Force-Generating Preparation from Human Atria as a Model for Studying Myocardial Uptake of Radiopharmaceuticals

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The aim of this study was to assess whether an in vitro preparation of force-generating human atrial trabeculae driven by external electrical stimulation is a suitable model for determining myocardial uptake of cardiotropic radiopharmaceuticals. **Methods:** Human atrial trabeculae were excised from specimens removed during cardiac surgery for insertion of heart–lung apparatus. Preparations were kept under physiologic conditions in a chamber continuously perfused by Tyrode's solution at 37°C under permanent oxygenation. Electrical stimulation was performed at a frequency of 1 Hz. Contractile response was continuously measured by a force transducer and registered by a lineacorder. The optimum length of the trabeculae was achieved by stepwise increases of 0.1 mm muscle length. A premixed solution containing 1.92–4.06 MBq ^{201}Tl-TlCl was added to the perfusate of the chamber. After 10, 30, and 60 min, respectively, of incubation with ^{201}Tl-TlCl, the atrial trabeculae were removed from the chamber and their activity was measured by a γ counter. These experiments were repeated with nonviable trabeculae pretreated by potassium cyanide (KCN). Myocardial uptake values were measured as cts/min, normalized to cts/min/mg, and expressed as percentages of cts/mL/min in the perfusate (RUP). **Results:** Thallium uptake was found to be dependent on the functional integrity of the tissue preparations and increased over time in intact atrial trabeculae. RUP was 325% ± 108% after 10 min of incubation and rose to 838% ± 160% and 1196% ± 493%, respectively, at 30 and 60 min of incubation ($P < 0.01$). After 30 min of incubation, RUP was significantly higher in viable than in nonviable trabeculae (838% ± 160% versus 90% ± 65%; $P < 0.01$). **Conclusion:** These preliminary results indicate that the model proposed is suitable for studying the mechanisms of uptake of cardiotropic radiopharmaceuticals by human myocardial tissue.

Key Words: ^{201}Tl; cardiotropic radiopharmaceuticals; in vitro models; myocardial trabeculae; Steiert organ bath

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Myocardial scintigraphy is a mainstay of diagnosis in clinical cardiology (*1,2*). To develop new radiopharmaceuticals to image the human myocardium it is important to predict the behavior of these agents in human tissue. For obtaining information on the factors governing the myocardial uptake of radiopharmaceuticals, animal models and isolated cells are in current use. The purpose of this study was to validate an experimental setup of intact force-generating myocardial units of human origin for the in vitro characterization of myocardial uptake of radiopharmaceuticals.

MATERIALS AND METHODS

Preparation of Human Atrial Trabeculae

Myocardial tissue used for this study was obtained from patients undergoing heart surgery, i.e., bypass surgery or valve reconstruction. We applied a procedure that has been used in many cardiophysiologic studies (*3–8*). Small pieces of the right atrial auricle that had been removed before connecting the venous crus of the heart–lung machine were stored in Tyrode's solution immediately after excision. The culture solution in the transportation vessel had been oxygenated by a gas mixture of 95% O_2 and 5% CO_2 and contained 30 mmol/L 2.3-butanedionemonoxime (BDM) to protect the myocardial cells from an overloading influx of calcium during transportation and preparation procedures (*9*).

The preparation of the tissue cultures was performed in a dissection chamber containing Tyrode's solution with 30 mmol/L 2.3-BDM under constant oxygenation. The piece of atrial auricle was fixed by 2 needles on a rubber plate covering the ground of the dissection chamber and visualized through a light microscope with a ×2 objective during the preparation procedure. Out of the subepicardial trabecular network, columna-shaped branches, usually approximately 2–6 mm in length, were excised using tweezers and fine surgical scissors.

The atrial trabeculae used for these experiments were prepared out of a total of 11 tissue specimens, each of which had been obtained out of a right atrial auricle during cardiac surgery. Single trabeculae weighed 1.0–6.7 mg (mean, 2.8 ± 1.8 mg) and their surface areas were in the 4.0–21.0 mm^2 range (mean, 12.0 ± 5.2 mm^2).

Integration of Trabecular Specimens into the Setup

Two Steiert organ-bath units were the core of the setup (Fig. 1). In each measurement, each of the 2 organ-bath units contained 1 trabecular preparation for simultaneous stimulation. Both chambers were superfused by approximately 80 mL circulating, BDM-free Tyrode's solution. The muscle-bath chambers were connected with the stimulation unit by 2 parallel platinum electrodes. Temperature was kept constant with an electronic feedback system. Each contractile response of the trabeculae was measured by a force transducer and recorded by a lineacorder.

Both ends of the atrial trabeculae were attached to fine steel hooks and placed into the chamber. Initially, the preparations were prestretched by a maximal load of 2.5 mN. Steady systolic force development was observed a short period after residual BDM had been washed out. The trabeculae had to be stretched to the optimum length at which, according to Frank–Starling's law, maximum force is developed (*5,10,11*). Optimum length was established stepwise by 0.1-mm stretches, using a micrometer. Optimum length was assumed when further stretching did not lead to additional force development.

As trabecular force declined within the incubation period, the average force represented by the mean value of the initial and the final contractions was designated to characterize trabecular force. Myocardial trabeculae exhibited a considerable variety in the extent of their force development, yielding values within the range of 0.04–5.55 mN. Data analysis revealed no correlation between the extent of force development and tracer uptake in viable preparations.

Tracer Experiments

Application of Radiopharmaceutical. Before thallium was added to the system, it had to be proven that the trabeculae were suitable for uptake measurements, i.e., morphologically and functionally intact. Using the microscope, morphologic integrity was verified by exclusion of fibrotic areas and injury in the myocardial tissue, e.g., injury caused by the preparation procedure. Functional integrity was assumed when the trabeculae performed 1 contraction after each stimulus and relaxation occurred without delay. Extrasystolic contractions were interpreted as signs of membrane disintegrity and led to the exclusion of trabecula.

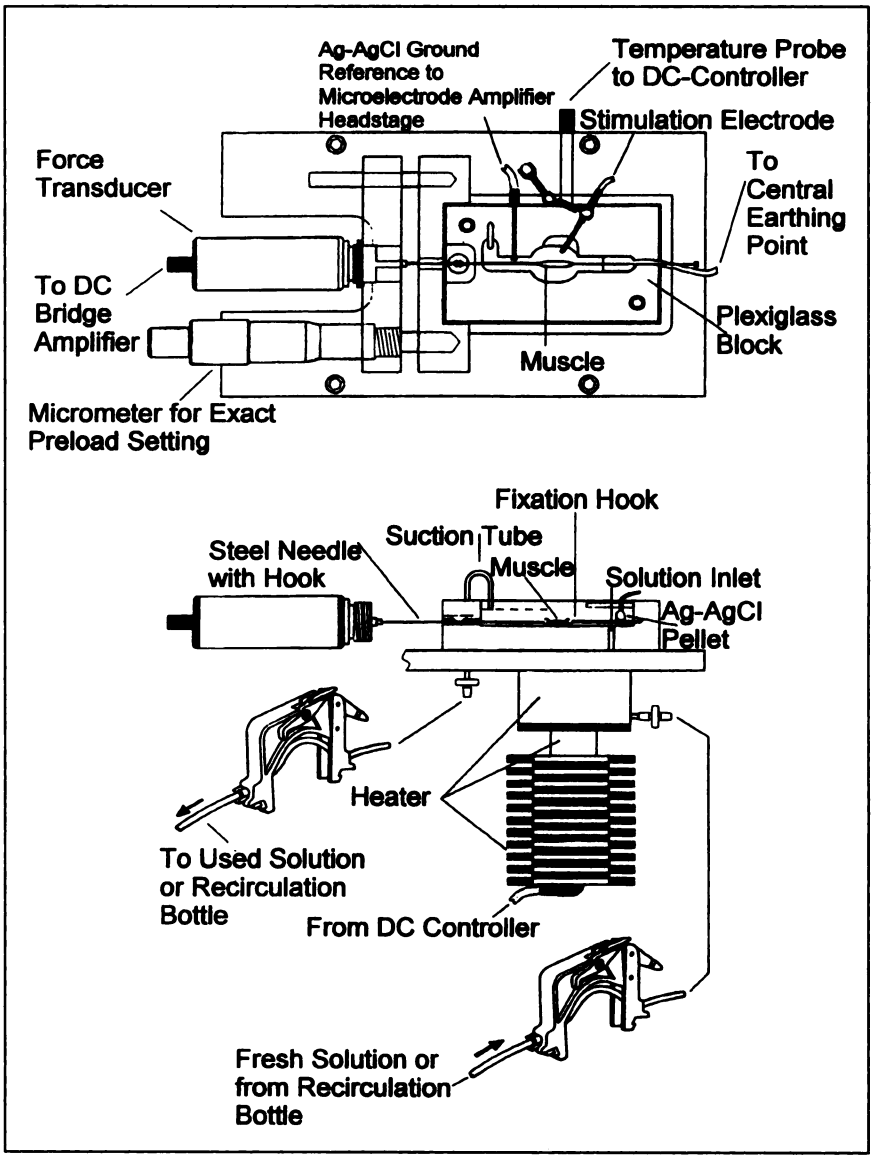

FIGURE 1. Force measuring system with force transducer (F30), slider for preload adjustment, and bath block with chamber for myocardial preparation. Various parts of apparatus are made mainly from plexiglass or stainless steel and are therefore largely inert and corrosion resistant. View from above (top). Lateral view (bottom).

When optimum length of the trabeculae had been established and force development had proven stable, the radiopharmaceutical was applied to the system. To avoid exposure of the trabeculae to activity peaks and the potential to influence net uptake, thallium was not injected as a bolus but was homogeneously distributed in the perfusate. It was injected into a flask allowing access into the circulatory system. At the time of injection the flask was disconnected from the tube that led the solution to the muscle-bath chambers. When thallium had distributed steadily in the solution, the flask was reconnected with the tube and the solution entered the bath chambers containing the trabeculae. At 1, 2, 3, 4, 5, and 10 min after administration of activity into circulation and at subsequent 10 min intervals thereafter, 200 μL of the solution were collected. Counting rates were integrated over time and average counting rates per minute, normalized to 1 μL solution, were calculated.

^{201}Tl had been applied into the volume of Tyrode's solution (approximately 80 mL) at predefined activities within the range of 1.92–4.06 MBq (mean, 2.72 MBq). After activity had been equilibrated in the volume, counting rates in the solution were 320–1092 cts/min/μL. Counting rates in the perfusate showed no significant loss within 60 min of perfusion, e.g., by adhesion to the inner surface of the setup (Fig. 2).

Measurements of Radiopharmaceutical Uptake. At the end of the experiments, after a predefined time of exposure to the radiopharmaceutical (10, 30, or 60 min, respectively), the trabeculae were removed from the organ-bath chambers. At this time, it was unavoidable that some of the activity-containing superfusate in which the preparations had been stored during the incubation period was covering the surface of the trabeculae. Thus, an uncertain quantity of activity that was not located intracellularly and thus not a part of myocardial thallium uptake would have been added to the results if the measurements were left uncorrected. The share of activity adherent to the surface of the tissue was intended to be removed by a washing procedure that was performed in a standardized way for every preparation. After removal from the bath chamber, each trabecula was transferred into a plastic tube, containing 400 μL activity-free Tyrode's solution. The tubes were deposited over a period of 5 min to remove the share of total activity adherent to the surface. The trabeculae were then each transferred into another plastic tube containing 200 μL activity-free Tyrode's solution. Both sets of tubes, the first with the activity removed from the trabeculae and the second with the activity-loaded trabeculae were analyzed by a γ counter.

The trabeculae were weighed, and their lengths and diameters were measured under the microscope using a cross table with a 0.1-mm scale. The trabeculae's surface areas were calculated according to the equation applicable to cylindrical figures:

$$A = 2\pi r(r + h),$$

where r = radius and h = height. Uptake values measured as cts/min were normalized to cts/min/mg myocardial tissue and expressed as a percentage of average activity in the solution (cts/min/μL).

Inhibition of Mitochondrial Respiratory Chain

To estimate the influence of viability on trabecular uptake, devitalization was performed by potassium cyanide (KCN) in a subset of trabeculae (*12,13*).

First, the trabeculae were integrated into the setup as described previously. When maximum force development had been achieved after consecutive prestretching, the perfusate was exchanged by a 10 mmol/L KCN-containing Tyrode's solution. This intervention was followed frequently by a rapid loss of contractility, indicating the metabolic damage caused by KCN. After 5 min incubation, the KCN-containing perfusate was replaced by a fresh Tyrode's solution without KCN. In the new perfusate, trabeculae did not regain their contractility, indicating that irreversible damage had occurred in the preparation after the administration of KCN.

After a perfusion period of 20 min, ^{201}Tl was added to the system and measurements were performed according to the protocol described above. Trabeculae exhibiting a residual contractile response after KCN had been applied to the system were excluded from the evaluation.

Statistics

Results were expressed as mean values ± SD. For statistical analysis, unpaired Student *t* tests were performed. Statistical significance was accepted at $P < 0.05$. Several correlations were quantified by calculation of Pearson's regression.

RESULTS

Intact, force-generating atrial trabeculae were found to accumulate ^{201}Tl in a time-dependent way. After an incuba-

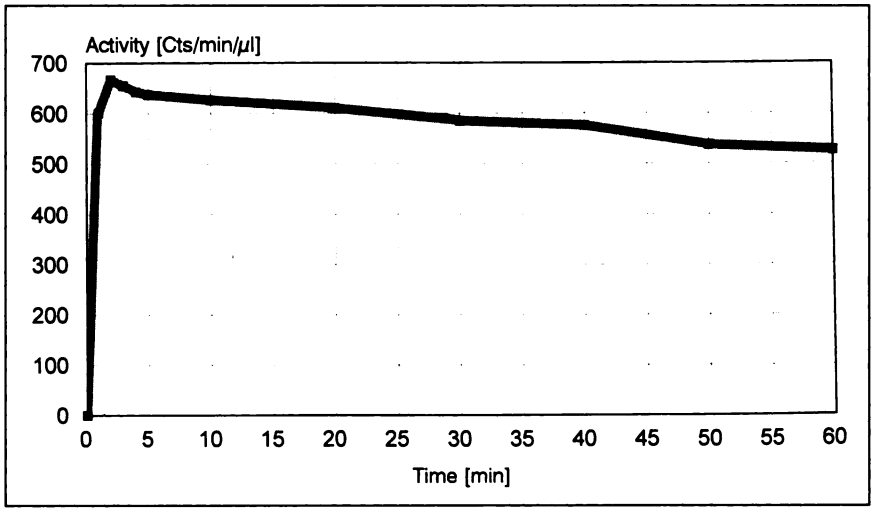

FIGURE 2. Consecutive measurements of ^{201}Tl activity (cts/min/μL) in perfusate surrounding trabecula. Maximum activity was measured 2 min after reservoir containing 2.05 MBq ^{201}Tl was connected with muscle bath chamber. Within 60 min, constant decline of activity was observed in medium. Calculation revealed average activity of 582 cts/min/μL. Time course of activity is representative for all experiments.

tion period of 10 min, activity in 1 mg trabecular tissue was found to be 325% ± 108% when compared with the activity (100%) in 1 μL perfusate. After 30 min, trabecular activity was 838% ± 160%, and, after 60 min, the trabeculae had gained 1196% ± 493% of the activity in the surrounding perfusate (Fig. 3).

A significant correlation was not found between the total weight of a trabecula and the radiopharmaceutical uptake per milligram of trabecular weight ($r = 0.50$ after 10 min exposure to ^{201}Tl; $r = -0.59$ after 30 min; $r = -0.60$ after 60 min).

In contrast, trabeculae pretreated by KCN did not exhibit accumulation of the radiopharmaceutical. Their activity after 30 min of incubation was 90% ± 65% of the activity in the perfusate. The difference between uptake in the viable and nonviable trabeculae proved to be highly significant (Fig. 4).

Using the washing procedure after removal of the trabeculae, a minor fraction of activity was separated from the myocardial tissue and was retained in the washing medium. The major fraction is described above and was found to increase with the time of exposure (Fig. 3). The minor fraction revealed a stable correlation with the surface areas of the trabeculae in all subsets ($r = 0.75$), independent of the time of exposure to the activity-loaded superfusate and not dependent on viability ($P > 0.05$; data not shown).

A quantitative correlation between force development and uptake of the radiopharmaceutical could not be determined ($r = 0.51$ after 10 min incubation of ^{201}Tl; $r = 0.43$ after 30 min; $r = 0.61$ after 60 min).

DISCUSSION

Numerous approaches to studying the uptake mechanisms of cardiotropic radiopharmaceuticals are in current use. Most use animal models or tissues obtained from animals, e.g., isolated rat or rabbit hearts (*14–17*) or myocytes obtained from adult, neonatal, or fetal animals (*18–23*).

All these approaches are eminently suitable for studying the biological behavior of cardiotropic radiopharmaceuticals, and each offers distinct experimental advantages. However, although most are well established, extrapolation of the results obtained using these animal models to the human heart may be questioned. Therefore, models using myocardial tissue obtained from humans are also under investigation. In this context, primary myocyte cultures obtained from human hearts have received some attention (*24–26*). However, human myocytes have been used only rarely to study the cardiac uptake of radiopharmaceuticals. This may be a consequence of the difficulties encountered in their preparation. Furthermore, cultured myocytes are devoid of structural organization and have lost their histiotypic architecture and, possibly, the biochemical properties associated with it (*27*). Their metabolism, then, may not necessarily be representative of myocardial metabolism in situ.

The model investigated in this publication uses viable, force-generating human myocardial units, thus not suffering from the limitations of the approaches discussed above. It has been extensively validated in pharmacologic studies (*3–8*) but has not been applied to the investigation of the cardiac uptake of radiopharmaceuticals. Because this approach allows the joint study of physiologic parameters, such as maximal force of contraction and the uptake of radiopharmaceuticals, it promises to yield results analogous to those obtained by the Langendorff preparation.

The material used, parts of the right atrial auricle, is routinely obtained during cardiac surgery, so the model could be established in principle in all institutions where cardiac surgery is performed. Trabeculae originating from ventricular tissue are also suitable for this experimental setup. Because ventricular myocardium is less frequently available and because physiologic studies show certain differences between atrial and ventricular trabeculae, we decided to exclude ventricular trabeculae from this initial evaluation of radiopharmaceutical uptake.

In vivo, the myocardial extraction of thallium is governed by regional blood flow and by cellular uptake mechanisms (*28–36*). In the present preparation, excitability and consecutive force generation represented viability of the atrial trabeculae. Perfusion was a constant parameter in this setup,

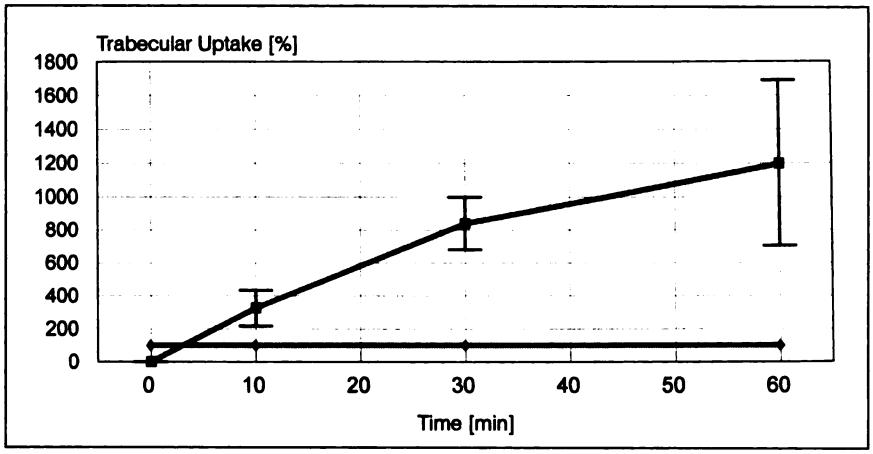

FIGURE 3. Correlation of ^{201}Tl uptake (%) with time of exposure to activity. Results are normalized to activity in superfusate (= 100%). Uptake values are significantly different from activity in superfusate (10 min versus 30 min, $P < 0.01$; 10 min versus 60 min, $P < 0.01$; 30 min versus 60 min, $P > 0.1$ [ns]). Error bars indicate SDs.

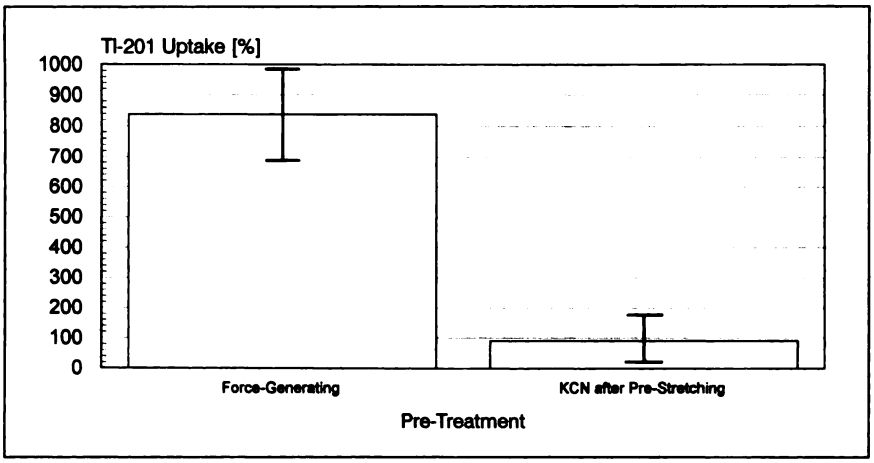

FIGURE 4. Accumulation of ^{201}Tl (%) in human atrial preparations after 30 min of incubation. Left = force-generating trabeculae. Right = force-generating trabeculae after exposure to KCN. The difference is highly significant ($P < 0.01$). Error bars indicate SDs.

because the trabeculae were superfused continuously by the solution, thus being exposed to an equilibrium thallium supply.

Concordant with our expectations, thallium uptake into myocardial preparations proved to increase with the amount of activity in the perfusate, the size of the tissue sample, and the time of exposure (Table 1). After normalization of the trabecular weights and the activities in the peritrabecular fluid, ^{201}Tl was found to accumulate in the atrial trabeculae as a function of time (Fig. 3). The time course registered in our model parallels that reported in myocytes (*19*). Furthermore, nonviable trabeculae do not concentrate the isotope (Fig. 4). This illustrates that ^{201}Tl uptake in the trabeculae reflects active metabolic processes, involving transport mechanisms against a transmembrane concentration or electrochemical gradient. In the case of ^{201}Tl, this is believed to be the action of the Na^+/K^+ adenosine triphosphotase (*37–40*).

A major limitation of the proposed model may be that radiopharmaceutical uptake is also dependent on the penetration time of the radiopharmaceutical into the trabeculae. Barriers to diffusion, such as the endothelium, therefore may influence the uptake measured. Furthermore, larger trabeculae, presenting greater distances to diffusion of the radiopharmaceutical, may exhibit smaller uptake values. Within the range of trabecular sizes studied, however, this may not represent a problem for ^{201}Tl, because there was no significant correlation between total trabecular weight and radiopharmaceutical uptake per milligram of trabecular weight in this study.

However, more data obtained by autoradiography of the trabeculae are needed to clarify this issue, in particular when

TABLE 1
Trabeculae and Radiopharmaceutical Distribution

Incubation period (min)	Viability ±	Weight of trabecula (mg)	Surface area of trabecula (mm^2)	Radiopharmaceutical uptake of trabecula (cts/min)	Activity removed by washing (cts/min)	Activity medium (cts/min/μL)	Applied activity (MBq)	Trabecular force (mN)
10	+	1.0	5.0	2,877	1,434	933	4.06	0.08
10	+	2.5	12.3	7,670	1,992	933	4.06	0.12
10	+	1.8	9.1	3,801	959	456	2.07	2.25
10	+	1.0	4.0	907	941	456	2.07	0.62
30	+	1.9	11.0	17,350	5,102	1,092	3.92	0.04
30	+	6.0	21.0	51,401	9,232	1,092	3.92	0.18
30	+	3.2	16.3	21,269	4,721	984	3.68	1.08
30	+	1.1	8.9	11,426	3,675	984	3.68	0.52
60	+	1.7	8.9	12,922	4,074	641	2.91	1.08
60	+	1.1	5.3	10,449	1,141	641	2.91	2.16
60	+	3.6	20.5	32,140	2,374	496	2.40	5.55
60	+	5.5	17.7	15,737	3,231	582	2.05	1.08
60	+	4.8	17.7	28,546	3,134	582	2.05	2.10
30	−	2.6	12.6	207	1,050	406	1.95	
30	−	1.6	9.8	219	726	406	1.95	
30	−	3.2	9.8	780	1,058	424	1.92	
30	−	2.0	9.8	896	1,275	424	1.92	
30	−	2.5	9.8	1,093	1,115	320	2.28	
30	−	6.7	18.6	3,988	2,898	320	2.28	

the uptakes of radiopharmaceuticals of larger molecular weights are studied.

It must be postulated that the amount of metabolic exchange between the cytoplasm of the myocardial cell and the perfusate is exclusively restricted by the diffusion barriers, i.e., by cell membranes and the connective tissue. Thus, the design of the setup rules out modification of metabolic exchange resulting from a component restricting the supply of the perfusate that could be attributable to a deficient vascular supply under in vivo conditions. Therefore, dissociating the contribution of flow from the influence of tissue viability on accumulation of ^{201}Tl is a potential of the setup. This makes our model particularly suitable to determine whether the uptake of a radiopharmaceutical is sensitive to tissue viability or other physiologic parameters in addition to blood flow.

It should be noted that we did not calculate the actual inward and outward transmembrane fluxes of ^{201}Tl between the tissue and the surrounding medium but, instead, the evolution of the net activities in both compartments at predefined times. It is not clear to what extent trabecular accumulation was superimposed by simultaneous washout. Therefore, it also is not clear if the activity that was removed by the washing procedure was identical with the amount that was covering the surface or if an additional outward shift of activity during the 5 min led to the addition of more activity. Regardless of these restrictions, the net activities of the trabeculae were clearly suitable for discriminating viable from nonviable tissue and even revealed a significant correlation with the incubation period.

In contrast, the amounts of activity that were retained in the washing media did not prove to be significantly different when the subsets were compared. The share of activity removed by the washing period reveals a constant correlation with the trabecular surface areas for all subsets of trabeculae. This finding suggests that the activity removed by the washing period may indeed reflect the share of activity that was located superficially.

CONCLUSION

These first data on radiopharmaceutical uptake into an in vitro model of force-generating viable human myocardium indicate that contracting human atrial trabeculae are a promising model for studying the mechanisms governing cardiac radiopharmaceutical uptake in vitro.

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