Gene Injection Into Canine Myocardium as a Useful Model for Studying Gene Expression in the Heart of Large Mammals

Rüdiger von Harsdorf, Robert J. Schott, You-Tang Shen, Stephen F. Vatner, Vijak Mahdavi, and Bernardo Nadal-Ginard

We have investigated the regulated expression of genes injected into the heart of large mammals in situ. Reporter constructs using the chloramphenicol acetyltransferase gene under the control of muscle-specific β-myosin heavy chain (β-MHC) or promiscuous (mouse sarcoma virus) promoters were injected into the canine myocardium. There was a linear dose–response relation between the level of gene expression and the quantity of plasmid DNA injected between 10 and 200 μg per injection site. The level of reporter gene expression did not correlate with the amount of injury imposed on the cardiac tissue. There was no regional variation in expression of injected reporter genes throughout the left ventricular wall. By use of both the mouse sarcoma virus and a muscle-specific β-MHC promoter, reporter gene expression was one to two orders of magnitude greater in the heart than in skeletal muscle. Expression in the left ventricle was threefold higher than in the right ventricle. Chloramphenicol acetyltransferase activity was detected at 3, 7, 14, and 21 days after injection, with maximal expression at 7 days after injection. Statistical analysis of coinjection experiments revealed that coinjection of a second gene construct (Rous sarcoma virus–luciferase) is useful in the control of transfection efficiency in vivo. Furthermore, using reporter constructs containing serial deletions of the 5′ flanking region of the β-MHC gene, we performed a series of experiments that demonstrate the utility of this model in mapping promoter regions and identifying important regulatory gene sequences in vivo. Thus, gene injection into canine myocardium has proven to be a powerful tool in the study of regulated gene expression in large mammals in vivo, with the potential of providing useful clues about the regulation of gene expression prevailing in human myocardium.

(\textit{Circulation Research} 1993;72:688–695)

**KEY WORDS** • in vivo gene transfer • large mammals • canine myocardium • β-myosin heavy chain • in vivo promoter mapping

Until recently, regulated gene expression in vivo has been studied by using transfection assays of cultured cells\textsuperscript{1–3} and/or by creating transgenic animals.\textsuperscript{4–7} Particularly in the case of terminally differentiated cells, such as the cardiac myocytes, the use of cultured cells has been hampered by low transfection efficiencies and the difficulty in reproducibly obtaining sufficient quantities of primary cells when phenotypically suitable established cell lines are not available. These problems are further accentuated in the study of genes expressed in the myocardium. Indeed, even in the best of cases, the interpretation and physiological significance of the results is weakened by the fact that the cells are not embedded in their natural environment. These reasons and the paucity of information available on cardiac gene regulation has stimulated the search for alternative approaches.

Introduction of exogenous DNA into germ cells and the creation of transgenic animals has greatly improved the study of gene regulation in a physiologically meaningful environment, as well as during development and organ differentiation,\textsuperscript{8} but for practical purposes, this approach is limited to small mammals, the mouse in particular, because of the significant effort and costs related to the creation of transgene carriers and the fact that the vast majority of genetic knowledge in mammals originates from the mouse. Thus, the recent demonstration of gene transfer in vivo by simple injection of pure plasmid DNA into skeletal muscle of living mice\textsuperscript{9} was a significant development that provided a convenient approach in the study of gene regulation and combined many of the advantages of the in vitro transfections and the transgenic models for short-term analyses. Varia-
tions of this principle for in vivo gene transfer have been used to deliver recombinant DNA\textsuperscript{2–11} or in vitro genetically altered smooth muscle cells\textsuperscript{12,13} to vascular endothelium via catheter, resulting in low levels of gene expression. In addition, this model of gene transfer in vivo offers a potential route for gene therapy in human disease.\textsuperscript{14}

The potential to take up and express naked DNA introduced by simple injection is not limited to skeletal muscle. In the recent past, several laboratories have demonstrated the expression of naked recombinant DNA injected into the rat heart.\textsuperscript{15–18} In this model, expression of the injected genes, as determined by histochemistry, appears to be localized around the injection channel,\textsuperscript{15,16} with a small number of cells actually expressing the gene product.\textsuperscript{17} The transfection efficiency appears to be higher with closed circular than with linear plasmid DNA.\textsuperscript{15} Southern blot analysis of DNA obtained from skeletal muscle of mice injected with plasmid DNA provided the first evidence of the episomal localization of the gene construct.\textsuperscript{8}

Rodents differ substantially in their cardiovascular physiology and pathophysiology from humans in the rate of metabolism, as reflected in the heart rate, in the pattern of cardiac contractile isoforms,\textsuperscript{19,20} and in the induction of isofrom switches during development and hypertrophy.\textsuperscript{21,22} Moreover, although small mammals have been the model of choice for genetic and molecular analyses, large mammals, particularly the dog, have been the model of choice for physiological studies, especially when a large heart is necessary for the analysis of cardiac performance. These facts, together with the closer similarities between canine and human cardiac physiology, make this species the animal of choice for the analysis of gene regulation under experimental conditions that are physiologically meaningful and that could provide relevant insights into expression patterns prevailing in humans.

For all these reasons, we have developed a methodology to study gene expression in large mammals by injection of plasmid DNA into canine myocardium. Our data, which show that the predominant isoform of contractile proteins in the canine myocardium is, as in humans, the \(\beta\)-myosin heavy chain (\(\beta\)-MHC), further support the use of this model for studies relevant to human cardiovascular biology. Several important parameters relevant to the expression of injected gene constructs in this model have been characterized. The results demonstrate that the canine myocardium is an excellent receptor for injected genes and provides an ideal system for the analysis of gene expression in vivo under carefully monitored physiological conditions.

**Material and Methods**

**Plasmids**

MSV-CAT was created by fusing the coding sequence of the chloramphenicol acetyltransferase (CAT) gene\textsuperscript{23} to the long terminal repeat of the mouse sarcoma virus (MSV). Rous sarcoma virus (RSV)–luciferase was described previously.\textsuperscript{24} The series of deletions of the 5’ flanking region of the \(\beta\)-MHC included the −3,300\(\beta\)-MHC–CAT, −667\(\beta\)-MHC–CAT, −354\(\beta\)-MHC–CAT, and −215\(\beta\)-MHC constructs, which are genomic fragments of the rat \(\beta\)-MHC (\(\beta\)-MHC) gene from −3,300, −667, −354, −215, and −186 base pairs (bp) to +38 bp relative to the transcriptional start site cloned in front of the CAT gene.\textsuperscript{25} Position −607 to +32 of the rat \(\alpha\)-myosin heavy chain (\(\alpha\)-MHC) promoter sequence linked to the CAT gene is termed −607–\(\alpha\)-MHC-CAT.\textsuperscript{26} Nucleotide sequence −256 to +500 of the 5’ flanking sequence of the rat apolipoprotein A-I (ApoAI) fused to the CAT gene is termed −256ApoAI-CAT.\textsuperscript{27}

**Animal Preparation and Injection of Plasmid DNA**

Fourteen adult mongrel dogs of either sex weighing between 20 and 26 kg were used for these experiments. Dogs were premedicated with xylazine (10 mg/kg i.m.), and general anesthesia was induced with thiayml (10–20 mg/kg i.v.) and maintained with halothane (0.5–1.5% [vol]). Sterile technique was used, and the pericardium was exposed through a lateral thoracotomy at the fifth intercostal space. The pericardium was opened, and the heart was anchored with a suture through the apex. Up to thirty 4-mm\textsuperscript{2} patches of Dacron were sewn to the epicardium to mark injection sites. After placement of the patches, circular plasmid DNA resuspended in 1× phosphate-buffered saline was injected through a 30-gauge needle inserted perpendicular to the epicardium. The incision was closed in layers, and the chest was evacuated. The animals were observed during recovery until fully conscious. Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and the “Guide for the Care and Use of Laboratory Animals” (Department of Health and Human Services, publication No. [NIH] 86-23).

**Tissue Preparation**

After 7 days, the animals were killed with an overdose of pentobarbital, and the heart was rapidly excised and placed in ice-cold saline. The labeled injection sites were excised as transmural blocks of myocardium, weighing 0.5–1.0 g, and immediately placed in liquid nitrogen. Tissue was stored at −80°C until further processing. Immediately before the CAT assay, tissue was homogenized in 1 ml homogenization buffer containing (mM) glycyl-glycine 25 (pH 7.8), MgSO\textsubscript{4} 15, EGTA 4 (pH 8.0), and dithiothreitol 1, as described previously.\textsuperscript{18} The suspension was centrifuged at 6,000g for 15 minutes at 4°C, and the supernatant was used for further analysis. The supernatant was normalized for protein content as determined by the Bradford assay (Bio-Rad Laboratories, Richmond, Calif.) by the appropriate dilution with homogenization buffer.

**CAT Assays**

CAT assays were performed as previously described.\textsuperscript{28} In brief, 10% of the supernatant normalized for protein content, 1 \(\mu\)l \(^{14}\)C-labeled chloramphenicol (0.25 \(\mu\)Ci), and 5 \(\mu\)l \(n\)-butyryl coenzyme A (5 mg/ml) were mixed and filled to a total volume of 125 \(\mu\)l with 250 mM Tris-HCl, pH 8.0. The reaction mixture was incubated at 37°C for 2 hours, which was in the linear range of the reaction. The acetylated chloramphenicol fraction of the suspension was extracted by adding 300 \(\mu\)l xylene. Suspensions were back-extracted twice with 250 mM Tris-HCl, pH 8.0. Aliquots of 200 \(\mu\)l were counted in scintillation fluid in a beta counter (model LS 600IC, Beckman Instruments).
Luciferase Assay

Luciferase assays were performed as described elsewhere.26 In summary, 10% of the supernatant normalized for protein content was brought to a volume of 100 µl with homogenization buffer (see “Tissue Preparation”) and mixed with 360 µl reaction buffer containing (mM) glycy1-glycine 25 (pH 7.8), MgSO4 15, EGTA 4 (pH 8.0), dithiothreitol 1, KPO4 15 (pH 7.8), and ATP 2, along with 0.3% Triton X-100. Light emission was measured in a monolight luminometer (1251 luminometer, LKE Wallac, Turku, Finland) immediately after the addition of 0.2 mM D-luciferin to the reaction mixture. Light units are expressed as the integral of activity measured over 20 seconds. Only values within the linear range were included for analysis.

Data Analysis

All data are reported as mean±SEM. For statistical comparisons of CAT activity across time and regionally within the left ventricle, analysis of variance (ANOVA) was used. ANOVA was also used for promoter comparisons and comparison of injection techniques. When significant, intergroup comparisons were performed by unpaired t tests with the Bonferroni adjustment. Linear regression analysis was used to examine the correlation between CAT and luciferase activities in the cotransfection experiments. All analyses were performed on a Macintosh computer using STATVIEW II (Abacus Concepts Inc., Berkeley, Calif.) with p<0.05 considered significant.

Results

Expression of Gene Constructs Injected Into the Myocardium Follows Dose–Response Kinetics

To determine the efficiency and kinetics of expression of DNA injected into canine myocardium, we injected a constant volume of 200 µl containing increasing amounts of MSV-CAT plasmid DNA ranging from 10 to 300 µg per injection site into one dog heart. As depicted in Figure 1, an amount as little as 10 µg DNA resulted in a CAT signal nearly 10 times the background signal. In the range of 10–200 µg, the dose–CAT activity relation appeared linear (y=0.2x+10.8; r2=0.54). Higher amounts of total DNA resulted in a plateau, reflecting a saturation kinetic of DNA uptake, transcription, or both. These results indicate that the canine myocardium has a large capacity for uptake of injected DNA over a very broad range of concentrations. However, the slope of the curve clearly indicates that the efficiency of expression is the highest at the lower concentrations. The reasons for this behavior are not known at this time. However, this finding stresses the requirement for internal standards when the efficiency of expression between different constructs and/or amounts of injected DNA are to be compared.

Amount of Injury Imposed on Injection Site Does Not Correlate With the Level of Gene Expression

Three different injection techniques were compared in one experiment to analyze the impact of injection-induced injury on the cardiac tissue and its relevance for the level of expression of the injected reporter gene constructs. Because it could be argued that the results presented here represent uptake by nonmyocyte cells and therefore are not meaningful for the study of cardiac muscle biology, we tested a reporter construct whose expression is restricted to muscle cells. Although, to date, there has been no published report identifying the pattern of myosin heavy chain expression in the canine myocardium, our RNA blot analysis indicates that, like in other large mammals, the normal adult isoform is β-MHC, with a low level of α-MHC (data not shown). For this reason, we chose to use previously characterized β-MHC promoter constructs for this analysis.27 In the first group, 200 µl of −667 β-MHC–CAT plasmid DNA solution were injected via one single injection. In the second group, 50 µl of the same concentration of DNA was injected four times per injection site. To account for differences in the distribution of the DNA solution in the tissue between these two groups, a third group was included in which 200 µl of the DNA solution containing the same amount of total DNA as the other two groups were injected via one injection, and three additional stabs with the needle (but without injection of DNA) were performed around the actual injection site. As depicted in Figure 2, there were no statistical differences in CAT activity between any of these groups (ANOVA, p>0.05). However, because of the apparent trend of higher expression in the group with 50 µl DNA injected four times and because of a lower standard deviation in this group, we used this injection technique for subsequent experiments.

Promiscuous and Tissue-Specific Reporter Gene Constructs Are Expressed Over Extended Periods of Time

To evaluate the stability and peak of the expression of injected recombinant gene constructs in canine myocardium, we killed animals at four different time points (days 3, 7, 14, and 21 after injection). Multiple injections of CAT gene constructs using either promiscuous
(MSV) or muscle-specific (−667rβ-MHC) promoters were performed (Figure 3). ANOVA for CAT activity within the MSV and −667rβ-MHC promoters was significant ($p<0.01$ for the MSV and −667rβ-MHC constructs). The overall temporal pattern of expression of the exogenous genes was similar between promiscuous and tissue-specific promoter constructs, with CAT activity already detectable 3 days after injection, a peak at day 7, and a subsequent decline in CAT activity throughout day 21 ($p<0.01$ for MSV and $p<0.0001$ for −667rβ-MHC by unpaired $t$ test). This pattern of expression at the protein level is likely to be an overestimate of duration of expression of the injected DNA because of the long half-life of the CAT protein, which is more than 50 hours in most cell types. Therefore, the levels of expression shown in Figure 2 not only reflect the activity of the driving promoter but also reflect phenomena beyond the transcriptional level, e.g., the half-life of the expressed protein as well as that of the injected episomal DNA.

**Reporter Gene Constructs Are Evenly Expressed Throughout the Left Venticular Wall**

Since it was the purpose of our study to establish a practical model to compare the regulation of different recombinant gene constructs within the same animal by injecting at numerous sites into the canine myocardium, we compared the expression of a given construct at different locations throughout the myocardium. To account for regional differences in uptake and/or expression of foreign DNA by the cardiocytes, we injected the muscle-specific construct −667rβ-MHC–CAT in 24 different sites of the left ventricle as depicted in Figure 4. There were no detectable regional differences in CAT expression (ANOVA, $p>0.05$); however, because of the apparent trend toward decreased expression at the extreme base and apex of the left ventricle, we elected not to inject at those locations for subsequent experiments.

**CAT Activity Is Approximately Threefold Higher in the Left Than in the Right Ventricle**

One of the advantages of the canine versus the rodent model is the possibility to perform multiple injections also into the right ventricle. As shown in Figure 5, the expression of promiscuous as well as tissue-specific promoter constructs was found to be a third that observed in the left ventricle. This phenomenon could be based on the difference in wall thickness, with a smaller number of cells being transfected along the injection tract in the right ventricle. Alternatively, this may reflect a higher chance of leakage of injected DNA into the myocardial cavity during injections into the right ventricle.

**The Heart Expresses Injected Reporter Gene Constructs One to Two Orders of Magnitude Higher Than Does the Skeletal Muscle**

To compare the level of expression of injected gene constructs in the canine heart with other organs and possibly detect organ-related differences in the expression of exogenous genes, we performed injections of the promiscuous MSV–CAT and the muscle-specific β-MHC–CAT constructs into the quadriceps, a skeletal muscle of mixed fiber types. The values in Figure 5 are expressed as percentage of the expression of the according construct in the left ventricle. The expression of both the muscle-specific and promiscuous promoter constructs was, respectively, approximately one and two orders of magnitude lower in the skeletal muscle than in the left ventricle. As demonstrated before on mRNA levels in the rat, the β-MHC is most abundant in the soleus, a skeletal slow-twitch muscle, and also in the cardiac ventricle in hypothyroid animals. Thus, the low level of expression of the β-MHC promoter construct may be due to the fact that we injected the DNA into a mixed-fiber muscle, in which the β-MHC protein is much less abundant than it is in slow-twitch fiber muscle. The reason for the lower expression of the promiscuous construct in the skeletal muscle compared with the heart is unknown.
Coinjection of a Control Gene Construct (RSV-Luciferase) Has Proven Useful in Monitoring the Transfection Efficiency in This Model

Cotransfection is used to control for transfection efficiency in vitro, and it has also been used in vivo, but its usefulness in vivo has not been evaluated before. This is particularly important since, in contrast to cell culture studies, the transfected cell pool consists of a heterogeneous cell population, which may express the two gene constructs in a diverse pattern, rendering the coinjection useless. Two representative experiments were analyzed for correlation of CAT versus luciferase expression in this model, as depicted in Figure 6. In one experiment, the muscle-specific -667r-B-MHC-CAT construct was coinjected with the RSV-luciferase gene (Figure 6A). The long terminal repeat (LTR) of RSV (RSV-LTR) functions as a relative promiscuous promoter as determined previously, where transgenes directed by the RSV-LTR were highly expressed in tissue of mesodermal origin. The correlation between CAT and luciferase activity was significant ($r^2=0.8$, slope $=0.8\pm0.2$, $p<0.01$). When two promiscuous promoter constructs (MSV-CAT and RSV-luciferase) were coinjected (Figure 6B), the linear regression analysis of CAT activity versus luciferase activity revealed an $r^2$ of 0.9, with a slope of 3.5$\pm$0.6 ($p<0.005, n=6$). This indicates that cotransfection is

**Figure 4.** Bar graphs showing regional expression pattern of injected gene constructs throughout the left ventricular wall. rB-MHC, rat B-myosin heavy chain; CAT, chloramphenicol acetyltransferase; A, anterior; AL, anterolateral; L, lateral; P, posterior. Twenty-four injections of -667r-B-MHC-CAT were performed with four columns around the left ventricle, each comprising six injection sites ranging from base to apex (see drawing). Mean$\pm$SEM values of each column are shown in the left panel ($n=6$). Mean$\pm$SEM values of each row are shown in the right panel ($n=4$).

**Figure 5.** Bar graph showing expression of promiscuous (mouse sarcoma virus [MSV]) or muscle-specific (-667 rat B-myosin heavy chain [-667 rat B-MHC]) promoter constructs in the right ventricle (RV) and in skeletal muscle (Sk.M.). CAT, chloramphenicol acetyltransferase. Values (mean$\pm$SEM) are depicted as percent expression of the same construct in the left ventricle (LV, 100%, solid bars). Open bar is RV ($n=10$ for MSV, $n=8$ for -667 rat B-MHC). Hatched bar is skeletal muscle ($n=10$ for MSV, $n=9$ for -667 rat B-MHC).

**Figure 6.** Correlation of chloramphenicol acetyltransferase (CAT) to luciferase activity in coinjection experiments. r667-CAT, -667 rat B-myosin heavy chain–CAT; MSV-CAT, mouse sarcoma virus–CAT. Scatterplots of CAT activity (counts per minute) versus luciferase activity (light units) are shown. One hundred micrograms of a tissue-specific (r667-CAT, closed circles, top panel) or a promiscuous (MSV-CAT, open circles, bottom panel) reporter gene construct was coinjected with 20 $\mu$g control gene construct (Rous sarcoma virus–luciferase). The regression functions are as indicated.
meaningful and necessary to account for transfection efficiency in vivo; therefore, differences in expression of gene constructs may be attributed to differences in the regulation of expression of these constructs as long as values are normalized for the activity of the cotransfected gene. This analysis also reveals the feasibility of coinjecting a tissue-specific promoter construct with a promiscuous promoter construct to control for transfection efficiency.

Injection of Reporter Gene Constructs Into Canine Myocardium as a Useful Method to Detect Regulatory Gene Sequences In Vivo

After characterization of many important parameters relevant for the regulated expression of injected reporter gene constructs, we addressed the question of the feasibility of this model for mapping promoter sequences and thus identifying regulatory gene sequences in vivo. Constructs using serial deletions of the 5' flanking region of the β-MHC gene cloned in front of the CAT reporter gene were used (Figure 7). As a negative control, we used a construct containing the nucleotide sequence −256 to +397 relative to the transcription start site of the ApoAl gene, which has been shown to be expressed in hepatocytes specifically.27 Activity of the different β-MHC constructs was compared by ANOVA (p=0.001). All six possible pairwise comparisons were made and found to be significant (p<0.005), except −354rβ-MHC versus −215rβ-MHC. The most active construct was the −667rβ-MHC−CAT reporter gene construct. The marked difference in activity compared with the other β-MHC−CAT constructs agrees with the presence of a positive regulatory element between positions −667 and −354 relative to the transcription start site. Further deletion of the β-MHC gene promoter to position −186 relative to the transcription start site (−186rβ-MHC−CAT) resulted in a sharp decline of the CAT activity to a level that was barely above that of the negative control construct (−256apoAI−CAT), indicating another positive regulatory element important for basal transcription between positions −215 and −186 relative to the transcription start site. A repressor element may be located further upstream, as implied by the drop of activity of the −3,300rβ-MHC−CAT construct to approximately one fourth of the activity of the −354 and −215rβ-MHC−CAT constructs. The −607rα-MHC−CAT construct, which is the most active tissue-specific construct in rat cardiocytes as demonstrated by transfection assays of primary cell cultures,35 was approximately sixfold less active than the −667rβ-MHC−CAT. This result was expected, given the relative level of endogenous β- and α-MHC mRNA expressed in large mammals versus small mammals.

Taken together, our observations demonstrate that direct DNA injection into the canine myocardium is a practical and efficient method to study gene regulation in the intact animal.

Discussion

Our study indicates the feasibility of investigating the regulated expression of injected gene constructs in vivo in the heart of large mammals, specifically the canine myocardium. We demonstrate that pure plasmid DNA can be injected into the myocardial wall of dogs without any side effects. Electrographic monitoring and intraarterial blood pressure measurements performed postoperatively over several subsequent days in a number of dogs revealed only transient tachyarrhythmias in the first 1–2 hours after surgery, but otherwise no cardiac malfunction due to the injection procedure was detected. The efficiency of expression of gene constructs injected into canine myocardium compares favorably with other transfection methods.

In the first series of experiments, we defined parameters important for direct injection of reporter gene constructs into the canine myocardium. We show that expression of injected gene constructs is dose dependent and has features of a saturation kinetic at doses above 200 µg per injection site. To some extent, this has been demonstrated before in the skeletal muscle of mice by injecting three different doses of plasmid DNA ranging from 10 to 100 µg,8 although no saturation kinetics were demonstrated.
The analysis of the time course of the expression of gene constructs in our model revealed a pattern similar to other investigations, although these other studies were performed in rodents. The reason for the decline in the level of gene expression after 7 days is not known, but Acsadi et al. have suggested that injected plasmid DNA remains episomal and that the DNA itself is lost with time because of rapid degradation. One also might speculate that cells harboring the CAT gene product might be eliminated by immunological processes. This hypothesis is favored by recent experiments conducted on nude (immunosuppressed) rats, which showed a prolonged expression of an injected luciferase construct compared with normal rats. Data obtained from transgenic animals also indicate that the expression of the transgene may induce immunological responses. However, other reports were able to demonstrate a rather long-lasting gene expression of 19 months in rodents after in vivo gene transfer into skeletal muscle and of 60 days after injection into rat hearts.

The local pattern of exogenous gene expression has not been addressed before because of the small size of the species investigated. In the present study, we find no regional differences in expression for a muscle-specific promoter construct throughout the left ventricular wall. This renders the canine model suitable for comparison of different injected gene constructs within one animal, thus reducing the interindividual variability, whereas a large pool of animals is required in studies undertaken on rodents to gain statistical significance. In contrast, we observed a reduction of expression in the right ventricle to approximately one third of the left ventricle, which is probably related to the differences in wall thickness and thus the number of cells, which can be transfected along the needle tract.

The histological distribution of cell transfection has been addressed in several reports either by analysis of expression in different areas around the injection site or by use of constructs containing the coding sequence of the Escherichia coli lacZ gene. Apparently only cells in the direct vicinity of the needle tract are transfected. This observation raised the question about the mechanism of DNA uptake in this model, since the localization around the needle tract favors the hypothesis of DNA uptake through leaking cell membranes induced by injury. However, our data reported here do not favor this hypothesis. The comparison of three different injection techniques with different degrees of injury imposed on the cardiac tissue did not reveal a positive correlation between the degree of injury and DNA uptake or expression. Since the expression pattern of injected reporter gene constructs seems to display tissue-specific gene regulation mechanisms (Figure 7), it is unlikely that macrophages, chemotactically attracted to the injection site and supposedly able to incorporate DNA molecules by phagocytosis, contribute significantly to the expression of the injected reporter gene constructs. However, the exact mechanism of uptake of injected DNA remains elusive. It has been reported elsewhere that administration of DNA through multiple injections decreased the expression or uptake of the injected gene constructs. Since this observation is not in agreement with our results, further studies have to be undertaken to elucidate the mechanism of uptake of exogenous DNA constructs into mammalian cells in vivo.

Both, promiscuous and muscle-specific promoter constructs demonstrated a much lower level of expression in the skeletal muscle than in the heart. This observation is consistent with another report in which the promiscuous RSV-CAT, used for coinjection, was expressed approximately 20-fold less in skeletal muscle than in the heart of rats. The reason for this difference in expression is not known and is the subject of speculation. The marked difference in expression of the promiscuous MSV-CAT promoter construct between cardiac and skeletal muscle is surprising if one bears in mind the generally known high level of expression of this construct in transfection assays of all cell types studied so far. In contrast to cells in culture, which do not undergo physiological control mechanisms, the expression of injected plasmid DNA in our model might reflect differences between certain organs, regarding the prevailing physiological regulation pattern. The rhythmic contraction of the heart with the concomitant alteration of the myocardial wall stress or other regional differences in the neurohormonal regulation of organ function might account for the observed difference of expression of both constructs in vivo. The differences in the structure of the tubule system between cardiac and skeletal muscle cells might be related to a different efficiency of DNA uptake between these two cell types. As for the B-MHC reporter construct, it also may reflect the difference in abundance of the B-MHC protein between cardiac and mixed fiber muscle as has been shown before. However, more work will be required to uncover the basis for this phenomenon.

Although coinjection of a second gene construct to account for transfection efficiency, thereby reducing the variability inherent in transfection assays of cell cultures, is very common, its usefulness in in vivo gene transfer experiments has not been analyzed before. Our results indicate a high degree of correlation between expression of both injected genes, independent of the tissue specificity of the driving promoter. Thus, cotransfection in this model has proven to be useful.

To assess the usefulness of direct injection of DNA into canine myocardium to identify regulatory gene sequences important for in vivo expression (promoter mapping), we injected constructs containing a series of deletions of the 5' flanking region of the B-MHC gene. Our data are in general agreement with results obtained from transfection assays of skeletal and cardiac muscle cells. Notably, the −667 to −354 B-MHC–CAT construct seems to be more active in the heart than in skeletal muscle cells, as one can see by comparing the relative activity of the −667 with the −354 and the −215 B-MHC–CAT construct in the heart and in transfected skeletal muscle cells. The reason for this observation is unknown, but it might reflect the existence of a positive regulatory element between nucleotide positions −667 and −354, which is recognized specifically in cardiocytes and acts cooperatively with other positive regulatory elements further downstream from the B-MHC gene promoter, one of which may be located between positions −215 and −186 relative to the transcription start site, as indicated by the marked drop of CAT activity induced by deletion of this sequence. This is in agreement with studies performed on cell cultures,
in which the same sequence has been found to be important for the basal activity of the β-MHC promoter in SolI8 myotubes. In general, these findings prove the usefulness of this method in the identification of regulatory gene sequences in vivo and possibly their importance in the induction of pathophysiological conditions.

In summary, injection of recombinant gene constructs into canine myocardium appears to be a practical and efficient model for studying the regulated gene expression in the heart of large mammals. It allows for better extrapolation to humans than methods using small mammals, as have been done so far for this type of analyses. Furthermore, this model holds promise to serve as a tool to manipulate the cardiac phenotype.

Acknowledgments

The help of Reid Thompson in providing us with numerous β-myosin heavy chain constructs is gratefully acknowledged. We thank Cheryl Miller, DVM, for her help in taking care of the animals.

References

31. Izumo S, Nadal-Ginard B, Mahdavi V: All members of the MHC multigene family respond to thyroid hormone in a highly tissue-specific manner. Science 1986;231:597–600
Gene injection into canine myocardium as a useful model for studying gene expression in the heart of large mammals.

R von Harsdorf, R J Schott, Y T Shen, S F Vatner, V Mahdavi and B Nadal-Ginard

Circ Res. 1993;72:688-695
doi: 10.1161/01.RES.72.3.688

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/72/3/688