Catecholamine-Induced Myocardial Potassium Uptake Mediated by β₂-Adrenoceptors and Adenylate Cyclase Activation in the Pig

Øyvind Ellingsen, Ole M. Sejersted, Severin Leraand, and Arnfinn Ilebekk

The myocardial potassium uptake during intracoronary isoproterenol stimulation was characterized in 12 anesthetized pigs. The β-receptor subtype specificity and the effect of adenylate cyclase activation were determined. Potassium concentrations were continuously recorded by PVC-valinomycin microelectrodes in the left atrial cavity and in coronary sinus blood diverted through a shunt to the right atrium. The difference in potassium concentration between the left atrial cavity and coronary sinus, and the accumulated myocardial potassium uptake were calculated after computerized data sampling. By intracoronary drug infusion, changes in heart rate and systemic effects were minimized. Isoproterenol (0.6-0.8 μg/min), a nonspecific β-receptor agonist, reduced coronary sinus potassium concentration transiently to a nadir of 0.28 (0.15-0.43) mM (median and 95% confidence interval) below control values (n = 12). The potassium uptake, which amounted to 140 (79-202) μmol/100 g tissue, corresponding to an intracellular potassium increase of about 3 mM, was abolished after selective β₂-blockade by pafenolol. The specific β₁-agonist dobutamine (40 μg/min) caused a similar potassium uptake before and after selective β₂-blockade by ICI 118,551. Salbutamol (2 μg/min), a specific β₂-agonist, induced a minor potassium uptake of 4 (1-20) μmol/100 g, blocked by pafenolol. After nonselective β-blockade by propranolol the adenylate cyclase stimulator forskolin caused a myocardial potassium uptake of similar magnitude to that of isoproterenol before β-blockade. We conclude that a myocardial potassium uptake ensues during β₂-adrenoceptor stimulation and adenylate cyclase activation. (Circulation Research 1987;60:540-550)

It is well known that β₂-stimulated K⁺ uptake in skeletal muscles can lead to hypokalemia. A low systemic extracellular K⁺ level also affects the myocardium and increases the risk for ventricular fibrillation. Therefore, part of the cardio-protective effect of β-adrenoceptor blockers has been attributed to their ability to prevent the catecholamine-induced cellular K⁺ uptake in skeletal muscles. However, the interstitial K⁺ concentration in the heart can also be lowered locally by K⁺ uptake in heart muscle cells. Recently, Sejersted et al demonstrated a pronounced K⁺ uptake during intravenous isoproterenol infusion, despite a considerable increase in heart rate that would otherwise cause a K⁺ release.

In the present study we address three questions: 1) how is the myocardial K⁺ balance changed by β₂-adrenergic stimulation when heart rate is constant; 2) which subtype of β-adrenoceptors is involved; and 3) is the myocardial K⁺ uptake mediated by the adenylate cyclase system? To answer these questions, we used open-chest pigs with an intact circulation. Increments in heart rate were avoided during β₂-adrenergic stimulation by infusing the β-agonists into the left coronary artery, thus stimulating the left ventricular myocardium selectively. Myocardial K⁺ balance was monitored continuously by ion-selective electrodes in arterial and myocardial venous blood. Access to venous blood from the heart and continuous coronary flow recording was provided by a shunt from the coronary sinus (CS) to the right atrium as described by Andersen et al.

In the porcine heart, the relative amounts of β₁- and β₂-adrenoceptors are nearly the same as in human ventricles. Intracoronary infusions of salbutamol (β₂-agonist) and isoproterenol (β₁ + β₂-agonist) before and after selective β₁-blockade by pafenolol and infusion of dobutamine (β₁-agonist) before and after β₂-blockade by ICI 118,551 allowed determination of the subtype of β-receptors causing the myocardial K⁺ uptake. Any non-β-receptor effects were examined by intracoronary infusion of salbutamol and isoproterenol after β₂-blockade by propranolol. Dose-response characteristics of isoproterenol and salbutamol were determined in a separate experiment, and a possible K⁺ uptake into erythrocytes was examined by in vitro application.

To study the effect of adenylate cyclase activation on myocardial K⁺ balance, we infused forskolin, a direct stimulator of adenylate cyclase, into the coronary artery after complete β-blockade.

Materials and Methods
Animal Preparation
Fifteen domestic pigs of both sexes (20-35 kg) were fasted for 36 hours and anesthetized by sodium pento-
barbital (starting dose 28–40 mg/kg i.p. and sustaining dose 5–20 mg/kg/hr i.v., according to the depth of anesthesia). The pigs were artificially ventilated through a tracheostoma by a volume-regulated respirator (Princeton Medical Instruments Inc., model 101, Natick, Mass.). Arterial blood samples were checked regularly on an automatic blood gas analyzer (AVL Biomedical Instruments, model 945, Graz, Austria). Oxygen was added and ventilation volumes adjusted to keep arterial $P_{O_2}$, $P_{CO_2}$, and pH within normal ranges. Constant body temperature was insured with wrappings and an electric heating pad. The urinary bladder was continuously drained through a cystostoma.

The heart was exposed through a midsternal split and an incision in the 5th left intercostal space and suspended in a pericardial cradle. The left azygos vein, which drains into the coronary sinus in the pig, was cannulated and a shunt line to the right atrium established (for details see Andersen et al). Myocardial venous blood was directed through the shunt by tightening a preset stitch ligature at the opening of the coronary sinus into the right atrium. Heparin (750 IU/kg) was given to prevent blood clotting. Blood draining from the shunt through a stopcock. For intracoronary infusions, a polyvinyl catheter (o.d. 0.7 mm) was inserted upstream into the left coronary artery and connected to a syringe pump. The left coronary artery supplies 70–80% of the left ventricle and all of the muscle mass drained by the shunt. All intravenous infusions were given through a polyethylene catheter in the right femoral vein. Glucose (5%) was infused intravenously during the surgical procedures and saline during the experimental periods to maintain stable hemodynamic conditions. Potassium chloride was added to obtain a 4-mM K$^+$ concentration in all infusates.

**Hemodynamic Measurements**

Left ventricular pressure (LVP) was recorded by a microtip pressure transducer catheter (Millar Instruments Inc., model PC-470, Houston, Tex.) introduced through the apical dimple of the heart. The frequency response of the catheter-tip transducer is 10 kHz while the response of the recorder is about 250 Hz; the latter determines the overall frequency response of the system. Arterial blood pressure was measured through a short polyethylene catheter (i.d. 2.0 mm) inserted into the right femoral artery and connected to a Statham pressure transducer (Gould Instruments, model P23 Gb, Hato Rey, Puerto Rico). This system has a frequency response flat to 40 Hz.

Aortic flow was recorded by an electromagnetic flowprobe fitted snugly to the ascending aorta. Both the aortic flow probe and a flow probe on the coronary sinus shunt were connected to a 2 channel square-wave electromagnetic flowmeter (Nycotron, model 376, Drammen, Norway). Calibration factors for the flow probes were determined in vitro by withdrawal of known volumes of blood at a constant rate. Aortic flow was regarded as 0 during diastole. Zero for shunt flow was obtained during brief periods when the polyvinyl tube of the shunt was clamped. Zero levels were determined regularly. Stroke volume was calculated from aortic flow and heart rate. Andersen et al found a high correlation ($r = 0.91$) of shunt blood flow measured electromagnetically and coronary flow determined by microspheres. There was also a high correlation ($r = 0.99$) of myocardial blood flow in the region drained by the shunt and adjacent areas. Diverting blood flow through the shunt exerted no adverse effects on the myocardium.

**Potassium-Sensitive Minielectrodes**

K$^+$ concentrations in arterial (Ka) and coronary sinus blood (Kcs) were measured continuously by PVC-valinomycin minielectrodes. The electrode for Ka measurements was inserted into the left atrium and secured by a purse-string suture. The electrode for myocardial venous blood K$^+$ recording was introduced into the coronary sinus shunt through a side hole. Previously described electrode systems were modified for continuous monitoring and on-line data sampling. A drawing of the measuring cell is presented in Figure 1.

**Electrode construction.** The K$^+$-sensitive membrane was composed as described by Hilt et al: valinomycin 4% (Sigma Chemical Co., St. Louis, Mo.), potassium tetrphenylborate 1% (Merck Sharp & Dohme, West Point, Penn.), dibutyl sebacate 72% (Sigma), and PVC-powder 23% (BDH Chemicals Ltd., Poole, England) (weight percentage). These components were dissolved in tetrahydrofuran to make a viscous mixture. A 0.2–0.5 mm thick K$^+$-sensitive membrane was made at the distal end of a 10-cm long PVC-tube (i.d. 0.9 mm, o.d. 1.5 mm) by dipping it repeatedly into the viscous mixture. The electrode was backfilled with 500 mM KCl saturated with AgCl and connected to a 1-ml syringe containing the same elec-

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**FIGURE 1. Potassium-selective electrode for measurements in left atrium, total length 25 cm. Shunt electrode has screw cap instead of sleeve near tip of electrode.**
trolyte solution and an Ag/AgCl inner reference electrode (In Vivo Metric Systems, model E255, Healdsburg, Calif.). A reference electrode with an open tip, otherwise identical to the K⁺-sensitive electrode, was filled with a 140-mM NaCl solution providing an open salt bridge. High input impedance preamplifiers were placed close to the electrodes and connected to a combined amplifier and antilogarithm unit in which gain and levels of the signal could be adjusted.

In vitro calibration and evaluation. The electrodes were calibrated in standard solutions of 1, 2, 3, 4, 5, and 10 mM KCl in 140 mM NaCl at room temperature. The correlation coefficient of the relation between electrode voltage and the logarithm of the K⁺ concentration exceeded 0.998 for all electrodes used. The voltage difference per decade at 37°C was 58.1 (56.4–59.4) mV (median and 95% confidence interval). Only 5 of the 26 electrodes deviated more than 10% from the theoretical value 61.5 mV, calculated from the Nernst equation. The impedance of the electrodes was 1–6 MΩ. The response time to reach a stable voltage after immersion in a new solution was less than 5 seconds, well below the time variations of K⁺ concentrations explored in this study.

The electrodes were insensitive to stirring and changes in shunt flow between 0 and 400 ml/min. Increasing hydrostatic pressure in the shunt stepwise from 20 to 200 mm Hg produced a linear change in voltage of —1.5 mV. This would not affect the measurements since the electrodes were never exposed to pressures exceeding 10 mm Hg during the experiments.

The PVC-valinomycin electrode is reported to be highly selective to potassium, with selectivity constants for Na⁺, H⁺, Ca²⁺, and Mg²⁺ ranging from 10⁻⁴ to 10⁻³.17 However, variations in serum composition can alter electrode voltage by changing the liquid junction potential of the Ag/AgCl-NaCl reference electrode.18 To determine the error induced by such changes, we varied concentrations to the major serum constituents beyond their normal limits at a constant K⁺ concentration of 4 mM. The corresponding changes in electrode voltage are presented in Table 1. Change in electrode voltage did not exceed 1 mV, which would account for an error in K⁺ readings of less than 4%. Hence the electrodes are highly selective for K⁺ and do not respond to major changes in other plasma constituents.

In vivo calibration. At the beginning of the experiment, the 0 levels of the electrodes were adjusted so that the K⁺ readings equaled the K⁺ concentration measured in plasma by flame photometry (Instrumentation Laboratory, S.p.A, model 943, Milan, Italy). This procedure corrected for the change in liquid junction potential of the reference electrode from standard solutions in vitro to blood in vivo, as well as for grounding potentials originating in electronic equipment attached to the animal.

To verify that the slope of the electrode was unchanged, 7.5 ml of a 160 mM KCl solution was infused intravenously by a syringe pump over a period of

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration change (mM)</th>
<th>Voltage change (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H⁺</td>
<td>7.6–7.0*</td>
<td>0.4</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>20–30</td>
<td>—0.7</td>
</tr>
<tr>
<td>Na⁺</td>
<td>110–170</td>
<td>±0.1</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>110–170</td>
<td>±0.1</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0–5</td>
<td>±0.1</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0–5</td>
<td>—0.5</td>
</tr>
<tr>
<td>HPO₄²⁻ /H₂PO₄⁻</td>
<td>0–5</td>
<td>—0.6</td>
</tr>
<tr>
<td>Albumin</td>
<td>0–100*</td>
<td>±0.6</td>
</tr>
</tbody>
</table>

*pH units; †bovine serum albumin g/l.

The changes in liquid junction potential of the Ag/AgCl-NaCl open saltbridge reference electrode were measured as the change in electrode voltage when plasma concentrations were increased from the lowest to the highest values indicated. All measurements were performed using 4 mM K⁺.

3 minutes at the beginning of experiments. Arterial blood and coronary sinus blood from the shunt were sampled before infusion and at the peak of the response.

Blood samples were drawn regularly from the femoral artery and the coronary sinus shunt to correct for drift of the K⁺-measuring system during the experiment. A linear drift in electrode voltage of 0.6 (0.2–1.0) mV/hr was observed. The resulting deviation in K⁺ recording was corrected in the computerized calculation program.

In all samples, hematocrit (Hct) was estimated as the relative volume of packed cells in a capillary tube after 3 minutes centrifugation in a Cellocrit 2 (AB Lars Ljungberg & Co, Stockholm, Sweden).

Experimental Procedures

Adrenoceptor stimulation and blockade. β₁- and β₂-adrenoceptors in the left ventricle were stimulated and blocked according to the following protocol. Salbutamol (10 μg/ml in saline) was infused into the left coronary artery at a constant rate of 0.2 ml/min, providing a dose of 2 μg/min for about 10 minutes. LV dP/dt declined to its preinfusion value 6.5 (4.25–9.25) minutes after the discontinuation of salbutamol, and a recovery period of 26 (14–36) minutes was allowed to provide adequate washout. Then isoproterenol (3–4 μg/ml in saline) was infused at the same rate, delivering a dose of 0.6–0.8 μg/min for 10–15 minutes. After the effects of isoproterenol had subsided, pafenolol (10 mg/ml in saline) was infused intravenously to block the β₂-adrenoceptors selectively. After this blockade, intracoronary salbutamol and isoproterenol infusions were repeated. A nonselective blockade of the β-adrenoceptors was obtained with 5–11 mg propranolol intravenously. Salbutamol and isoproterenol infusions were repeated as before. Finally, forskolin (Hoechst AG, Frankfurt (M), Federal Republic of Germany), 1 mg/ml dissolved in equal amounts of ethanol and saline, was infused into the left coronary artery at a
Constant rate of 0.2–0.4 ml/min (0.2–0.4 mg/min). Estimated drug concentrations are presented in Table 2. The protocol was completed in 5 pigs, and each procedure was accomplished in 6 or more animals.

$K^+$ concentrations and shunt flow were recorded by the computer system every second. Hemodynamic data were recorded at stable conditions before the intracoronary infusions were started and during drug infusion after a new steady state in $K^+$ concentration had been reached.

Immediately after euthanasia, 20–40 ml 2,3,5-triphenyltetrazolium chloride (20 mg/ml) was injected into the shunt, staining the myocardium drained by the shunt brick red. After dissection, the weight of the stained myocardium was determined.

**Dose-response characteristics.** In 1 animal, the dose-response characteristics of peak myocardial $K^+$ uptake and contractility increase were determined by 4 separate intracoronary infusions of salbutamol and isoproterenol. We gave 0.6, 1.2, 2.4, and 4.8 $\mu$g/min of salbutamol and 0.08, 0.16, 0.32, and 0.8 $\mu$g/min of isoproterenol with washout periods of 15–37 minutes.

Crossover to $\beta_1$-adrenoceptors under high doses of salbutamol was assessed in another animal by determining effects on peak myocardial $K^+$ uptake and contractility increase after $\beta_1$-selective blockade by 70 mg pafenolol during infusions of 2.4 $\mu$g/min and 9.6 $\mu$g/min. For reference, isoproterenol was given in doses of 0.8 and 4.2 $\mu$g/min after the same $\beta_1$-blockade. Washout periods were 60 minutes after 9.6 $\mu$g/min salbutamol and 20–28 minutes in other cases.

**Selective $\beta_1$-stimulation.** In 1 animal, myocardial $K^+$ balance was determined during intracoronary infusion of dobutamine 40 $\mu$g/min before and after selective $\beta_1$-blockade by ICI 118,551 (385 $\mu$g/kg i.v.).

**Myocardial glucose uptake.** In 6 experiments, the concentration of plasma glucose was determined by an enzymatic method (Boehringer Mannheim GmbH, Mannheim, Federal Republic of Germany) in arterial and coronary sinus blood sampled in the control immediately before drug infusion and under steady-state conditions after 8.5 (7.25–10.0) minutes of isoproterenol infusion. Myocardial glucose uptake was calculated as the product of the arterial-coronary sinus (a-cs) difference and plasma flow per 100 g tissue.

**In vitro isoproterenol administration.** To determine the magnitude of a possible $K^+$ uptake in erythrocytes during isoproterenol stimulation, 100 ml arterial blood was withdrawn from the arterial catheter into a beaker with 1 ml heparin (500 IU/ml) before any adrenoceptor stimulators or blockers were given to the animal. The blood was stirred continuously by a magnetic stirrer at 37°C. Oxygen supply, 50 ml/min, was provided through a 5-mm silicon tubing by a dispenser in the blood. After a 3-minute control period, isoproterenol was added to give final concentrations 45 and 450 nM, which is about the same dose and 10 times the intracoronary dose, respectively. Whole blood $K^+$ concentration was monitored continuously by a PVC-valinomycin electrode, and the recordings were adjusted using blood samples taken every 2 minutes.

**Computerized Data-Sampling and Calculations**

$K^+$ concentrations and hemodynamic parameters were continuously monitored on an 8 channel galvanometric recorder (Hewlett Packard, model 7758 B). Separate $K^+$ and shunt flow readings were also monitored on a Rikadenki potentiometer recorder. Output signals (range ± 2 V) from the Hewlett Packard recorder were amplified to ± 8 V and fed into a NORD 10/S computer (Norsk Data, Oslo, Norway) through an 8 channel analog-to-digital converter. Data were stored on flexible disks during the experiment and then transferred to hard disks in a NORD 10/VS computer for further calculations.

**Hemodynamic parameters.** The following hemodynamic parameters were sampled during 3–10 second periods at a frequency of 100 Hz: LVP, aortic pressure (AP), aortic flow (AF), and shunt flow (SF). LVP pulses were analyzed to detect the start of a cardiac cycle. The cycle commenced when the pressure exceeded the minimal pressure plus 10% of the difference between its maximal and minimal value. A cardiac cycle was accepted for further calculations if 2 criteria were satisfied: its duration deviated less than 2% from the median of the period and its LVP amplitude exceeded 95% of the maximum value of the sampling period. If a cycle was discarded because of either criterion, then its preceding and succeeding cycles were also rejected. An average cardiac cycle was computed from the remaining acceptable cycles. The LV $dP/dt$ was calculated from the difference in average pressure of 2 consecutive sampling intervals. The maximum positive and negative LV $dP/dt$ values were determined, defining the limits of the systole. Mean systolic LVP was computed by averaging the LVP over the systole, and the mean aortic pressure and cardiac output were found by averaging the entire cardiac cycle of AP and AF, respectively. Heart rate was calculated from the mean duration of all accepted cycles.

**Potassium measurements.** $K^+$ concentrations in arterial and myocardial venous blood and averaged shunt flow (SF) were recorded at 1-Hz sampling fre-
quency during the intracoronary infusions. The linear drift in electrode voltage was compensated by a time-dependent correction factor. To minimize the influence of transient noise potentials, signals representing K+ concentrations were smoothed by a digital low-pass filter with a frequency response flat up to 0.05 Hz. The subsequent calculations were performed on data-points representing the mean over 15 seconds.

The a-cs K+ concentration difference at steady state immediately before intracoronary infusion (Ka-cs0) was subtracted from the succeeding concentration differences (Ka-cs') to obtain the net changes (Ka-cs = Ka-cs' − Ka-cs0) caused by the experimental interventions. To correct for transit time, Ka measurements were compared to Kcs registrations with a 30-second delay. This transit time was determined from the time elapsed between the K+ peak in left atrium and the K+ peak in the shunt during in vivo calibration by intravenous potassium chloride infusion.

Instantaneous myocardial K+ uptake ($\mu$mol/100 g tissue/min) was calculated as 1.04 (1-Hct)SF(Ka-cs)/MW, where the factor 1.04 corrects for the underestimation of the plasma fraction by the centrifugation method, Hct is the hematocrit in the coronary sinus blood, and MW is weight of myocardium drained by the shunt. Initial K+ release and accumulated K+ uptake were calculated by integration of the instantaneous myocardial K+ uptake from the start of drug infusion to the establishment of a new steady state of the K+ levels (i.e., when Ka-cs changed less than 0.02 mM from one 15-second datapoint to the next). Numerical integration was performed by the trapezoid rule.

Statistics

Data are presented as the median and a nonparametric 95% confidence interval for the median (in brackets), based on Walsh numbers. The Wilcoxon signed rank test for paired observations was applied to estimate the two-sided significance level of changes in hemodynamic measurements. The Friedman test was used for comparison of hemodynamic control values as well as hemodynamic responses and changes in K+ balance during intracoronary drug infusions. 21 A probability value < 0.05 was considered statistically significant. Estimation of median values, confidence intervals, and Wilcoxon p values were based on the maximum number of observations (6–12); each animal was represented once. The Friedman test was performed on the data from the 5 complete sets of interventions.

Results

Isoproterenol Infusion

Intracoronary isoproterenol infusion caused a biphasic change in myocardial K+ balance. The original recordings in Figure 2 show changes in Ka, Kcs, and shunt flow from one experiment.

Over the first minute of isoproterenol infusion, Kcs transiently increased by a maximum of 0.05 (0.03–0.07) mM (p < 0.05). Thereafter, Kcs fell abruptly, reaching a nadir value of 0.28 (0.15–0.43) mM below control level (p < 0.001) 3.0 (2.5–4.0) minutes after the start of isoproterenol infusion. During continued isoproterenol infusion, Kcs rose again, reaching a steady-state level 0.07 (0.03–0.12) mM below control (p < 0.01) after 6.5 (5.0–8.0) minutes. Kcs was 4.12 (3.89–4.39) mM before infusion and 4.05 (3.84–4.26) mM under steady-state conditions.

Ka was 4.06 (3.86–4.28) mM before isoproterenol infusion and 4.05 (3.87–4.25) mM under steady-state conditions. Ka rose by 0.1 mM or more in 3 animals (as seen in Figure 2) and declined by 0.1 mM or more in 3 animals during isoproterenol infusion. Kcs was 0.06 (0.03–0.07) mM higher than Ka in the control period (p < 0.05) but was not significantly different under steady-state conditions.

Figure 2. Tracings from an original porcine experiment (weight, 21 kg) showing changes in arterial and coronary sinus K+ concentration and shunt flow during isoproterenol infusion into left coronary artery. In this animal, arterial K+ concentration increased 0.2 mM during drug infusion, whereas median value of 12 experiments was unchanged. More pronounced noise on the arterial recording was probably due to movement of left atrium during myocardial contraction.
A computer record of myocardial $K^+$ balance is presented in Figure 3 (data are from the experiment shown in Figure 2). Left upper panel demonstrates arterial (a) and coronary sinus (cs) $K^+$ concentrations averaged over 15-second periods. As shown in the left lower panel, control a-cs $K^+$ differences were normalized to 0 in the control condition to obtain net changes in $K^+$ balance caused by drug infusion. This correction is justified since the Ka-cs difference in the control period is probably due to the small increase in red cell volume caused by the $\text{HCO}_3^-/\text{Cl}^-$ shift of the erythrocytes during passage of the capillary bed. Right upper panel displays the instantaneous $K^+$ uptake, calculated as the product of the normalized a-cs difference for $K^+$ and coronary plasma flow. The right lower panel demonstrates accumulated myocardial $K^+$ uptake, acquired by integration of instantaneous uptake from the start of drug infusion until new steady state in Ka-cs was reached.

The peak instantaneous myocardial $K^+$ uptake amounted to $37 (26-48) \mu$mol/100 g/min ($p < 0.001$). The accumulated $K^+$ uptake was $140 (79-202) \mu$mol/100 g ($p < 0.001$), whereas the initial release amounted to $2.5 (1.5-4.0) \mu$mol/100 g ($p < 0.001$). The steady-state Ka-cs during continued isoproterenol infusion was $0.07 (0.03-0.12) \text{mM}$ over a period of $3.25 (2.5-6.25)$ minutes after the transient response was over.

Isoproterenol caused shunt flow to rise by $30\%$ during the first minute and $40\%$ during the first 2 minutes, eventually reaching a level $93\%$ above control. No changes in a-cs hematocrit differences were observed.

**Comparison of $\beta$-Adrenergic Stimulation Before and During Receptor Blockade**

An initial $K^+$ release of the same magnitude and duration as during isoproterenol infusion also occurred during intracoronary salbutamol infusion before and after selective $\beta_1$-blockade by pafenolol and also during isoproterenol infusion after $\beta_1$-blockade. After complete $\beta$-blockade by propranolol, no such changes occurred.

Figure 4 summarizes the changes in myocardial $K^+$ balance during intracoronary infusions of salbutamol (S) and isoproterenol (I) before any $\beta$-receptor blockade, after $\beta_1$-selective blockade by pafenolol, and during nonselective $\beta$-blockade by propranolol. The effects of forskolin (F) are also presented.

$\beta_2$-Stimulation by salbutamol caused only a minor myocardial $K^+$ uptake, amounting to $8\%-15\%$ of the accumulated uptake elicited by isoproterenol ($\beta_1 + \beta_2$-stimulation). Both agents induced significant hemodynamic changes. The contractility parameter LV $dP/dt$ rose by $80\% (50-120\%)$ during salbutamol and by $200\% (130-260\%)$ during isoproterenol infusion (Figure 5). Steady state in myocardial $K^+$ balance was reached after $2.5 (1.75-3.0)$ minutes, before any changes in arterial $K^+$ concentration occurred.
Table 3. Hemodynamic Values Before Intracoronary Infusions of Salbutamol (S), Isoproterenol (I), and Forskolin (F)

<table>
<thead>
<tr>
<th></th>
<th>LVSP (mm Hg)</th>
<th>LV dP/dt (mm Hg/sec)</th>
<th>HR (beats/min)</th>
<th>AP (mm Hg)</th>
<th>SV (ml)</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>83-99</td>
<td>1,600-2,250</td>
<td>112-135</td>
<td>78-101</td>
<td>12-18</td>
<td>10</td>
</tr>
<tr>
<td>I</td>
<td>82-94</td>
<td>1,600-2,450</td>
<td>113-137</td>
<td>78-98</td>
<td>12-18</td>
<td>12</td>
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<tr>
<td>After pafenolol</td>
<td></td>
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</tr>
<tr>
<td>S</td>
<td>71-99</td>
<td>1,160-1,300</td>
<td>88-118</td>
<td>82-103</td>
<td>9-18</td>
<td>7</td>
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<tr>
<td>I</td>
<td>76-97</td>
<td>1,150-1,600</td>
<td>90-126</td>
<td>82-103</td>
<td>9-18</td>
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<td>After propranolol</td>
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<tr>
<td>S</td>
<td>83-98</td>
<td>1,030-1,540</td>
<td>73-115</td>
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<tr>
<td>I</td>
<td>84-101</td>
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<td>F</td>
<td>75-96</td>
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<td>70-121</td>
<td>81-102</td>
<td>9-19</td>
<td>6</td>
</tr>
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</table>

Median values and their nonparametric 95% confidence intervals (on line below) are given for LVSP, mean systolic left ventricular pressure; LV dP/dt, max positive LV dP/dt; HR, heart rate; AP, mean aortic pressure; SV, stroke volume; n, number of experiments.

Pafenolol reduced LV dP/dt by 30% (0-50%) when injected after the effects of isoproterenol had subsided (Table 3). During β₁-blockade by pafenolol, neither salbutamol nor isoproterenol caused any K⁺ uptake (Figure 4). The relative increase in LV dP/dt during salbutamol was unchanged, whereas the inotropic response to isoproterenol was reduced by 80% (60-90%) (Figure 5).

Nonselective β-blockade by injection of propranolol during persisting β₁-blockade caused small hemodynamic alterations (Table 3) and reduced heart rate by 17 (2-24) beats/min. After propranolol, no changes were found in K⁺ balance by isoproterenol and salbutamol (Figure 4). Hemodynamic responses were also small (Figure 5).

**Dose-Response Characteristics**

The dose-response characteristics of peak K⁺ uptake and changes in maximal positive LV dP/dt during intracoronary infusions of isoproterenol and salbutamol before and after selective β₁-blockade are presented in Figure 6. The isoproterenol dose routinely employed was about twice the dose causing half-maximum response in peak instantaneous K⁺ uptake. The β₁-blockade of pafenolol could be overcome by increasing the isoproterenol dose about 10 times. The standard dose of salbutamol was chosen to cause a significant increase in LV dP/dt. Again the β-blockade of pafenolol could be overcome by increasing the salbutamol dose about 4 times.

**Selective β₂-Stimulation**

Changes in myocardial K⁺ balance during intracoronary infusion of dobutamine (40 μg/min) before and after selective β₂-blockade by ICI 118,551 (385

**FIGURE 5.** Changes in hemodynamic parameters by intracoronary infusions of salbutamol, isoproterenol, and forskolin before and after β₁-selective and nonselective β-blockade. Abbreviations and number of experiments as in Figure 4.
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Isoproterenol Salbutamol

• before, ○ after Pafenolol

Figure 6. The ● = the dose-dependent peak myocardial K+ uptake (upper panel) and the LV dP/dt-increase (lower panel) for isoproterenol and salbutamol before any receptor blockade in 1 pig (weight, 27 kg). ○ = values obtained after selective β2-adrenoceptor blockade by pafenolol (3.5 mg/kg i.v.) in another pig (weight, 20 kg). Note displacement of the dose-response curve to the right after β2-blockade both for isoproterenol and salbutamol. Note also that effects of low dose salbutamol on K+ uptake and LV dP/dt increase were almost completely inhibited after β2-adrenoceptor blockade. Arrows indicate doses routinely applied.

μg/kg) are presented in Figure 7. During both infusions, the response was qualitatively and quantitatively the same as that of isoproterenol infusion. After a small initial myocardial K+ release, a considerable uptake ensued, peaking after about 2 minutes and reaching a new steady state in a-cs K+ difference at 5.25 minutes. Although the maximum values of a-cs K+ concentration difference and instantaneous K+ uptake were greater before than after β2-blockade (0.24 mM vs. 0.16 mM and 40 μmol/100 g/min vs. 29 μmol/100 g/min, respectively), the accumulated K+ uptake at steady-state a-cs difference 5.25 minutes after the start of infusion was practically the same (90 μmol/100 g vs. 88 μmol/100 g) because of the more protracted time course after blockade. The β2-blockade decreased heart rate from 125 to 103 beats/min and reduced LV dP/dt from 1,100 to 800 mm Hg/sec. Mean arterial pressure declined from 73 to 65 mm Hg, while mean systolic LVP remained unchanged at 81 mm Hg. During dobutamine infusion the rise in contractility before and after β2-blockade was about the same, namely, 3,200 and 2,800 mm Hg/sec, respectively.

Myocardial Glucose Uptake

In the control situation, arterial and coronary sinus plasma glucose concentrations were 5.43 (4.67–6.39) mM and 5.03 (4.55–6.35) mM, respectively. During isoproterenol infusion, the corresponding values were 5.35 (4.63–6.10) mM and 5.19 (4.60–6.07) mM. A-cs glucose concentration difference was 0.19 (–0.03–0.61) mM before infusion and 0.09 (–0.08–0.43) mM at steady state, whereas myocardial glucose uptake was 10.5 (–2.1–57.6) μmol/100 g/min and 11.5 (–8.0–68.8) μmol/100 g/min, respectively. Neither a-cs difference nor myocardial glucose uptake changed significantly from control to steady state.

In Vitro Isoproterenol Administration

Isoproterenol 45 nM in heparinized and oxygenated blood caused a slight rise in K+ concentration from 4.33 mM to 4.36 mM at 3.25 minutes after drug administration and a decline to 4.32 mM after 9 minutes. When isoproterenol concentration was raised to 450 nM, K+ concentration fell further to 4.26 mM after 5.75 minutes and then rose to 4.34 after 13 minutes.

Effects of Forskolin

Intracoronary infusion of forskolin during β2-blockade by propranolol induced a peak change in a-cs K+ concentration difference of 0.32 (0.10–0.58) mM (p < 0.05) and an accumulated K+ uptake of 128 (73–375 μmol/100 g. Ka was 4.26 (3.77–4.93) mM.

A-cs K+ uptake (μmol/100 g/min)

LV dP/dt change (mmHg)

Drug dose (g/min)

FIGURE 7. Computer recording of myocardial K+ balance and myocardial blood flow during infusion of dobutamine 40 μg/min into left coronary artery, before (1) and after (2) selective β2-adrenoceptor blockade by ICI 118,551 (385 μg/kg i.v.). Infusions were started at time zero. Left upper panel shows arterial (--) and coronary sinus (—) concentrations, averaged over 15-second periods. Left lower panel shows myocardial blood flow. Right upper panel displays myocardial K+ uptake, calculated as the product of a-cs K+ difference and coronary plasma flow. Right lower panel shows the accumulated myocardial K+ uptake acquired by integration of the instantaneous uptake from start of drug infusion.
before drug infusion, 4.37 (3.95–5.01) mM at peak K⁺ uptake, and 4.22 (3.80–4.91) mM when steady-state conditions were reached. The corresponding values of Kcs were 4.30 (3.89–5.01) mM, 4.23 (3.48–4.97) mM, and 4.16 (3.87–4.90) mM. An initial rise in Kcs occurred in 2 of 6 experiments, in one of them probably because of an abrupt increase in heart rate.

The increase in LV dP/dt was 190% (160–220%) (p < 0.05). These changes were similar to those elicited by isoproterenol before any β-blockade (Figure 4). The significant increase in heart rate and decline in mean arterial blood pressure (Figure 5) was probably due to spillover and recirculation of forskolin.

Discussion

Myocardial K⁺ Uptake

The main effect of intracoronary isoproterenol and forskolin infusions was a transient reduction in cs K⁺ concentration that amounted to as much as 0.6 mM in 2 animals. Such a decrease in cs K⁺ could only be explained by a K⁺ uptake in myocardial cells.

Alternatively, the decrease in cs K⁺ could have been caused by shift of K⁺-free fluid from erythrocytes or myocardial cells to plasma, but this is not likely since a decline in cs hematocrit was not observed. A 10% decline in cs K⁺ concentration by a volume shift would require a cs hematocrit to be lowered by 0.03–0.04 units, which was also not found.

K⁺ uptake in erythrocytes was ruled out by the in vitro administration of isoproterenol to freshly sampled arterial blood. Virtually no change in plasma K⁺ occurred even at very high concentrations. Hence, erythrocytes were unable to account for the observed decline in Kcs, which we conclude is caused by K⁺ uptake in the myocardium.

Normally, extracellular and plasma K⁺ concentrations are similar. Theoretically, a K⁺ gradient from plasma to interstitial fluid could be created by movement of K⁺-free water out of the myocytes. However, to account for a net disappearance of 140 μmol/100 g K⁺ from plasma, the interstitial volume would have to be nearly doubled, which is unlikely to occur. Therefore, we conclude that isoproterenol stimulates K⁺ uptake in the myocardial cells themselves.

The amount of K⁺ taken up was 140 (79–202) μmol/100 g, which is similar to the uptake observed by Sejersted et al during intravenous isoproterenol administration. In both studies, LV dP/dt more than doubled. However, during intravenous isoproterenol infusion, heart rate rose abruptly by 68 beats/min as compared to a gradual rise of 8 (2–15) beats/min in the present study. It is therefore clear that changes in heart rate have no or only marginal influence on the magnitude of the K⁺ uptake induced by isoproterenol. It is also clear that the small initial K⁺ release during intracoronary isoproterenol infusion could not result from increased beating frequency since the rise in heart rate did not develop until after K⁺ uptake commenced.

The biphasic change in myocardial K⁺ balance was not a secondary effect of increased cardiac contractility per se. According to unpublished experiments from our laboratory, raising contractility to the same extent as during isoproterenol infusion by an intracoronary infusion of calcium chloride (25–50 mg/min) induced a completely different change in K⁺ balance. In fact, a slow progressive K⁺ release ensued 1–2 minutes after contractility was fully developed, eventually resulting in a myocardial K⁺ release of comparable magnitude to the observed K⁺ uptake during β-stimulation. It is also unlikely that myocardial K⁺ uptake during isoproterenol infusion is associated with increased glucose transport into cells under the present experimental conditions since glucose uptake did not change significantly. This finding is in accordance with data from Breull and Rubart who reported that isoproterenol caused only a small increase in myocardial glucose metabolism.

The membrane events associated with β-adrenoceptor-stimulated changes in cardiac K⁺ balance are still uncertain. The prevailing hypotheses comprise increased K⁺ conductance of the sarcolemma and stimulation of the Na-K pump. Boyden et al suggested that noradrenaline-induced hyperpolarization in canine coronary sinus cells was caused by a β-stimulated increase in K⁺ permeability. This mechanism could probably explain the initial net K⁺ efflux observed in the present experiments.

Myocardial K⁺ uptake could be caused by increased Na-K pump activity. Results consistent with catecholamine-induced Na-K pump stimulation in cardiac muscle were reported by Wasserstrom et al and more recently in isolated myocytes by Desilets and Baumberg. Both groups found a decline in intracellular Na⁺ concentration of about 2 mM after 5–10 minutes of isoproterenol and norepinephrine stimulation. If this decline in intracellular Na⁺ is mainly caused by increased Na-K pump activity, a corresponding rise in intracellular K⁺ concentration is likely to occur. In the present experiments, the accumulated myocardial K⁺ uptake amounted to 140 μmol/100 g tissue after about 6.5 minutes. According to Page and Page, 80% of wet tissue weight in the mammalian heart is water, and about 60% of the water is intracellular. That is, 48–50% of wet tissue weight is intracellular water. Hence, the observed uptake would correspond to an increase in intracellular K⁺ concentration of about 3 mM, which corresponds both in time and magnitude with the reduction in intracellular Na⁺ during β-stimulation in isolated preparations.

The peak instantaneous K⁺ uptake was 37 μmol/100 g/min. The number of Na-K pumps measured as ouabain-binding sites is close to 700 pmol/g tissue. A reasonable maximum K⁺-pumping rate for Na⁺,K⁺-ATPase would be 200/sec. Hence, the peak K⁺ uptake corresponds to an increase in pump activity of 4–5% of its maximum pump rate.

However, the mechanism of a possible increase in Na-K pump rate remains to be determined. Sejersted et al found no indication of a higher number of pumps, comparing the number of ouabain-binding sites in biopsies from the left ventricle before and during isopro-
terenol infusion. One possibility for Na-K pump stimulation could be increased substrate sensitivity, but direct evidence for altered enzyme kinetics is still lacking.

Irrespective of the mechanism of a possible pump stimulation, the transience of myocardial K⁺ uptake could be explained in two ways. First, the reported decline in intracellular Na⁺ would counteract Na-K pump stimulation. Second, after an initial K⁺ uptake, new electrochemical gradients would be established for K⁺ and Na⁺. In addition, membrane permeabilities are probably altered. Hence, a new steady state condition, probably with increased membrane Na⁺ and K⁺ fluxes, is reached.

β-Adrenoceptor Specificity

One of the main purposes of the present study was to determine the specificity of the β-adrenoceptor inducing K⁺ uptake in heart muscle. The experiments showed that intracoronary isoproterenol infusion caused a myocardial K⁺ uptake that could not be elicited after administration of pafenolol. This was not due to unresponsiveness of our preparation. First, the blockade was overcome by increasing the isoproterenol dose. Second, the response to isoproterenol could be repeated several times in succession after short recovery periods.

Since pafenolol is a highly selective β₁-adrenoceptor blocking drug (β₁:β₂ affinity 70:1), these results indicate that the K⁺ uptake was elicited by β₁-stimulation. The additional experiment with dobutamine strongly supports this conclusion, especially since the response was also present after selective β₂-blockade by ICI 118,551.

Because the K⁺ balance of skeletal muscle is significantly modified by β₂-stimulation, it was also pertinent to examine whether β₂-stimulation in addition to β₁-stimulation could cause myocardial K⁺ uptake. However, the amount of β₂-adrenoceptors in the porcine myocardium is low. Even a large dose of salbutamol, causing a significant increase in cardiac contractility, induced only a minor K⁺ uptake that was abolished by pafenolol. Hence, the salbutamol effect on K⁺ balance most likely was due to stimulation of β₁-receptors. Again the experiments with dobutamine indicate that any β₂-effect must be of minute quantitative importance. These data taken together, and considering the dose-response characteristics of salbutamol and isoproterenol before and after pafenolol, lead to the conclusion that the β-adrenergic K⁺ uptake in the porcine myocardium is a consequence of β₁-adrenoceptor stimulation. However, a minor β₂-mediated K⁺ response cannot be entirely excluded.

Adenylate Cyclase Activation

Forskolin infusion served two purposes. First, it showed that the myocardium was still capable of accumulating K⁺, thereby excluding the possibility that lack of response to isoproterenol after β-blockade was merely caused by deterioration of the preparation. Second, forskolin activates adenylate cyclase independent of the β-adrenoceptors. In the isolated perfused rat heart, 0.2 μM forskolin increased cyclic adenosine monophosphate (cAMP) content to a greater extent than epinephrine. Therefore, it seems reasonable that the K⁺ uptake during forskolin is a consequence of increased intracellular cAMP.

The fact that forskolin and isoproterenol exerted similar effects on cardiac K⁺ balance and contractility is further evidence that the β-adrenoceptor-stimulated myocardial K⁺ uptake is closely associated to adenylate cyclase activation and a rise in cAMP.

The substantial rise in beating frequency during forskolin infusion may have induced some changes in myocardial K⁺ balance. Ilebekk et al reported that a sudden increase in heart rate of 54 beats/min induced a transient K⁺ efflux of 13 μmol/100 g tissue. In the present study, an initial K⁺ efflux was observed in 2 of 6 experiments, one in which beating frequency increased abruptly. These small changes do not significantly alter the main response, which is a substantial K⁺ uptake. Any effect of sympathetic stimulation due to hypotension was prevented by nonselective β-blockade by propranolol. Forskolin was first infused after the patency of the β-blockade had been tested by isoproterenol infusion.

Conclusion

The purpose of the present experiments was to study the changes in myocardial K⁺ balance during β-adrenoceptor stimulation at constant heart rate and to determine its β-receptor subtype specificity and possible relation to adenylate cyclase activation. After an initial K⁺ efflux, a cardiac K⁺ uptake with a major transient and a minor persistent component ensued, resulting from β₁-adrenoceptor stimulation and adenylate cyclase activation. The almost negligible effect of β₂-stimulation was probably due to the small proportion of β₂-receptors in porcine myocardial membranes. Thus, K⁺ uptake in muscle cells seems to be a general effect of β-adrenoceptor stimulation, dominated by β₁ in the heart and β₂ in skeletal muscle according to the subtype preponderance in either tissue.

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