Original Research

Beta2-Adrenergic Activation Via Administration of Atenolol/Formoterol Combination Increases Contractility and Coronary Blood Flow in Isolated Rat Hearts

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Introduction: Commonly used adrenergic agonists in low cardiac output scenarios rely primarily on beta1-adrenergic activation to stimulate cardiac function. Little is known about the use of beta2-adrenergic agonist administration for this purpose, although the associated vasodilation may be beneficial. This study was conducted in order to assess the efficacy of one such beta2-adrenergic agonist, formoterol, in augmenting cardiac function.

Methods: The hearts of 8 anesthetized female Wistar rats were excised, and subsequently kept functional in an isolated heart preparation (Langendorff apparatus). After placement on the apparatus, hearts were subjected to the beta1-blocker, atenolol, and then to a combination of formoterol/atenolol. Left ventricular developed pressure (LVDP), heart rate (HR), and coronary flow (CF) were monitored.

Results: CF showed a median increase of 16% ($p<0.05$) after formoterol/atenolol administration, with this effect lasting 20 min post-administration. Furthermore, statistically significant differences included an early 26% increase in LVDP and a late 21% increase in HR. The CF increase was independent of HR and LVDP changes.

Conclusions: Our results indicate that the beta2-agonist formoterol not only successfully increases heart rate and contractility, but also increases coronary flow, most likely by means of beta2-mediated coronary vasodilation. This pharmacological profile may prove to be especially beneficial in situations where cardiac output must be increased, while adequate myocardial oxygen delivery needs to be maintained.

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Adrenergic agonists such as adrenaline are commonly used for the management of acute, low cardiac output and other situations characterized by end-organ hypoperfusion (anaphylaxis, sepsis, etc.).[1-3] Most adrenergic agonists used clinically act on the myocardium mainly via their affinity to $beta_1$ adrenergic receptors, through which the heart's contractility and heart rate are increased, thereby increasing cardiac output. They rely on a combination of central (cardiac) inotropic, and peripheral (vascular) constrictive effect to improve the perfusion of certain end organs, especially the brain and the kidneys.

Adrenergic stimuli also have a direct impact on coronary flow. The early studies of Gaal et al[4] showed that intracoronary adrenaline and noradrenaline injections induce a short-lived 20 s to 2 min coronary flow increase in dogs, independently of aortic pressure. Studies of the physiological analogue of adrenergic stimulation (exercise) have shown that the approximately sixfold increase in the oxygen demands of the left ventricle during intense exercise is met principally by an augmen-

tation of coronary blood flow (~5-fold), as hemoglobin concentration and oxygen extraction (which is already 70-80% at rest) increase only modestly in most species.[5] In fact, via adrenaline injections in dogs, Creates and Grayson showed that oxygen extraction ratio and blood flow are inversely related.[6]

Following adrenaline administration, metabolic demand and oxygen "hunger" lead to an increase in coronary flow, so that oxygen supply matches demand. However, inotropic therapy in patients hospitalized with acute decompensated heart failure often fails to reduce the mortality rate.[7,8] It appears that one of the major shortcomings of this adrenaline-induced myocardial workload is the resultant hypoxia, originating from a detrimental outmatch between an enhanced cardiomyocyte metabolism and an insufficiently increased coronary flow.

In an attempt to create a fine balance between the vasodilatory capacity of inotropes and counterbalance the side-effects of metabolic overload (when an adrenergic agonist is used), we experimented with formoterol, a $beta_2$-selective adrenergic agonist, currently used as an inhaled bronchodilator in asthma and chronic obstructive pulmonary disease.[9] We used the isolated rat heart model and sought to assess whether we could reverse the ratio between myocardial contractility and coronary flow. Formoterol fumarate has a more than 200-fold greater agonist effect (FORADIL Aerolized Datasheet, Novartis-Global) on $beta_2$ receptors than on $beta_1$ receptors, and it was much more likely to favor coronary vasodilatation over myocardial contractility, in a beneficial manner.

One concern with this approach was that formoterol, despite its high $beta_2$-adrenergic specificity, might also display some $beta_1$-adrenergic receptor activity, expressed as tachycardia, palpitations and ischemia, in humans.[10,11] In an attempt to further suppress the $beta_1$ activation, we tapered it down by using atenolol. Atenolol's intrinsic sympathomimetic activity has been shown not to alter coronary flow.[12]

Methods

Surgical procedure and heart perfusion

Animal treatment was in compliance with our institution's Policy on Animal Experimentation and all procedures were carried out in the most humane way possible. Eight female Wistar rats (weight 225-300 g), were used. The rats were pre-anesthetized with a continuous flow of an isoflurane-oxygen mixture for 30 s and subsequently anesthetized with an isovolemic ketamine (10 mg/mL) and xylazine (25 mg/mL) mixture, at 1.2 mL/kg body weight, injected intraperitoneally. After complete anesthesia was achieved, a mid-sagittal section was made on the anterior abdominal wall, followed by an injection of 1000 IU heparin into the exposed abdominal vein, 10 s prior to harvesting the heart. The hearts were excised and arrested in ice-cold Krebs–Henseleit (KH) buffer. The aorta was cannulated and the hearts retrogradely perfused from a reservoir 90 cm above the heart. Special care was taken in order to maintain the perfusate's upper level at 100 cm. Hearts were perfused with non-recirculating KH buffer containing 120 mM NaCl, 5.9 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 1.25 mM $CaCl_2$, 25 mM $NaHCO_3$, and 11 mM glucose at pH 7.4. The perfusate was equilibrated with 95% O_2 and 5% CO_2 and maintained at a temperature of 37°C. To monitor left ventricular developed pressure (LVDP), a latex water-filled balloon connected to a pressure transducer (Datascope, New Jersey) was inserted into the left ventricle and inflated with water in order to conform to the left ventricular cavity. This pressure sensor provided constant left ventricular pressure and heart rate monitoring, which was recorded using ABI software (ABI Instruments, Patras, Greece). The volume of the balloon was set to give an end-diastolic LV pressure of 5-10 cm H_2O and a peak systolic LV pressure of >80 cm H_2O, and LV volume was maintained constant thereafter. The isolated rat hearts were perfused for at least 30 min with KH buffer or until baseline functional parameters were established prior to the start of the experiment. The coronary flow and rate were monitored volumetrically, through continuous collection of the perfusate coming out of the pumping heart. This gave us an indirect measure of coronary resistance and coronary flow, under constant pressure.

Experimental protocols and measurements

All hearts were given at least 30 min to stabilize and mean coronary outflow was measured over 1-2 min periods. A 7.8 μg/mL solution of atenolol diluted in oxygenated KH buffer was prepared and 50 mL were added directly into the perfusate, via a 3-way connector, above the beating heart's cannulation site, at a constant infusion rate of 1 mL/s. The atenolol so-

lution was equilibrated with perfusate temperature before the time of infusion. We subsequently monitored three main outcome measures: heart rate (HR), changes in LVDP, and coronary flow (CF). Coronary flow was measured 1-2 min post-atenolol infusion, and then at 5 min (when all 50 mL of KH buffer had flowed through the isolated heart). Immediately after this final measurement, 50 mL of a 7.8 μg/mL atenolol + 1.2 μg/mL formoterol solution in oxygenated KH buffer were added into the perfusate at a rate of 1 mL/s. CF was measured at 1-2 min post-infusion, and then at 5, 10, 15, and 20 min.

It should be noted, that unlike atenolol, formoterol is not available in IV form; thus, the solution was made by performing 6 controlled-dose sprays with the FORADIL inhaler (formoterol fumarate inhalation powder, 12 μg metered-doses; Foradil® Aerolizer®; Novartis; Athens, Greece) in a 60 mL syringe. Furthermore, the pharmacokinetics of formoterol aerolized powder (Novartis Global) are quite different from other typical $beta_2$ intravenous agonists.

Five main characteristics must be emphasized (items 1-3 from Foradil Aerolizer datasheet, Novartis-Global):

1. Formoterol fumarate is a highly lipophilic substance. Formoterol fumarate enters the plasma cell membrane in the form of a depot and is gradually released into the aqueous phase to react with the $beta_2$ receptor, resulting in a long duration of action. The aqueous phase activity is responsible for the rapid onset of action of Formoterol fumarate, whereas the lipophilic phase corresponds to a longer duration via a slow release mechanism.
2. Formoterol fumarate exhibits significant *in vitro* binding to plasma proteins.
3. The rabbit and rat plasma levels of formoterol toxicity (μg/mL scale) that have been observed *in vivo* are 1000-1,000,000 times higher compared to the average human therapeutic plasma levels (pg/mL scale).
4. Regardless of the dose and concentration of infused formoterol (range 12-72 μg per 50 mL), we monitored a long lasting (>20 min) effect on CF. (Data not shown).
5. Given the pharmacokinetic specifics of formoterol, the experimental concentrations used by previous researchers in animals, and the mean isolated heart CF rate (~15 mL/min), we arbitrarily used an initial high concentration (1.2 μg/mL) of formoterol (in the form of a single 50 mL formoterol solution in oxygenated KH buffer), assuming that this dose is spread over 20 min and achieves the *in vivo* analogue of a high distribution volume.

Data analysis and statistical procedures

Data analysis was split into two series of multiple tests, with strict internal correction for multiple comparisons within each series.

The first series of analyses involved the immediate and sustained effect of formoterol on CF, LVDP, and HR. With the exception of CF, our experiments showed that the response to formoterol did not display a Gaussian distribution, with some hearts even having opposite responses. Thus, non-parametric statistical analyses were applied for all variables. This consisted of three separate Friedman analyses of variance for each of the variables, with data being inputted as multiple measurements for each experiment at baseline, 2-3 min post-atenolol, 1-2 min post-formoterol/atenolol, and 20 min post-formoterol/atenolol. The null-hypotheses were rejected with a modified significant p-value, based on the sequentially rejective Bonferroni test, in order to keep the overall alpha-error below 0.05.[13]

Within each Friedman analysis, Dunn's *post hoc* test (with built-in multiple comparison correction) was performed to assess the statistical significance of observed changes in variable values between different time points of the experiment. For LVDP and HR, comparisons were made between each of the two post-formoterol/atenolol times and baseline. For CF, an additional comparison between the two post-formoterol times was made with post-atenolol CF, to assess the effect of the $beta_1$-blocker on CF increase.

The second series of analyses involved the quantification of the correlation between CF and each of LVDP, and HR, using the Spearman rank test for data values at the same time points used in the Friedman tests described above. Again, the significance of p-values was corrected for multiple comparisons.

Materials

Chemical compounds for KH buffer were purchased from Sigma Aldrich. Atenolol was acquired from Astra Zeneca, Athens, Greece (brand name TENORMIN, IV ampule). Formoterol was acquired from Novartis, Athens, Greece (brand name FORADIL, inhaler).

Results

Figure 1 displays the changes in CF, LVDP, and HR in the 8 isolated hearts over the entire experimental protocol.

Atenolol effects

To examine the sole effect of $beta_1$ blockade on the isolated perfused hearts, we infused 7.8 μg/mL atenolol and collected data before, during and after atenolol infusion. The HR response to atenolol was quite variable. We define "change" as a difference of more than 10%. In 6/8 hearts the HR was unchanged, 1/8 showed bradycardia and 1/8 tachycardia. A more pronounced response was observed with LVDP measurements. In 3/8 hearts the LVDP was unchanged, in 2/8 hearts the LVDP increased, and in 3/8 hearts it decreased. The CF showed no change in all hearts. As the atenolol was washed out, there was a complete recovery of all measurements to baseline, within 1-5 min, in 7/8 hearts.

Formoterol/atenolol effects

A significant change in CF was observed in 8/8 hearts when they were infused with formoterol/atenolol. The CF increased 40 s to 2 min after the initiation of the perfusion and the response was persistent for about 20-30 min after formoterol administration. This finding indicates that formoterol's $beta_2$-receptor activation effect is sustained over time, perhaps by means of a retention, slow transformation and release of formoterol from the lipophilic to the aqueous phase.

In 3/8 hearts the HR remained unchanged. However, a significant change in HR was observed in 5/8 hearts after formoterol administration, compared to the pre-atenolol values. This indicates the presence of a strong chronotropic effect. The HR response increased dramatically just after atenolol's short-lived effect wore off. That indicates a sufficient rate control effect of atenolol over formoterol which is abrogated after atenolol's washout.

A transient increase in LVDP, in parallel with the CF increase, was observed in all 8/8 hearts during formoterol/atenolol infusion.

Table 1 provides a summary of the statistical analysis of the experimental data. Column p-values for each of CF, LVDP, and HR were all significant following analysis with Friedman's test of variance. This implies that the observed changes in each of these variable during experimentation (overall) were not likely to be due to chance.

Subsequent testing with Dunn's *post hoc* test revealed a statistically significant ($p<0.05$) increase in CF compared to either baseline or atenolol values, both during formoterol/atenolol mixture administration and 20 min later. This corresponds to a lasting increase in coronary flow due to formoterol $beta_2$ activation, which is irrespective of atenolol's direct $beta_1$ blocking properties.

Analogous testing that compared LVDP and HR during and post-formoterol/atenolol to baseline values led to more interesting results. Given with atenolol, formoterol produced an initial statistically significant ($p<0.05$) increase in LVDP, but not in HR. As the short-acting atenolol effect wore off, HR increased significantly ($p<0.05$), while LVDP returned to baseline. Thus, atenolol appears to protect the LVDP increase associated with formoterol.

Correlation of CF with LVDP, and HR

Another question that we wanted to answer was whether what we observed as an independent increase in CF due to $beta_2$ activation was in fact due to variations in LVDP and/or HR. To check for this, we performed Spearman's rank (correlation) test on our data (Table 2). The CF increases showed only a weak correlation with both LVDP and HR, and this did not reach statistical significance. We consider the weak correlation to be the result of non-specific $beta_1$ activation of formoterol, which may be susceptible to titration with varying doses of atenolol.

Discussion

Coronary flow is determined by aortic pressure, the vasomotor state of the coronary bed, and the passive constrictive effect of myocardium on the coronary vasculature.

Patients with acute, low cardiac output often experience a need for CF increase, in order to marginally shift the balance between myocardial oxygen supply and demand. In heart failure, nitrates, beta-blockers and calcium channel blockers are used. These agents are effective in the management of CF augmentation, mainly via inducing coronary vasodilatation. However, their use is often complicated by a diminished cardiac output due to either a negative inotropic effect on an already diseased myocardium, or preload-afterload changes, which may compromise aortic pressure and

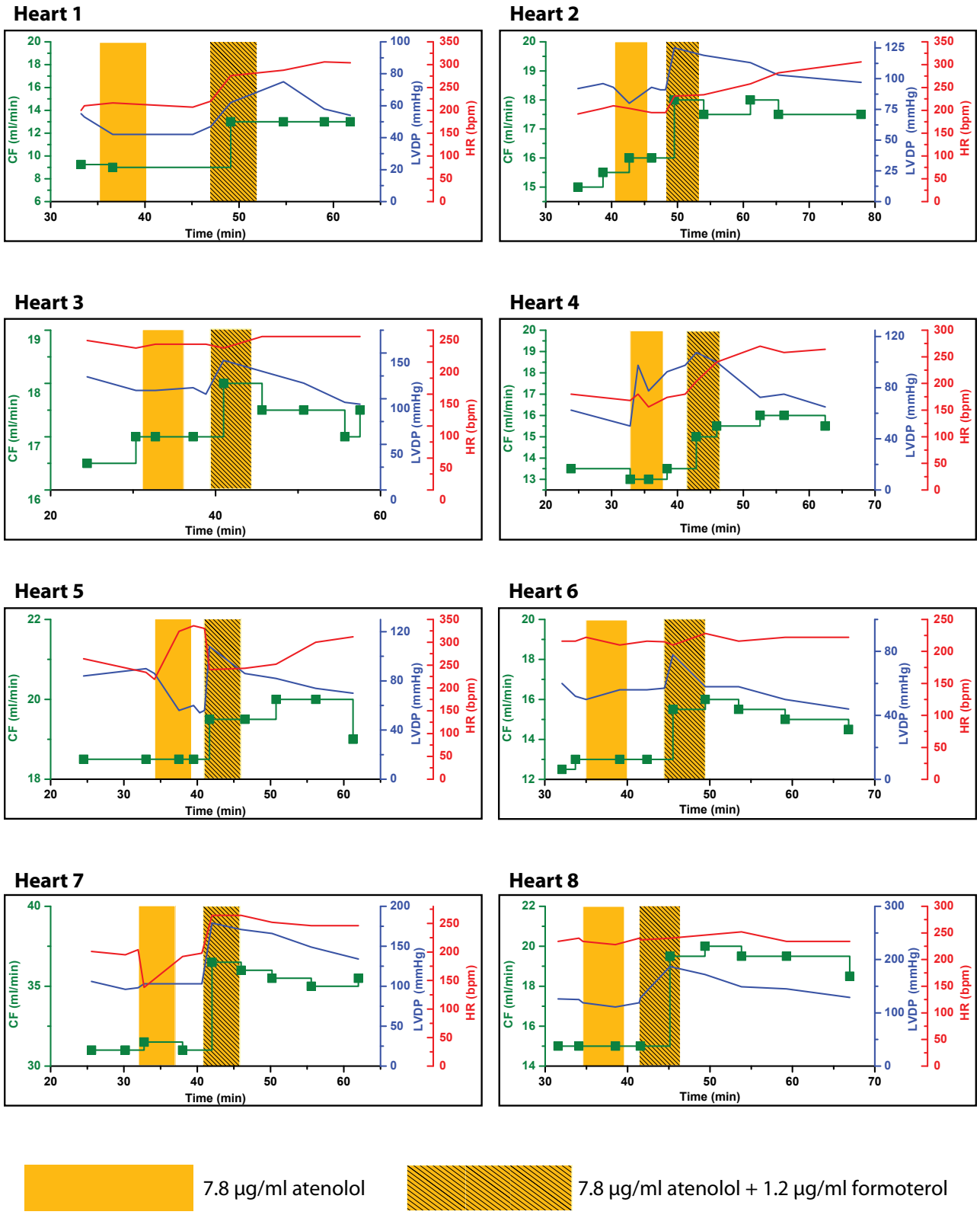

Figure 1. Graphical summary of coronary flow, left ventricular developed pressure, and heart rate changes throughout the experimental protocol; CF – coronary flow; LVDP – left ventricular developed pressure; HR – heart rate.

Table 1. Results summary (median values ± standard error of variables and analysis for statistical significance).

	Coronary flow (mL/min, n=8, p<0.0001*)	Left ventricular developed pressure (mmHg, n=8, p=0.002*)	Heart rate (bpm, n=8, p=0.032*)
Baseline	15.3 ± 2.3	93.0 ± 10.1	210 ± 8.2
During atenolol	15.5 ± 2.4	78.8 ± 9.4	213 ± 19.7
During formoterol/atenolol	18.0 ± 2.6†,‡	117 ± 15.7†	236 ± 8.8
20 min post-formoterol/atenolol	17.5 ± 2.5†,‡	82 ± 11.8	255 ± 12.8†

*Significant p-value for variable variance overall. †p<0.05 compared to baseline. ‡p<0.05 compared to post-atenolol.

Table 2. Correlation tests between experimental variables.

	Correlation coefficient (r_s)	p-value*
Coronary flow vs. left ventricular -pressure (n=8)	0.397	ns
Coronary flow vs. heart rate (n=8)	0.447	ns

*Significance of p-value based on sequentially rejective Bonferroni test (overall alpha-error <0.05). ns – non-significant.

subsequently CF. If this occurs, counteractive supportive measures (inotropic support and volume load) have to be applied promptly to compensate. Inotropes instantly increase cardiac output, but evidently they also raise the myocardial oxygen consumption of an often already ischemic heart. The perfect balance may be hard to find, especially with the imminent possibility of a cardiac arrest looming.

Using the Langendorff apparatus, we addressed the relationship between the long acting $beta_2$ agonist formoterol and coronary flow. We used a combination of a high-dose $beta_2$ agonist (formoterol) and a low-dose $beta_1$ blocker (atenolol) and studied the vasodilatory effect of formoterol on the coronary bed of the isolated rat heart. We further directed our assessment towards answering the clinical question: Can a combination of a $beta_2$ agonist and $beta_1$ blocker be a pharmaceutical alternative to inotropes in the ischemic/hypoperfused patient, when CF augmentation is desired?

Our constant pressure experiments showed that the addition of formoterol to the KH buffer decreased coronary flow resistance, irrespective of its effect on myocardial contractility and heart rate.

Moreover, in 8/8 hearts following the formoterol/atenolol administration there was a parallel course between CF and LVDP, with a latency period for LVDP following the CF increase. We hypothesized that coronary flow augmentation impacts directly on LVDP as oxygen supply increases. In 3/8 hearts (hearts 2,4,5) the parallel CF and LVDP change was interrupted after the effect of atenolol wore off and HR subsequently increased. In 3/8 hearts (hearts 3,6,8) neither atenolol nor formoterol/atenolol induced HR alterations, although they did induce CF and resultant LVDP changes. This indicates that atenolol and formoterol probably rely on the same receptor for their HR modifying properties.

Similar parallel rate-responses were observed with salmeterol and atenolol/salmeterol infusions during initial standardization experiments (data not shown). This would be consistent with the hypothesis that $beta_1$ receptor (rate control) properties share polymorphisms that are independent of beta-2 receptors (CF and resultant LVDP augmentation). This implies that formoterol has important therapeutic potential.

In 3/8 hearts the parallel CF/LVDP course was also characterized by a progressive increase in HR, shortly after the formoterol/atenolol infusion was stopped. Our results seem to fit with the concept that the effect of CF on LV pressure depends on the HR exhaustion. As soon as the atenolol effect wears off, this leads to inadequate HR control that exhausts the heart and, as in the case of CF decline, restrains it from producing an elevated LV pressure.

Our results indicate that the $beta_2$-mediated increase in coronary flow occurred rapidly following formoterol infusion and lasted for at least 20 min. These findings are in accordance with previous pharmacological studies of this drug, which show both an immediate and long-lasting effect of formoterol, as explained by the presence of

an intracellular, lipophilic drug depot that gradually enters a pharmacologically active, aqueous phase.[14] Contrarily, our corresponding studies with salmeterol (unpublished data) failed to consistently induce, or sustain, a statistically significant CF increase, regardless of the concentration used. This may be due to the experimentally unfavorable pharmacology of salmeterol, whose long-lasting effect has a slow onset.[14] Additional studies to understand the effect-responsiveness curves, the capacity for re-stimulation, and differences that might exist between different $beta_2$ agonists are warranted.

Our results could have important and novel clinical implications. First, they suggest that formoterol may play a valuable role in addressing the therapeutic need for a combined inotrope/coronary vasodilatory agent, compared to traditional inotropes, as it may circumvent the detrimental effects of both a reduced cardiac output and an increased metabolic demand. Second, they provide appealing physiological evidence that direct $beta_2$ targeting and moderate increases in CF by 10-20%, seem to cause a direct secondary rise of LV pressure. Third, they indicate that atenolol seems to share common receptors and HR modifying properties with formoterol.

Further *in vivo* animal experiments are warranted to ascertain formoterol's systemic flow effects, as well as its impact on myocardial oxygen adequacy.

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References

1. Hollenberg SM. Vasopressor support in septic shock. Chest. 2007; 132: 1678-1687.
2. Jessup M, Abraham WT, Casey DE, et al. 2009 focused update: ACCF/AHA Guidelines for the Diagnosis and Management of Heart Failure in Adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation. Circulation. 2009; 119: 1977-2016.
3. Simons FE. Anaphylaxis. J Allergy Clin Immunol. 2010; 125: S161-181.
4. Gaal PG, Kattus AA, Kolin A, Ross G. Effects of adrenaline and noradrenaline on coronary blood flow before and after beta-adrenergic blockade. Br J Pharmacol Chemother. 1966; 26: 713-722.
5. Duncker DJ, Bache RJ. Regulation of coronary blood flow during exercise. Physiol Rev. 2008; 88: 1009-1086.
6. Creates MS, Grayson J. The effect of adrenaline and noradrenaline on coronary vascular reserve in the dog. J Physiol. 1980; 309: 557-568.
7. Abraham WT, Adams KF, Fonarow GC, et al. In-hospital mortality in patients with acute decompensated heart failure requiring intravenous vasoactive medications: an analysis from the Acute Decompensated Heart Failure National Registry (ADHERE). J Am Coll Cardiol. 2005; 46: 57-64.
8. Teerlink JR, Metra M, Zacà V, et al. Agents with inotropic properties for the management of acute heart failure syndromes. Traditional agents and beyond. Heart Fail Rev. 2009; 14: 243-253.
9. Prenner BM. Formoterol dry-powder inhaler for the treatment of asthma and chronic obstructive pulmonary disease. Expert Opin Pharmacother. 2007; 8: 3069-3084.
10. Guhan AR, Cooper S, Oborne J, Lewis S, Bennett J, Tattersfield AE. Systemic effects of formoterol and salmeterol: a dose-response comparison in healthy subjects. Thorax. 2000; 55: 650-656.
11. Salpeter SR, Ormiston TM, Salpeter EE. Cardiovascular effects of beta-agonists in patients with asthma and COPD: a meta-analysis. Chest. 2004; 125: 2309-2321.
12. Berdeaux A, Giudicelli JF. Intrinsic sympathomimetic activity and coronary blood flow. Brit J Clin Pharmacol. 1982; 13(Suppl 2): 175S-180S.
13. Gordi T, Khamis H. Simple solution to a common statistical problem: interpreting multiple tests. Clin Ther. 2004; 26: 780-786.
14. Sears MR, Lötvall J. Past, present and future—$beta_2$-adrenoceptor agonists in asthma management. Respir Med. 2005; 99: 152-170.