rate.

The βARs are excellent targets for directed gene expression in the murine heart. The human β_2AR is a particularly attractive aim as it is immunologically distinct from, yet structurally and functionally similar to the murine receptor (9–11). Transgenic mice constructed with the α-MHC promoter fused to human β_2AR result in mice overexpressing β_2AR throughout the heart (12). These transgenic mice manifest enhanced myocardial function with increased heart rate and cardiac inotropy. This approach is somewhat limited because the β_2AR-mediated increase in cardiac inotropy as well as chronotropic responses cannot be separated. Therefore, it is difficult to determine whether a local gene delivery approach that selec-

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1. *Abbreviations used in this paper:* βAP, β actin promoter; βAR, β adrenergic receptor; bpm, beats per minute; ECG, electrocardiogram; GFP, green fluorescent protein.

tively elevates βAR density in right atrial and sinus nodal tissue would selectively increase cardiac rate in the intact heart.

The purpose of this study is to determine whether the local elevation of β_2AR density in the right atrium by direct gene transfer increases the rate of the heart. In addition, we plan to establish a set of model systems of increasing complexity to assess other candidate genes under in vitro, ex vivo, and finally in vivo conditions. This integrated approach should permit the prolongation/optimization of the effect of transferred exogenous genes on basal heart rate and cardiac rhythm, which will be helpful in most contemplated practical usage.

Methods

Plasmid constructs. The human β_2AR cDNA was a kind gift from Dr. Robert J. Lefkowitz (Duke University Medical Center, Durham, NC). A 2.25-kb Sal1-BamH1 fragment of the human β_2AR cDNA was ligated into a Sal1-BamH1 site 3′ to the β actin promoter (βAP) in a pBR322 vector to generate pBR322-βAP-β_2AR-SV40. In similar fashion, the bacterial β-galactosidase gene (LacZ) was ligated to the βAP in a pBR322 vector and served as a control expression vector. The plasmid phGFP-S65T encoding the green fluorescent protein (GFP) was purchased from Clontech (Palo Alto, CA).

Myocyte harvest. Cultured fetal murine myocardial cells were prepared as described previously (13, 14). Myocytes from ventricles of 17.5-d-old B6D2F1 fetal mice were fragmented with a straight-edge razor. The tissue was then digested with 0.5 mg/ml collagenase II (Worthington Biochemical Corp., Freehold, NJ) and 1.0 mg/ml pancreatin (Sigma Chemical Co., St. Louis, MO) in ADS buffer (116 mM NaCl, 20 mM Hepes, 1 mM NaH_2PO_4, 5.5 mM glucose, 5.4 mM KCl, 0.8 mM $MgSO_4$, pH 7.4) at 37°C for 10 min. The cells were centrifuged at 700 *g* at 4°C for 5 min. The cells were then plated onto 48-well plates (Falcon Labware, Cockeysville, MD) precoated with 1% gelatin or onto 25-mm^2 square coverslips precoated with 1% gelatin and 20 μg/ml laminin at a density of 10^5 cells/ml in DME supplemented with 10% FCS, streptomycin 100 μg/ml, and penicillin 100 U/ml. The myocytes were grown at 37°C in 5% CO_2.

In vitro myocyte transfection. The transfection of myocytes with lipofection was optimized. Myocytes were plated as described above and grown overnight. The GFP and β_2AR or LacZ encoding plasmids (1:2.5 M/M; 0.125 μg total DNA/well) were incubated with Lipofectamine (1 μl/well) in Opti-Mem I (GIBCO BRL, Gaithersburg, MD) reduced serum medium (12.5 μl/well) for 30 min at 25°C. After incubation, DME (100 μl/well) was added. The myocyte cultures were washed with PBS twice, the Lipofectamine–DNA mixtures were added, and the cultures incubated at 37°C. After 4 h, an equal volume of DME with 20% FCS was added to the cultures. The cultures were incubated overnight and the medium was changed to DME with 10% FCS.

The myocytes were assayed 48 h after transfection. Myocytes cotransfected with GFP were identified by inverted microscopy using epifluorescence filters for FITC $_{(\text{excitation 405 nm/emission 490 nm})}$. Expression of either human β_2AR or LacZ was confirmed by immunostaining or X-gal staining, respectively. Immunostaining for the human β_2AR was performed with a rabbit anti–human β_2AR polyclonal antibody (Santa Cruz Research, Santa Cruz, CA), which does not cross-react with the murine receptor. The myocyte cultures were washed with PBS and blocked with 10% normal serum in PBS for 20 min. Samples were then incubated with the primary antibody at 1.0 μg/ml in PBS with 1% BSA for 1 h in a humid chamber at 25°C. The sample was then washed with PBS three times and then incubated with the secondary donkey anti–rabbit Cy3 polyclonal antibody (Jackson ImmunoResearch, West Grove, PA) at a 1:1,000 dilution in PBS with 1% BSA for 1 h in a humid chamber at room temperature. The samples were washed with PBS three times and mounted with 90% glycerol in PBS.

Myocyte contraction rate determination. The percentage of beating myocytes was determined for cells transfected with either the β_2AR expression vector or the control construct. The myocytes were identified by GFP as described above. The total percentage of beating cells (≥ 1 contraction/min) was estimated visually from cotransfected GFP-positive myocytes (> 100 cells/point). In addition, the percentage of myocytes that were beating faster than 60 beats per minute (bpm) was determined in identical fashion. Similar measurements of the percentages of both total and fast beating myocyte were conducted at various concentrations of isoproterenol (control, 10^{-5}, 10^{-4}, and 10^{-3} M). Both the total percentage of beating myocytes and those with rates > 60 bpm were used as a measure of automaticity.

The average rate of myocyte contraction was quantitated by motion detector under both baseline and 10^{-3} M isoproterenol. Inverted microscopy with epifluorescence filters for FITC $_{(\text{excitation 405 nm/emission 490 nm})}$ and a video edge motion detector (Cresecent Electronics, Sandy, UT) were used to determine the average rate of the contractions of GFP-positive myocytes. The statistical significance of the increased rate of myocyte contraction was determined by Student's *t* test analysis.

Heart transplantation and DNA injection. Neonatal B6D2F1 murine hearts were transplanted into the pinneas of adult mice as described previously (15, 16). Briefly, recipient 6-wk-old adult B6D2F1 mice were anesthetized with avertin 2.5% (vol/vol). After cleaning the dorsum of the pinnea of the mouse ear with 70% ethanol, an incision penetrating only the epidermis, 2–5 mm in length, was made with a scalpel transverse to the longitudinal axis of the ear, 3–4 mm distal to its implantation into the skull. A small pocket between the skin and cartilage was blunt dissected toward the tip of the ear with delicate curved forceps. The total donor neonatal heart was excised without the pericardial sac and inserted into the ear pocket. Gentle pressure with the tips of the forceps was applied to the ear to express air from the pocket and facilitate the adherence between donor and recipient tissues. 4–6 wk after transplantation, the transplanted hearts were assayed for visual pulsation and electrocardiographic activity. Visual pulsation of the transplanted tissue was observed in the anesthetized host mice under stereoscopic microscopy. Electrocardiograms (ECGs) of the transplanted hearts were also recorded. Host mice were anesthetized and ECG limb leads were clipped to the ear surrounding the transplanted heart. ECGs were recorded with a Silogic EC-60 monitor (Silogic, Stewartstown, PA). Approximately 80% of the transplanted hearts were observed to have visual pulsations and electrocardiographic activity.

Transplanted hearts with both visual pulsations and electrocardiographic activity were then used in DNA injection experiments. After baseline ECGs were recorded from the transplanted hearts, expression vectors were injected into the atrium of the transplanted hearts similarly as previously described in murine skeletal muscle injection (17). Briefly, the β_2AR expression vector or the control construct (5 μl of DNA [2 μg/ml] in 20% sucrose, 2% Evans blue, in PBS) was injected into the transplanted hearts with a 33-gauge needle. Electrocardiographic activity was recorded daily for up to 7 d after the injections. The statistical significance of increased heart rates was determined by Student's *t* test analysis.

Native heart DNA injection. The right atria of 6-wk-old adult B6D2F1 murine hearts were injected with the expression vectors. Adult mice were anesthetized with avertin 2.5%, and baseline ECGs were recorded. The heart was exposed as described previously (18, 19). Briefly, the mice were then intubated and mechanically ventilated with a rodent ventilator (model 683; Harvard Apparatus, Inc., South Natick, MA) with room air. A right anterolateral thoracotomy was then performed and the heart was visualized. The β_2AR expression vector or the control construct was then introduced into the right atrial wall with a 30-gauge needle, as described above. The lungs were reexpanded and the chest was closed in three layers with 4-0 silk sutures. The mice were then allowed to recover spontaneous respiration. Electrocardiographic activity was recorded daily for up to 7 d after the injections. The statistical significance of increased heart rate was determined by Student's *t* test analysis.

β_2AR immunostaining. Sections of injected hearts were immunostained for the human β_2AR as described above. Briefly, β_2AR and control expression vector injected transplanted or intact hearts were sectioned to 8-μm sections and fixed with cold acetone for 10 min. The sections were then washed with PBS and blocked with 10% normal serum in PBS for 20 min. Samples were then incubated with rabbit anti–human β_2AR polyclonal antibody at 1.0 μg/ml as described above. Additionally, sections through the right lateral atrium, the periinjection site, were scored for the frequency of specific immunostaining in hearts injected with the control and β_2AR constructs.

Results

Myocyte contractile recruitment. The percentage of cardiac myocytes that beat under baseline conditions was higher in the population of cells transfected (3–5% efficiency) with the β_2AR expression vector, as compared with the control LacZ expression vector. Fig. 1 *A* shows that under baseline conditions 67% of the β_2AR transfected myocytes exhibit spontaneous contractions as compared with 42% in the control LacZ transfected cells. The addition of increasing concentrations of isoproterenol elevated the percentage of control LacZ transfected myocytes to 69%, which is similar to the percentage of β_2AR transfected cells contracting at baseline. Moreover, the addition of isoproterenol failed to increase the number of contracting β_2AR transfected myocytes.

The percentage of myocytes with chronotropic rates > 60 bpm (fast beating cells) was higher in the β_2AR transfected myocytes as compared with the control transfected cells. Fig. 1 *B* shows that in the absence of isoproterenol 37% of the β_2AR transfected cells beat fast as compared with 15% of the control transfected cells. As compared with the total percentage of contracting cells, the number of fast beating cells increased in both the β_2AR and control populations with addition of isoproterenol. At 10^{-3} M isoproterenol, the percentage of β_2AR transfected cells increased to 57%, which was not different from the response of the control transfected cells (54%).

Direct rate measurements. The average rate of contraction determined by motion detection was higher in the β_2AR transfected myocytes, as compared with control transfected cells under both baseline conditions and in the presence of 10^{-3} M isoproterenol. Fig. 2 reveals that under control conditions the average rate of contraction of β_2AR transfected myocytes was significantly higher as compared with control transfected cells (71±14 vs. 50±10 bpm; $P < 0.001$). The average rate of contraction increased by a similar proportion in both populations with the addition of isoproterenol. In the presence of 10^{-3} M isoproterenol, the average rate of contraction of β_2AR transfected myocytes was significantly higher as compared with the control cells (98±26 vs. 75±18 bpm; $P < 0.05$).

Heart transplant DNA injections. Injection of transplanted hearts with the β_2AR expression vector, as compared with control constructs, generated an increased heart rate. ECGs recorded from the pinnea surrounding the transplanted neonatal hearts demonstrate that β_2AR injected hearts exhibit faster cardiac rates, as compared with hearts before injection and to hearts injected with control constructs (Fig. 3 *A*). Recordings before injection revealed consistent electrocardiographic activity with an average heart rate of 180±20 bpm (Fig. 3 *B*). 2 d after injection with the β_2AR expression vector, the heart rate increased to 220±20 bpm, which was significantly higher than the preinjection heart rate ($P < 0.001$) or the heart rate observed with control constructs ($P < 0.005$). The increased heart rate was sustained for 3–4 d, after which the heart rate returned to baseline levels.

Adult heart DNA injections. Injection of the β_2AR expression vector increased the density of receptor in the right atrium of endogenous hearts. Immunostaining for the human β_2AR 3 d after injection revealed right atrial expression in the hearts injected with the β_2AR expression vector, but not in the hearts injected with control construct (Fig. 4, *A–D*). Expression of the

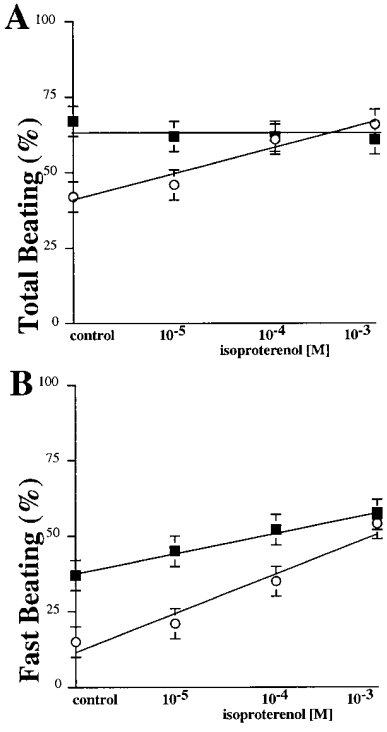

Figure 1. In vitro cardiac myocyte chronotropic recruitment. (*A*) The percentage of cardiac myocytes contracting, in the β_2AR transfected cells (*black boxes*) and control cells (*white boxes*), in the presence of increasing concentrations of isoproterenol (0–10^{-3} M) was determined as described in Methods. (*B*) The percentage of cardiac myocytes with a chronotropic rate > 60 bpm, in the β_2AR transfected (*black boxes*) and control cells (*white boxes*), in the presence of increasing concentrations of isoproterenol (0–10^{-3} M) was determined as described in Methods.

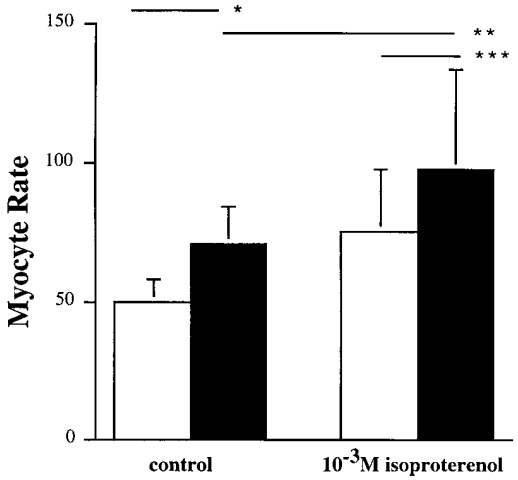

Figure 2. In vitro cardiac myocyte chronotropic rates. The average chronotropic rate of β_2AR transfected cardiac myocytes (*black bars*) and control cells (*white bars*) in the presence of 10^{-3} M isoproterenol, was determined as described in Methods. *$P < 0.00001$; **$P < 0.001$; ***$P < 0.05$.

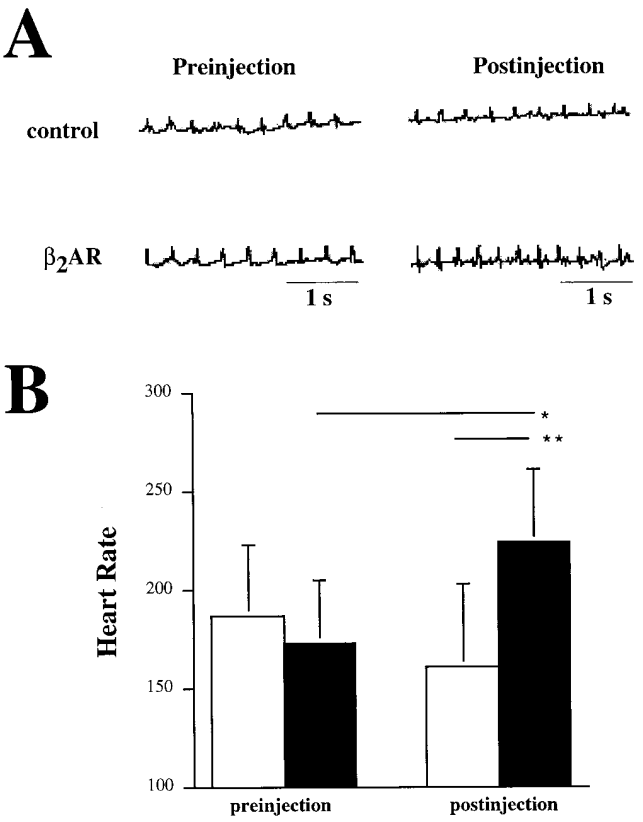

Figure 3. Transplanted hearts injected with the β_2AR expression vector. (*A*) Representative ECG tracings of the transplanted neonatal murine hearts before and 2 d after injection with either the β_2AR construct ($n = 10$) or the control construct ($n = 10$). (*B*) The average basal rate of the transplanted hearts before and 2 d after injection with the β_2AR construct (*white bars*) or the control construct (*black bars*). $*P < 0.001$; $**P < 0.05$.

human β_2AR was detected in 81±13% of the myocytes in the periinjection site of the targeted hearts, with no specific staining in the control injected atria. Surface ECGs recorded from adult mice demonstrate that hearts injected with the β_2AR expression vector exhibit a higher rate of contraction as compared with hearts before injection as well as to the hearts injected with the control construct (Fig. 4 *E*). The average heart rate of the anesthetized adult mice was 370±20 bpm before injection (Fig. 4 *F*), similar to previously reported rates in resting and anesthetized mice (12). 2 d after injection with the β_2AR expression vector, the heart rate increased to 550±42 bpm, which was significantly higher as compared with the preinjection heart rate ($P < 0.01$), as well as to the control construct postinjection heart rate ($P < 0.05$). The increased heart rate was sustained for 2–3 d, after which time the heart rate returned to baseline levels.

Discussion

The experiments outlined in this paper were directed at assembling a combination of in vitro, ex vivo, and in vivo gene transfer techniques to identify and study genes that can be used to selectively upregulate heart rate and alter cardiac rhythm in the intact heart. Our goal is to eventually locally deliver DNA and and/or molecularly engineered myocytes which can activate the sinoatrial or atrioventricular nodes or serve as foci of automatic activity for varying periods. We have chosen to focus our initial efforts on β_2AR since considerable accumulated evidence as outlined in the Introduction and summarized below suggests that selective overexpression of the receptor in the immediate vicinity of the sinoatrial node might accomplish the above goal.

Previous investigations have developed a variety of approaches for expressing exogenous genes in cardiac myocytes under in vitro and in vivo conditions. Transient transfection of isolated cells by the calcium phosphate or lipofection technique with the subsequent determination of the rate of contraction allows initial screening of candidate genes which might upregulate cardiac chronotropic rate. Cardiac myocytes were derived from fetal hearts in order to take advantage of their enhanced viability and transfection frequency relative to adult-derived cells (20). Unfortunately, the efficiency of transfection is relatively low (3–5%) which requires cotransfection with markers that permit selective monitoring of transfected cells. In our case, we used GFP in conjunction with inverted microscopy and epifluorescence filters to manually count the contraction rates of myocytes (spontaneously beating or fast beating cells) or with a video edge motion detector to obtain the average rate of contraction of all transfected cells. Prior studies have also used an adenoviral system to achieve virtually complete transfection of isolated myocytes which obviates the need for marking transfected cells (21). This latter approach has been used to overexpress the human β_2AR in rabbit cardiac myocytes with a documented enhancement in the relevant signaling pathway (22). In similar fashion, Johns and co-workers transfected cultured rat cardiac myocytes with an adenoviral-like vector to express a voltage-gated potassium channel (23). Although we have not used this approach for in vitro assessment of candidate genes that upregulate heart rate or alter cardiac rhythm, the above technique represents a powerful approach to achieve this goal.

Transgenic mice have also been constructed that overexpress human β_2AR throughout the murine heart, which results in a significant augmentation in heart rate and inotropy (12). This technique is of limited value for our purpose because the widespread expression of the candidate gene throughout the heart prevents us from determining whether local delivery of DNA or cells will upregulate heart rate and alter cardiac rhythm. Atrial targeting of a transgene may be achieved with previously described atrial specific promoters (24) and has been used to overexpress the human β_1 adrenergic receptor (25). Interestingly, these mice, unlike the β_2AR transgenic mice, lacked enhanced chronotropy, potentially due to pronounced downregulation of the constitutively overexpressed receptor. Using inducible elements in concert with the atrial-specific promoter may decrease such downregulation, and may be valuable in the final evaluation of candidate genes, particularly if their expression could specifically be targeted to the sinus node or other critical conduction tissue. However, the transgenic approach as an intermediate step in the evaluation of candidate gene was significantly limited by the time period necessary for in vivo generation of the mice.

The adenoviral viral vectors as described above allow for a much more rapid assessment of candidate genes under in vivo conditions. This approach has been used with high efficiency for transduction of exogenous genes into intact or transplanted

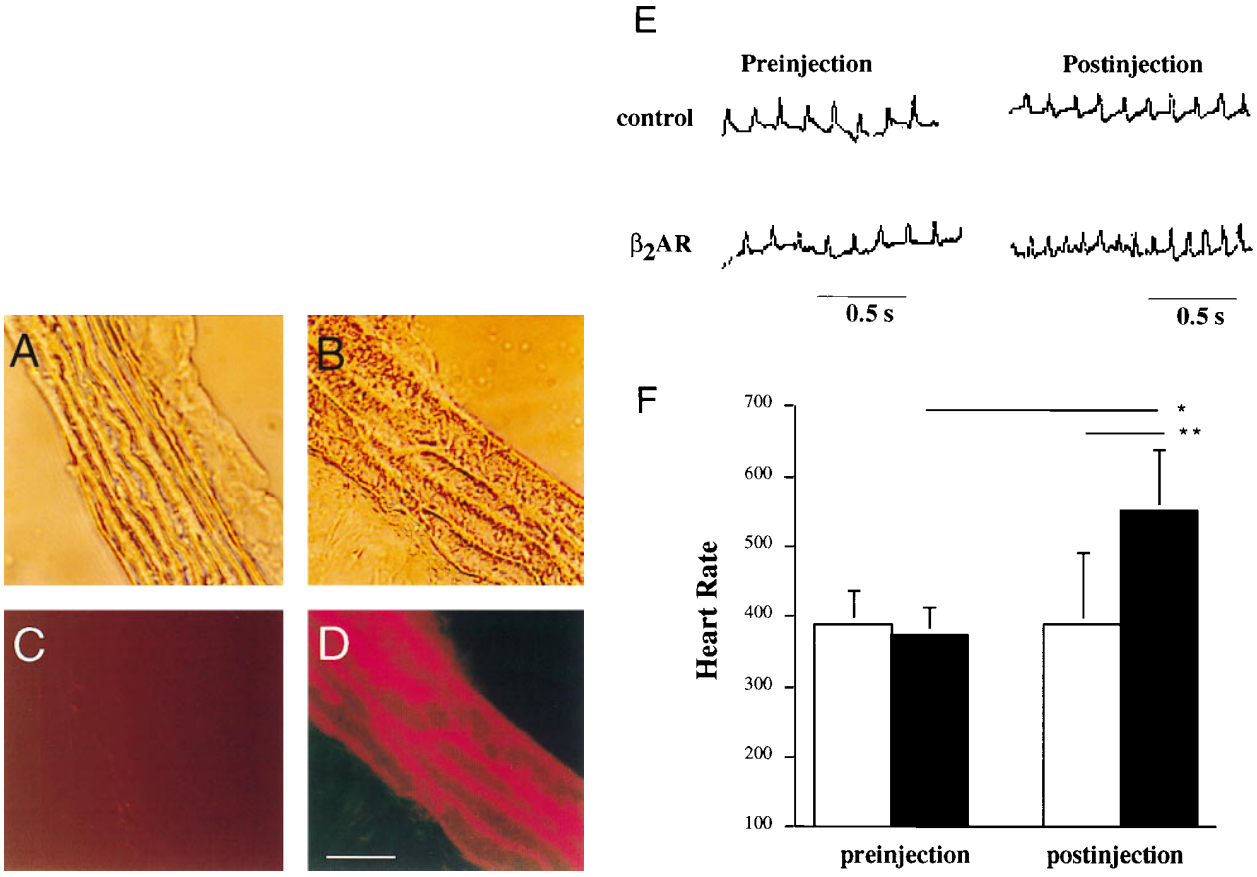

Figure 4. Endogenous hearts injected with the human β_2AR expression vector. Phase and immunofluorescent immunostaining for human β_2AR in 8-μm sections through the right atrium injected with control construct (*A* and *C*) or (*B* and *D*) the β_2AR expression vector. (*E*) Representative ECG tracings of murine hearts before and 2 d after injection with either the β_2AR construct ($n = 7$) or the control construct ($n = 8$). (*F*) The average rate of murine hearts before and 2 d after injection with the β_2AR construct (*black bars*) or the control construct (*white bars*). Bar, 70 μm. $^*P < 0.01$; $^{**}P < 0.05$.

hearts (26, 27). Unfortunately, similar to the transgenic approach, the cardiac expression of adenoviral-mediated genes is widespread and cannot be used to evaluate local effects of targeted constructs or engineered cells. However, transfection of expression vectors with injection or lipofection has been extended recently to intact or transplanted hearts, allowing local delivery of exogenous genes to the intact organ. The direct introduction of DNA with these methods has successfully modified the function of the heart in vivo, although the latter data have not always completely agreed with in vitro results (19, 28, 29). Similarly, intravascular transfection of DNA as outlined above has also been used to achieve local coronary arterial expression in native as well as transplanted hearts (30). The discrepancies observed between in vivo data and in vitro results may be due to low level expression of exogenous genes in restricted areas of the heart.

Given the limitations of the available methods, we chose to use transient transfection of cultured myocytes with expression vectors and Lipofectamine as the initial screen for assessing candidate genes that upregulate heart rate or alter cardiac rhythm. We then used a similar approach to locally deliver exogenous genes to the intact contracting murine heart transplanted into the mouse ear which permits a rapid appraisal of the action of the candidate gene at the whole organ level that can be used for rapid evaluation of multiple constructs. Finally, we injected the exogenous gene into the right atrium of the intact murine heart to determine its effect on heart rate and cardiac rhythm under conditions approaching the situation under which it will be ultimately used.

We demonstrated initially that expression of the human β_2AR in isolated murine fetal myocytes leads to a significant recruitment of cardiac cells to both spontaneously contract as well as to beat at a higher rate. This suggests that the expression of β_2AR leads to both increased automatic depolarization of myocytes as well as a higher steady state signaling through the receptor. The latter result is in accordance with recent studies in which adenoviral-mediated overexpression of human β_2AR in rabbit cardiac myocytes generates raised adenylate cyclase activity (22). We then showed that addition of the adrenergic agonist isoproterenol recruited a higher percentage of control myocytes to the contractile state, but did not alter the percentage of contracting β_2AR transfected cells. Moreover, saturating levels of isoproterenol, 10^{-3} M, raise the same percentage of myocytes to the higher contractile rate in both β_2AR transfected and control myocytes. The concentration of isoproterenol required to achieve maximal stimulation is similar to levels of agonist used in previous studies using embryonic cardiac myocytes (31, 32). Finally, the above level of agonist increased the average contractile rate of the β_2AR transfected myocytes to significantly higher levels as compared

with control cells. These data suggest that enhanced signaling through β_2AR overexpression leads to an increased rate of spontaneously cardiac myocyte beating, as well as an increased extent of steady state signaling which further augments the rate of contraction. These two effects are induced in control cells by addition of isoproterenol and the employment of the agonist in combination with transfection allows an augmented response to the drug.

The in vitro experiments outlined above served as an excellent foundation for extending the investigation to the next phase of the evaluation. The results obtained with cultured cardiac myocytes transfected with the β_2AR suggested that expression of the receptor in cardiac tissue should result in an increased heart rate. The above hypothesis was initially tested in an ex vivo model. The transplanted neonatal hearts served as an intermediate test in the progression from in vitro to in vivo models of β_2AR gene transfer. The subdermally transplanted heart, as compared with the native heart, possesses the advantage of being easily accessible, which permits injection of constructs under direct observation without the need for complex surgical procedures. Furthermore, ECGs of the transplanted hearts can be recorded from leads attached to the host ear, and are electrically isolated from the host heart which can be used as a control.

The injection of transplanted hearts with constructs encoding the β_2AR elevated the basal rate of cardiac contraction of the transplanted heart for several days, presumably during the expression of the β_2AR construct with no other additional alterations in the ECG. These results demonstrate that the in vitro observations of β_2AR-mediated enhancement of myocyte chronotropy are predictive of the ability of the transferred exogenous gene to increase basal heart rate in the whole organ. We speculate that the effects of β_2AR on the spontaneous depolarization of myocytes are of particular importance in this regard. Moreover, the above results suggest that injection of candidate genes into transplanted hearts should serve as an excellent model for testing cardiac gene therapy targets.

Based on the ex vivo and in vivo data, we injected β_2AR constructs into the right atrium of native murine hearts which generated a marked increase in cardiac rate as compared with control plasmids for several days, presumably during the expression of the β_2AR construct. Similar to observations made with the ex vivo model, minimal changes were noted in the ECGs of β_2AR transfected hearts except for the increased basal rate. The expression of the encoded human β_2AR is confined to the right atrium of the injected hearts, as demonstrated by immunohistochemical analyses which suggest that β_2AR-enhanced stimulation is initiated in the right atrium and then proceeds through the normal conduction system of the heart.

In summary, these studies demonstrate that the basal rate of the heart can be enhanced by local delivery of exogenous genes which can be used to improve our understanding of the regulation of cardiac automaticity. In addition, these investigations also provide an integrated experimental approach for identifying candidate genes and developing local delivery approaches for maximizing/prolonging the effects of these candidate genes in upregulating heart rate and altering cardiac rhythm. Indeed we envision that the above approaches may eventually be useful in the potential development of both transient molecularly mediated and stable cellular-based cardiac pacemakers.

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