Effects of a Bradycardic Agent (DK-AH 269) on Haemodynamics and Oxygen Consumption of Isolated Blood-Perfused Rabbit Hearts

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A. Granetzny, U. Schwanke, E. Gams, J. D. Schipke

Objectives: Pharmacologic bradycardia is a promising strategy to improve myocardial energetic balance. We evaluated the effects of the novel sinus node inhibitor DK-AH 269 (DK) on ventricular function and perfusion in isolated rabbit hearts.

Methods: To differentiate between the effects of the negative force-frequency relation and a direct negative inotropic action of DK, measurements of haemodynamic and metabolic parameters were made during electrical pacing (EP), after application of DK (DK+EP), and after termination of pacing (DK–EP).

Results: Heart rate was significantly reduced by DK (EP: 161 ± 20, DK+EP: 161 ± 19 min⁻¹, mean ± SD, p = 0.02). In parallel, diastole significantly lengthened without pacing (EP: 241 ± 35, DK+EP: 234 ± 38, DK–EP: 459 ± 220 ms, p = 0.04). Aortic flow was decreased in the presence of DK (EP: 40.6 ± 21.7, DK+EP: 32.8 ± 17.9 ml/min) and even further in the absence of pacing (DK–EP: 22.8 ± 24.6 ml/min). However, stroke volume (EP: 0.31 ± 0.16, DK+EP: 0.26 ± 0.14 ml) and peak isovolumic left ventricular pressure (EP: 109 ± 19, DK+EP: 92 ± 20, mmHg) were only moderately reduced. DP/dtmax remained essentially unchanged after DK (EP: 1355 ± 545, DK+EP: 1390 ± 830 mmHg/s), but decreased without pacing (DK–EP: 890 ± 500 mmHg/s). DP/dtmin, as a measure of early relaxation, had a tendency to decrease after DK with and without pacing (EP: −1245 ± 625, DK+EP: −1055 ± 410, DK–EP: −725 ± 340 mmHg/s), while the left ventricular end-diastolic pressure remained unchanged after DK and significantly decreased without pacing (EP: 10 ± 7, DK+EP: 9 ± 6, DK–EP: 5 ± 5 mmHg, p = 0.03). Coronary blood flow (CBF) decreased from 204 ± 29 to 156 ± 21 ml/min/100 g with DK and remained almost constant without pacing (148 ± 33 ml/min/100 g). The relation between subendocardial and subepicardial flow (colored microspheres) decreased slightly with DK (EP: 1.46 ± 0.39, DK+EP: 1.40 ± 0.28, DK–EP: 1.36 ± 0.41). The myocardial oxygen consumption (MVO₂) decreased with DK and further without pacing (EP: 9.8 ± 3.0, DK+EP: 8.7 ± 3.0, DK–EP: 6.2 ± 3.8 ml/min/100 g).

Conclusions: DK effectively reduced HR and prolonged diastole. The drug has no major negative inotropic effect, reduces MVO₂ and permits, in parallel, CBF to fall. In consequence, this novel bradycardic agent could prevent tachycardia in the experimental setting or could prevent unwanted, postoperative tachycardia. In addition, it could be an effective approach to induce bradycardia for off-pump coronary operations without compromising left ventricular function. J Clin Basic Cardiol 2000; 3: 191–6.

Key words: isolated blood-perfused heart, systolic and diastolic function, bradycardia, rabbit, oxygen consumption
For assessing aortic flow, an ultrasonic flow probe was used to respectively mimic aortic and mitral valves. An artificial systemic circuit that contained a windkessel and a cannula was placed into the pulmonary artery. The balloon was connected to an artificial respirator (Ugo-Basile, 505L). A 3F microtip-manometer (TC 500, Millar) was inserted into the balloon for measurement of left ventricular pressure. A cannula was placed into the pulmonary artery for collecting the coronary venous blood. The coronary circuit contained a servocontrolled roller pump (505L, Watson Marlow) that provided a constant coronary pressure of 80 mmHg. The flow within the coronary circuit was measured by a flow meter (T 206, Transonic System) connected to a flow meter. The blood was oxygenated (22% O2, 72% N2, 6% CO2) using a hollow fiber baby oxygenator (Masterflo 51, dideco). After muscular relaxation, the heart was excised and without intermittent ischemia, a small-animal respirator (Ugo-Basile, 7025) was used for electrical stimulation. Both electrodes were attached to the right atrium.

Methods

Experiments were performed on 10 isolated hearts of male New Zealand White rabbits with an average age of 7 months; body mass ranged from 2500 to 3500 g. The rabbits were handled according to the animal welfare regulations of the German federal authorities which are in accordance with the Guide for the Care and Use of Laboratory animals (NIH publication #85–23, revised 1985). The animals were anesthetized with 25 mg/kg i.m. ketaminhydrochloride and 5 mg/kg xylazine. After muscular relaxation, the heart was rapidly excised and without intermittent ischemia, the heart was connected to a modified Langendorff apparatus (Fig. 2). They were perfused with a modified Krebs-Henseleit buffer that contained bovine erythrocytes to give a hematocrit of 30%. To prevent edema formation, bovine albumin was added. After opening the left atrium and cutting all chordae tendineae, a thin-walled latex balloon (HSE #12–14) was inserted into the left ventricular cavity through the mitral valve. The balloon was connected to an artificial systemic circuit that contained a windkessel and two valves to respectively mimic aortic and mitral valves. For assessing aortic flow, an ultrasonic flow probe was used that was connected to a flow meter (T 206, Transonic Systems). In addition, this systemic circuit permitted changing preload and afterload. For the assessment of preload throughout the protocol, left intraventricular diameter was measured by sonomicrometry (System 6, Triton) using a pair of ultrasonic piezocrystals glued on either side of the balloon. A 3F microtip-manometer (TC 500, Millar) was inserted into the balloon for measurement of left ventricular pressure. A cannula was placed into the pulmonary artery for collecting the coronary venous blood. The coronary circuit contained a servocontrolled roller pump (505L) that provided a constant coronary arterial pressure of 80 mmHg. The flow within the coronary circuit was measured by using a second ultrasonic flow probe connected to the same flow meter. The blood was oxygenated (22% O2, 72% N2, 6% CO2) using a hollow fiber baby oxygenator (Masterflo 51, dideco).

Experimental Protocol

Measurements were performed after 20 min stabilization with electrical pacing to avoid negative inotropic effects of pacing. DK-AH 269 (DK) was administered at a concentration of 10^-6 M, and measurements were repeated after 30 min. After termination of pacing, the measurements were repeated. To evaluate the regional myocardial flow, colored microspheres were injected after vigorous mixing (Vibrofix, IKA-Labortechnik) during each of the three experimental steps, ie during electrical pacing, after DK, and without pacing. The temperature of the heart was kept constant at 38.5 °C. At the end of each experiment, the hearts were arrested using saturated potassium chloride. To normalize blood flow both left and right ventricles were weighed.

Data analysis, calculations and statistics

Variables were recorded on a forced ink chart recorder (Brush 481). At periods of interest, the paper speed was 50 mm/s. The variables were stored simultaneously on a magnetic disc using a custom-made computer program (EASYDAT [17] and a personal computer (Pentium, 100 MHz). The time constant of pressure decay was calculated using a monoexponential equation with an asymptote [18]. Coronary blood flow was normalized to 100 g wet myocardial mass. Myocardial oxygen consumption was calculated according to Fick’s principle using the normalized coronary flow and arterio-venous difference in oxygen content. Statistical analysis was performed using a computer program (SYSTAT [19]). A one-way analysis of variance with a subsequent posthoc correction according to Bonferroni was performed to exhibit differences between the three experimental steps. A p value < 0.05 was considered to represent statistical significant differences. All data are mean values ± SD.

Results

The heart rate during electrical pacing before (EP) and after (DK+EP) administration of 10^-6 M DK-AH 269 (DK) was 161 ± 20 and 161 ± 19 min^-1 respectively. Without pacing (DK-EP), heart rate significantly decreased to 101 ± 32 min^-1 (Fig. 3, p = 0.02). In parallel, at unchanged length of systole,
the duration of diastole was maintained or increased re-
220 ms, p = 0.04). Aortic flow decreased by 19% from 40.6 ±
21.7 to 32.8 ± 17.9 ml/min in the presence of DK and with-
out pacing by an additional 30% to 22.8 ± 24.6 ml/min (n.s.).
Stroke volume was moderately reduced (EP: 0.31 ± 0.16,
DK+EP: 0.26 ± 0.14 ml) and was further reduced to 0.21 ±
0.25 ml without pacing. Peak left ventricular isovolumic
pressure exhibited a tendency to decrease during the three
experimental steps from 109 ± 19 to 92 ± 20.3 to 75 ± 25
mmHg (Fig. 4, top). dP/dt max as measure of contractile
state, remained unchanged after administration of the agent
(1355 ± 545 vs. 1390 ± 830 mmHg/s) and was decreased
without pacing (890 ± 500 mmHg/s; n.s.) (Fig. 4, bottom).
dP/dt min was decreased with DK and furthermore without
−725 ± 340 mmHg/s). The time constant of pressure decay
(τ) was slightly reduced with DK (EP: 40.6 ± 9.0, DK+EP:
35.3 ± 4.9 ms) and almost recovered without pacing (38.7 ±
6.5 ms). The left ventricular end-diastolic pressure was 10 ±
7 mmHg during control and remained almost unchanged in
the presence of the agent (9 ± 6 mmHg). The decrease to 5 ±
5 mmHg after termination of pacing was significant (Fig. 5,
p = 0.03). In parallel, the end-diastolic left ventricular di-
ameter was decreased (EP: 10.0 ± 2.9, DK+EP: 9.39 ±
22, DK–EP: 9.39 ± 9.3 mm), ie preload was reduced. The
global coronary blood flow was reduced from 204 ± 29 to
156 ± 21 to 148 ± 33 ml/min/100 g in the three experimen-
tal steps (Fig. 6, top). No significant transmural redistribu-
tion paralleled these changes: the ratio between subendo-
cardial and subepicardial flow only slightly decreased (EP:
1.46 ± 0.39, DK+EP: 1.40 ± 0.28, DK–EP: 1.36 ± 0.41).
The coronary blood flow per beat was reduced with DK
(EP: 62 ± 20, DK+EP: 45 ± 12 µl/100 g) and reached
predrug level after termination of pacing (DK–EP: 61 ± 10
µl/100 g) (Fig. 6, bottom). Conversely, the coronary resist-

![Figure 3](image1.png)  
**Figure 3.** The heart rate (HR) was significantly reduced with DK. Mean ± SD during pacing (EP), DK-AH 269 10^-6 M (DK+EP) and
without pacing (DK–EP), *p = 0.02

![Figure 4](image2.png)  
**Figure 4.** The peak left ventricular pressure (LVP max) was
reduced with DK (top); dP/dt max was constant with DK+EP and
was diminished without pacing (bottom). Mean ± SD during
pacing (EP), DK-AH 269 10^-6 M (DK+EP) and without pacing
(DK–EP)

![Figure 5](image3.png)  
**Figure 5.** The end-diastolic left ventricular pressure (LVP )
was significantly decreased with DK after pacing was terminated.
Mean ± SD during pacing (EP), DK-AH 269 10^-6 M (DK+EP) and
without pacing (DK–EP), *p = 0.03

![Figure 6](image4.png)  
**Figure 6.** The global coronary blood flow (CBF) was reduced
with DK+EP and remained constant after pacing was terminated (top)
while the coronary blood flow per beat (CBF/beat) was increased in
the last experimental step (bottom). Mean ± SD during pacing
(EP), DK-AH 269 10^-6 M (DK+EP) and without pacing (DK–EP)
Discussion

Heart rate
Together with wall stress and contractile state, heart rate is a major determinant of myocardial oxygen demand [2]. Therefore, tachycardia in patients with coronary artery disease will likely induce detrimental ischaemia. Conversely, a decrease in heart rate may prove beneficial. With this rationale, we examined the haemodynamic effects of a novel bradycardic agent of the benzazepinone-type, DK-AH 269 (DK), that is reported to solely act on sinus node cells [20].

In former studies with buffer-perfused rabbit hearts [13] or on hearts of anaesthetized pigs [12], heart rate was significantly reduced and diastole prolonged after administration of DK. In other [11] and in our own [13] studies, DK was shown to reduce heart rate dose-dependently. Thus, only one dose was used in this study that was known to reduce heart rate by about one third. The bradycardic effect of 10^{-6} M DK on heart rate (37 % in this study) compares well with former studies using UL-FS 49 (zatebradine), a specific bradydycardic agent of the benzazepinone-type [8-10,21].

Bradycardic agents have no major effect on the duration of systole and, thus, prolongation of the cardiac cycle results in a prolonged diastole [22]. In this study, the length of diastole was almost doubled after DK. This is consistent with the mechanism of action of such If channel blocking agents, since these channels open only during diastole and therefore do not influence membrane potential during systole. Blockade of If channels results in a selective slowing of the spontaneous diastolic depolarization and therefore in a prolongation of diastole.

In particular, in the ischaemic heart two advantages should be associated with a prolonged diastole: the ventricular can more completely relax, resulting in a better end-diastolic filling and/or a lower end-diastolic pressure. On the other hand, myocardial perfusion should be improved since myocardial inflow – which occurs predominantly in diastole – should be increased [23].

Systolic function
The force-frequency relation (= staircase phenomenon) relates the contractile state to heart rate [15, 16, 24]. As a consequence, heart rate reduction can cause a reduction of myocardial contractility [11]. At a constantly held heart rate, systolic function was only moderately reduced after administration of DK and further without pacing (EP: 9.8 ± 3.0, DK+EP: 8.7 ± 3.0, DK–EP: 6.2 ± 3.8 ml/min/100 g) (Fig. 7), and the MVO2 per beat was reduced after DK with pacing but not without pacing (EP: 61 ± 28, DK+EP: 54 ± 33, DK–EP: 61 ± 30 µl/beat/100 g).

Diastolic function
The effect of heart rate on myocardial relaxation has been carefully studied, but the results are not consistent. Whereas heart rate changes only slightly affect the time constant of left ventricular pressure decay (τ) [30], this measure was decreased if heart rate was increased [31]. Similarly, the impaired dP/dt_{min} after DK was more likely due to the reduced heart rate rather than to a direct drug effect on early relaxation. In contrast to the further reduction of dP/dt_{min} after termination of pacing, τ remained nearly constant in this study. Likely, τ is less heart rate-dependent than dP/dt_{min} [30]. Additional results from
a study on isolated pig hearts treated with UL-FS 49 support the notion that the benzazepine-type agents do not affect early diastole in that study, neither dP/dt, nor \( \tau \) were significantly affected [8].

End-diastolic ventricular filling is inversely related to heart rate, as shown by vagal or atrial stimulation [32]. The prolonged diastole positively influences left ventricular filling [33], which, in turn, will affect end-diastolic pressure. Bradycardic agents must hereby not necessarily change diastolic pressure [8] and, therefore, the variable findings suggest that methodological aspects (experimental model, working point on the end-diastolic pressure-volume curve, degree of bradycardia) represent important factors in determining the effects of bradycardic agents on the end-diastolic pressure. Thus, the decreased end-diastolic pressure after DK without pacing in this study fits the concept that a reduction in heart rate is associated with a more complete late ventricular relaxation.

Global and regional coronary blood flow, myocardial oxygen consumption

Heart rate reduction with sinus node inhibitors such as alinidine [34], zatebradine [21, 23], or DK-AH 269 [11–13] is associated with a prolonged diastole, and thus, should allow an increase in coronary blood flow if it is limited by a stenosis. However, with almost intact vasculature, coronary flow in buffer-perfused hearts was decreased by 16 % (DK-AH 269 at 10^{-6} M; [13]) and in blood-perfused hearts even by 30 % [1].

The effects of bradycardia, however, look different, when coronary flow per beat is analyzed. It was significantly increased in our buffer-perfused hearts and remained unchanged in our blood-perfused hearts. In the present study, coronary blood flow per beat was considerably increased (+36 %) without pacing.

The relation between subendocardial and subepicardial flow remained nearly constant over the three experimental steps. This finding is in concert with our two preceding studies [13, 14]. From the previous studies and the present study on non-ischaemic myocardium, however, we cannot conclude whether bradycardia produces a selective increase in subendocardial blood flow in the ischaemic myocardium as described in other studies [35, 36].

After administration of 10^{-6} M DK, the coronary resistance was increased by 43 % in this study. It was similarly increased (49 %) in blood-perfused hearts [13] and less pronounced (21 %) in buffer-perfused hearts [14]. At first sight, the increased resistance would argue for a vasoconstrictive potency of DK. We suggest, however, an indirect effect that was due to the reduced oxygen requirements, i.e. vasoconstriction was initiated via autoregulation [21, 35].

Ventricular function was reduced in the non-paced hearts after administration of DK. In parallel, MVO_{2} was decreased, a fact that might already be beneficial in the normal myocardium. Ischaemic myocardium should, however, benefit much more from reducing heart rate and the concomitantly reduced oxygen requirements; heart rate in this study was reduced by 37 % in the DK-treated hearts compared to control.

In summary: Although the heart rate was reduced by 37 % by the novel sinus node inhibitor DK-AH 269, systolic function in the isolated rabbit hearts was not significantly impaired. Similarly, early relaxation was not affected much and end-diastolic left ventricular pressure was diminished as a consequence of the considerably prolonged diastole. In parallel with these changes, the myocardial oxygen consumption was decreased.

Conclusion: The agent seems useful in the treatment of various heart diseases by improving myocardial energetic balance, and it seems of value in the operating theatre by decreasing unwanted tachycardia after cardiac surgery or by inducing bradycardia during coronary artery bypass procedures on the beating heart.

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References


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