대한생리학회지 : 제23권 제 2 호

□ SPECIAL LECTURE □

Myogenic Autoregulation of Coronary Vessels and Heterometric Autoregulation of the Myocardium

H.J. Döring*, I. Schlicht, V. Hiller and X. -R. Jiang

Physiological Institute of the University of Freiburg, Freiburg, Germany

INTRODUCTION

Basically contractile function of muscular organs can be tested in 2 ways: First by measurement of absolute force or shortening at steady state, and second by challenging cellular intrinsic regulatory mechanisms, for example subsequent to mechanical or metabolic loading. Differentiated information, however, can only be achieved through the latter method of applying specific load or provocation tests to the muscle. At the intact beating heart such investigations can not be performed in an easy way because of the well known interaction between myocardial contractile force and coronary flow. Therefore both muscle types involved, namely the myocardium and the coronary vasculature, usually must be investigated separately. In recent years a combined load test for the *simultaneous* analysis of cardiac contractile activity and coronary vascular behavior was developed which can be applied at intact isolated perfused hearts (Döring and Dehnert (1988); Döring (1989)). This method depends on the intrinsic autoregulatory properties of the myocardium and of the coronary vasculature.

METHODS

Hearts from guinea-pigs (BW 280~350 g) and rats (BW 250~320 g) were perfused according to Langendorff at constant pressure (50 mmHg) with modified Krebs-Henseleit solution** and electric stimulation of 270/min. The following parameters were measured under steady conditions (see Fig. 1): isovolumetric left ventricular pressure by means of a balloon catheter connected to a mechano-electrical pressure transducer, coronary flow above the aortic cannula using an electromagnetic flowmeter (Biotronex BM 402) and perfusion pressure at a side port of the aortic cannula.

The perfusion pressure was kept constant at 50 mmHg by an electronic perfusion pressure control unit (Hugo Sachs Electronik, D-7806 march (FRG)). This unit not only controls the preset constant perfusion pressure but in addition allows stepwise changes in perfusion pressure.

By this means pressure loads to the heart could be applied by suddenly raising perfusion pressure. This causes two reactions in the heart (Fig. 2).

Firstly, the increased coronary perfusion pressure induces an immediate increase in the contractile force of the myocardium obviously caused by increments of the prestretch of the myocardial fibres (Gregg,

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**Original composition with the addition of 2 mmol/l Na-pyruvate

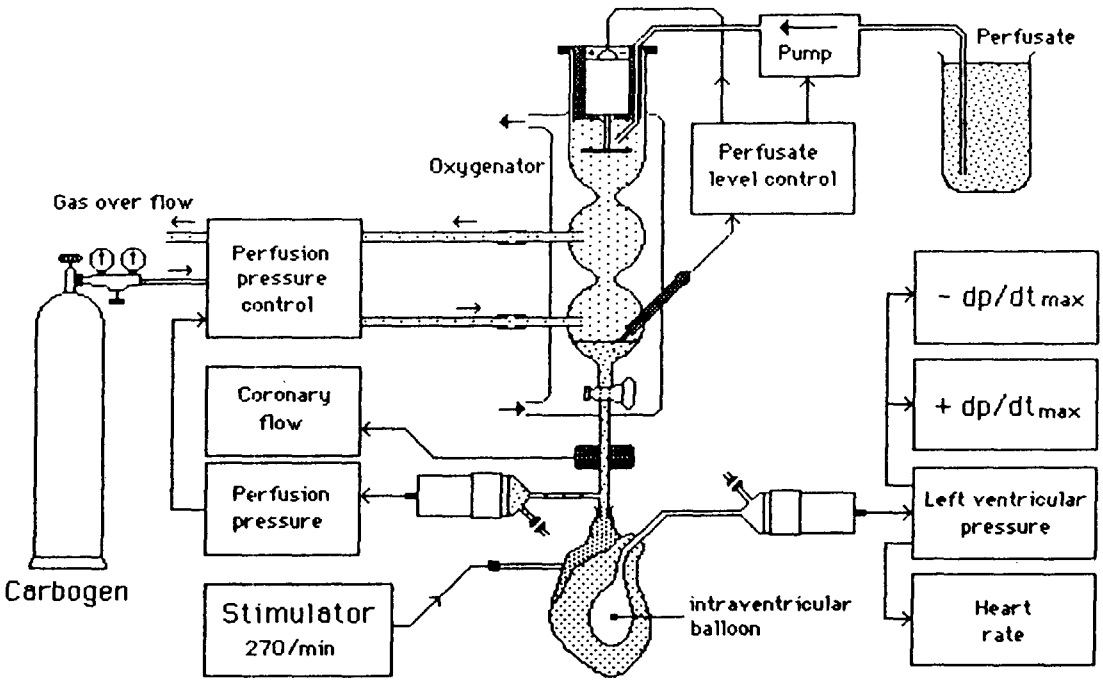

Fig. 1. Block diagram of the measuring devices of the isolated perfused heart.

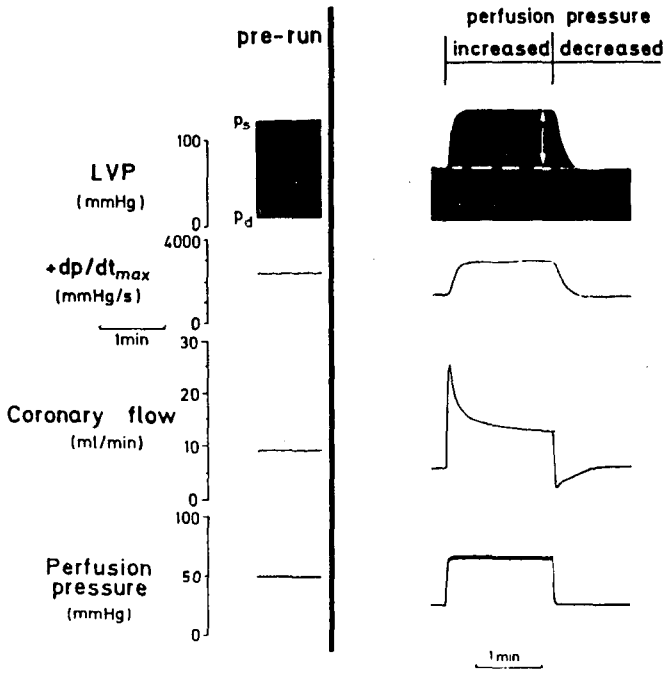

Fig. 2. Heterometric autoregulation of the myocardium (Gregg effect) and myogenic autoregulation of the coronary vessels (Bayliss effect) induced by a perfusion pressure step of 40 mmHg.

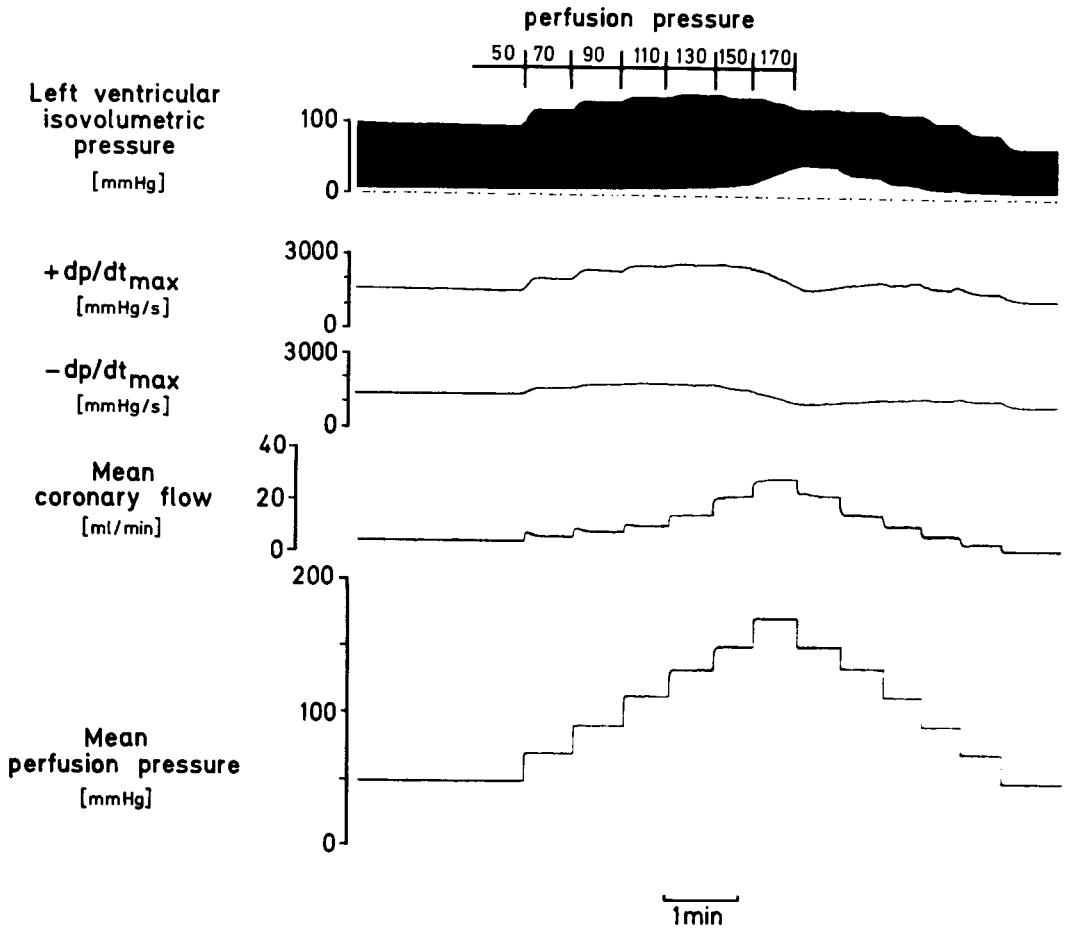

Fig. 3. Influence of stepwise increase of perfusion pressure on the myocardial contractile force and the coronary flow of an isolated perfused guinea-pig heart (BW 350 g).

1963). According to Sarnoff (1960) we termed this prestretch-dependent increase in contractile force heterometric autoregulation.

Secondly, the intracoronary pressure increase induces the myogenic autoregulation of the smooth vasculature known as the BAYLISS effect. Following the pressure increase, there is an initial pressure-passive expansion of the vessel immediately followed by a reactive vasoconstriction. An abrupt pressure decrease produces the opposite effects.

In place of a single pressure step a series of small steps of 10 or 20 mmHg can be applied such as shown in Fig. 3. It is evident that the systolic ventricular pressure increases rapidly at the first two pressure steps, much less so for the next two steps, and even drops again above a perfusion pressure of 150 mmHg. The diastolic pressure remains constant up to a perfusion pressure of 110 mmHg. The effects of myogenic autoregulation on the coronary flow can be observed, too, however, due to the lower pressure steps only to a smaller extent (for details see Döring (1989)).

The control and on-line evaluation of the experiments was performed by means of an IBM-PC with corresponding software.

The cause of the increase in contractile force is the

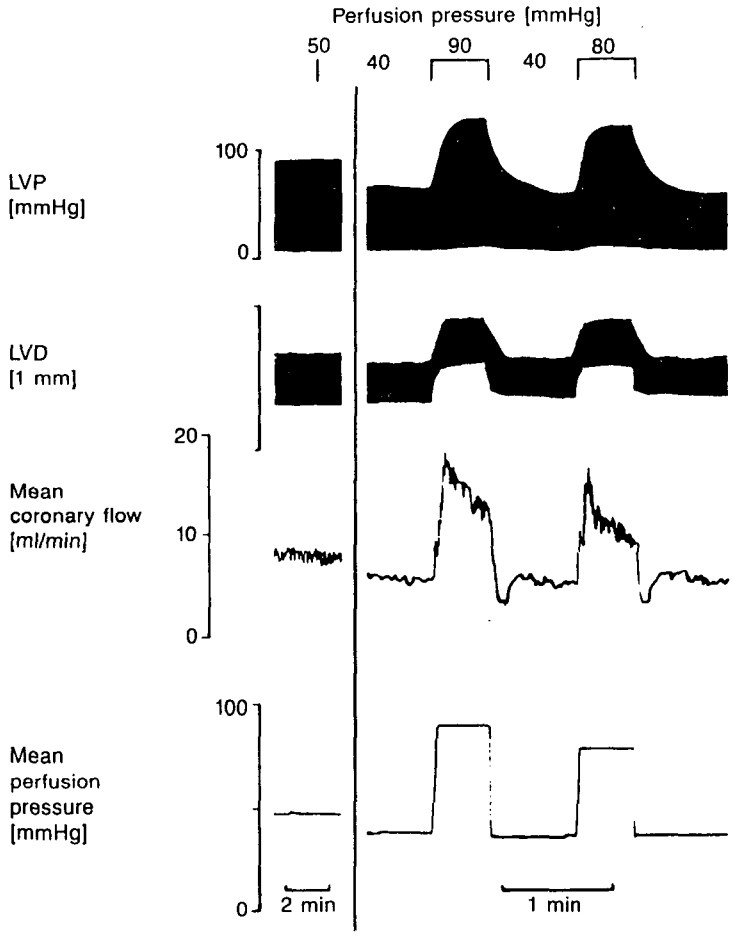

Fig. 4. Influence of coronary perfusion pressure on the change in left ventricular cross setional diameter (LVD) of an isolated perfused guinea-pig heart. An abrupt increase in perfusion pressure from 40 to 90 mmHg results in:

a) a left ventricular pressure (LVP) increase from 70 to 130 mmHg.

b) an increase in the diastolic and systolic left ventricular cross sectional diameter of 0.3 mm each (outer diameter at the level of the heart's base: 10 ~13 mm):

US-crystals 2 mm diameter, Sonomicrometer Triton Company. The ultrasonic transmitter was positioned at the epicardium at the largest cardiac diameter. The corresponding ultrasonic receiver was inserted through the right atrium into the right ventricle.

The upper level of the LVD recording represents with diastolic diameter, the lower level is the systolic diameter of the left ventricle.

myocardial fibre stretch induced by a rise in intracoronary perfusion pressure. Fig. 4 is an original registration of the change in left ventricular cross sectional diameter (measured by piezoelectric ultrasonic transducers) during such a rise in perfusion pressure. The left ventricular pressure increases simultaneously to the diameter. However, the change in diameter is not consistent with the change in coronary flow.

A graphic evaluation of 8 experiments on guinea-pig hearts is given in Fig. 5. The curve peaks at a perfusion pressure of 130 mmHg on the average. The developed pressure of the left ventricle at this point is about 120 mmHg. Since obviously stretch of the myocardial fibres—induced by raising perfusion pressure— causes the rise of the left ventricular pressure, the curve represents an equivalent of the Frank-Starling function curve of the left ventricle. Instead of the enddiastolic pressure the stretching force is replaced here by the perfusion pressure. At perfusion pressures of 30 to 110 mmHg we are therefore in the ascending branch of the Frank -Starling function curve. But this is also the optimum working range of the heart. Above a perfusion pressure of 120 mmHg the descending branch of the function curve starts.

When plotting coronary flow (the actual autoregulatory adjusted values) instead of developed ventricular pressure against the perfusion pressure,

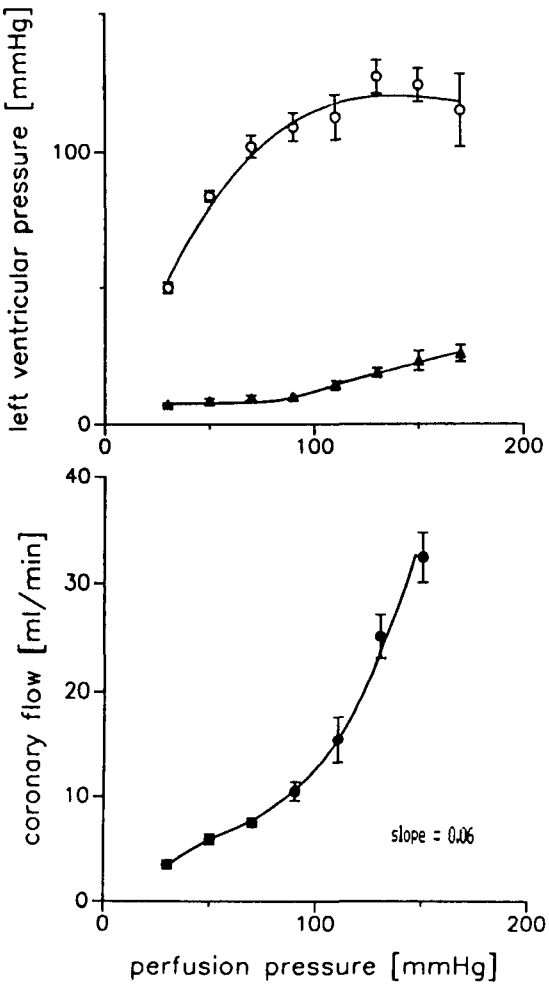

Fig. 5. Graphic evaluation (means +/−S.E.M.) of 8 experiments like in Fig. 3 (guinea-pigs).

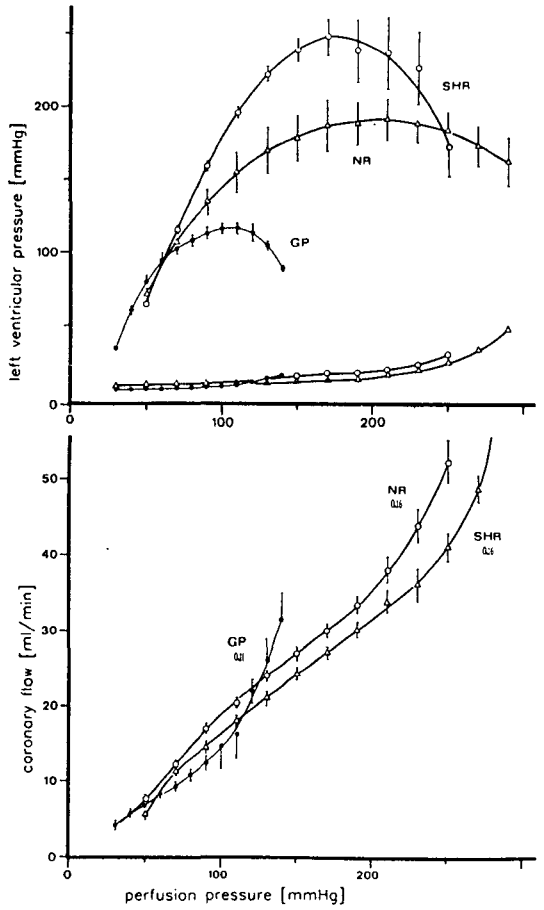

Fig. 6. Left ventricular function curves (upper pannel) and coronary pressure-flow curves (lower pannel) from guinea-pig and rat hearts. Numbers at the pressure-flow curves represent the slope in the perfusion pressure range given in chap. "method". NR=normotensive rats; SHR= spontaneously hypertensive rats.

pressue-flow curves of the coronary bed are obtained (lower part of Fig. 5). The slightly flat part of the resulting curve in the pressure range from 30 to 100 mmHg indicates the autoregulatory range of the coronary vascular bed. Above 100 mmHg the curve bends over and then continues as a straight line.

As an additional measure of the degree of the myogenic autoregulation the slope of the pressure-flow curves was calculated in the section which normally shows autoregulation, i.e. 40~60 mmHg in guinea-pigs and 90~190 mmHg in rats. In the curve of Fig. 5 the slope is 0.06.

RESULTS

The Frank-Starling function curves as well as the pressure-flow curves differ appreciably between species. Both types of curves from guinea-pigs, normotensive rate (NR) and, in addition, from spontaneously hypertensive rats (SHR) are combined in Fig. 6. Concerning the myocardial function curves those of rat hearts are appreciably extended to higher values

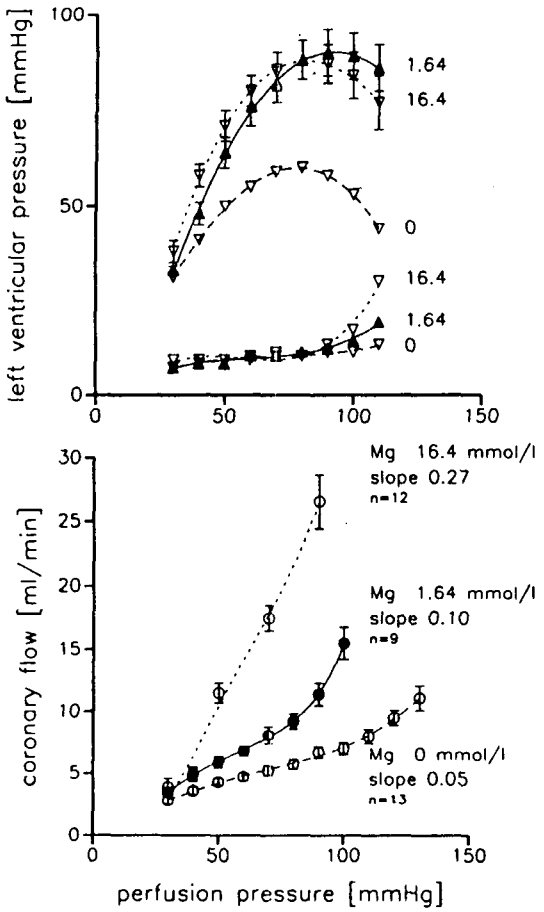

Fig. 7. Influence of different magnesium concentrations on left ventricular function curves and coronary pressure-flow curves from guinea-pig hearts. means +/− S.E.M.

when compared with those of guinea-pigs: firstly, the maximum pressure devoloped by the left ventricle is 190 mmHg on the average, and secondly the maximum perfusion pressure loading is of the order of 170 ~190 mmHg. SHR only showed higher developed left ventricular pressures as compared with NR.

The lower part of the figure shows the respective pressure-flow curves of the coronary vessels. Here, too, significant differences between the different groups of animals were found. For example the autoregulatory range of the rat coronary vessels is in the range of 90 to 190 mmHg. The slope of both types of rat coronary vessels is identical at 0.16 and thus higher compared with guinea-pigs.

The shape of the myocardial function curves and of the coronary pressure-flow curves also depends decisively on the composition of the perfusate. A very important factor is the calcium/magnesium ratio. Fig. 7 shows the influence of changes in the magnesium content of the perfusion solution at a constant Ca^{2+} concentration of 2.5 mmol/l. With magnesium-free solution myocardial contractile force is appreciably reduced by 33% compared with the control curve at 1.64 mmol/l Mg^{2+}. Surprisingly, contractile force under 16.4 mmol/l Mg^{2+} is not affected.

In the bottom curve (lower panel)—these are the experiments with magnesium-free solution —myogenic autoregulation is enhanced. At 0.05 the slope is only half of that of the curves with normal Mg-content of 0.10 shown in the middle. On the other hand the slope of the pressure-flow curve under the 10-fold Mg-concentration (16.4 mmol/l) is increased 3-fold to 0.27.

From these experiments we learn that high Mg concentrations can depress an increased spastic activity of the coronary vessels without major inhibition of myocardial contractile force.

Now the question of the molecular causes of myogenic autoregulation of the coronary vessels and the heterometric autoregulation of the myocardium is to be addressed.

Myogenic autoregulation

Different hypotheses try to explain that passive stretch or shear stress causes contraction of vascular smooth musculature:

1) Endothelium hypothesis: Davies (1989) postulated that endothelial cells release, flow-or pressure-induced and mediated by ion-channels and endothelial-derived relaxing or contracting factor. The latter should cause the smooth muscle cells to relax or contract. The nature of these factors is not known by now. Katusic et al. (1987) found at brain

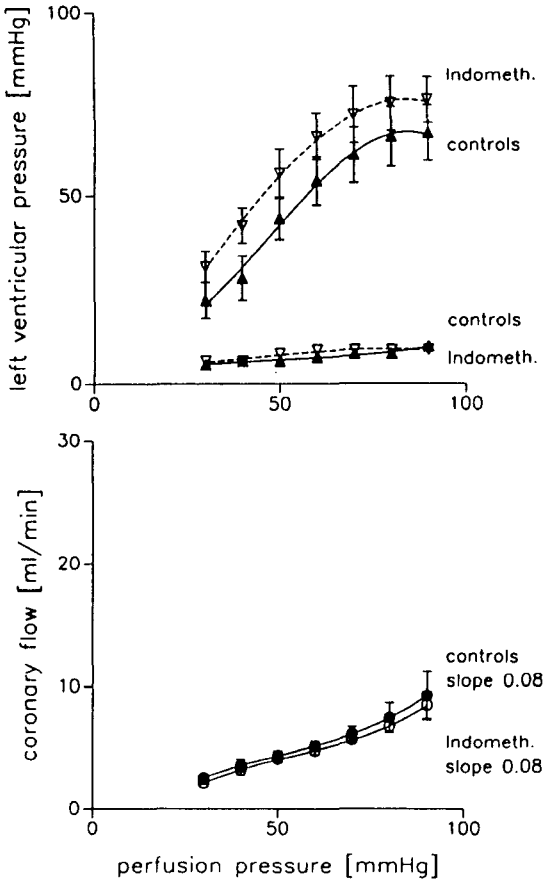

Fig. 8. Influence of indometacin (5 ml/l=10^{-5} mol/l) on left ventricular function curves and coronary pressure-flow curves from guinea-pig hearts. means +/−S.E.M. n=3.

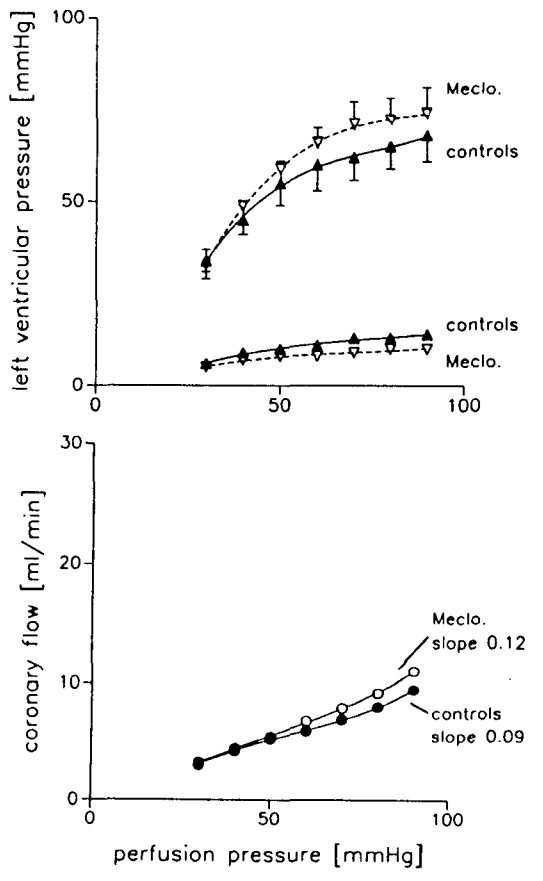

Fig. 9. Influence of meclofenamate (1 mg/l=3.4×10^{-6} mol/l) on left ventricular function curves and coronary pressure-flow curves from guinea-pig hearts. means +/−S.E.M., =4.

vessels in vitro that removal of endothelium or application of the cyclooxygenase inhibitor indometacin (but not of the leucotriene synthetase inhibitor diethylcarbamazine) blocks myogenic autoregulation. The authors concluded that prostaglandins may, at least in part, be involved in myogenic autoregulation (see Vanhoutte (1987)).

2) Myogenic hypothesis: Mellander (1988) considered vascular smooth muscles as a mechanoreceptor-mechanoeffector unit. Deformation of "receptor" cells causes facilitation of spike discharge or membrane depolarization without spikes (probably via Ca channels) which induces contraction of the neighbouring "effector" cells. This hypothesis s consistent with results of Govyrin (1989) who found myogenic autoregulation to be not endothelium-derived.

Relevant results from our own laboratory obtained with the method introduced above will be demonstrated in Figs. 8 and 9. Fig. 8 shows that the cyclooxygenase inhibitor indometacin in the very high concentration of 10^{-5} mol/l had no effect on myogenic autoregulation. The slope before and after application of the substance is identical, namely 0.08. Also meclofenamate, another cyclooxygenase inhibitor proved to be ineffective (Fig. 9). Thus the results of

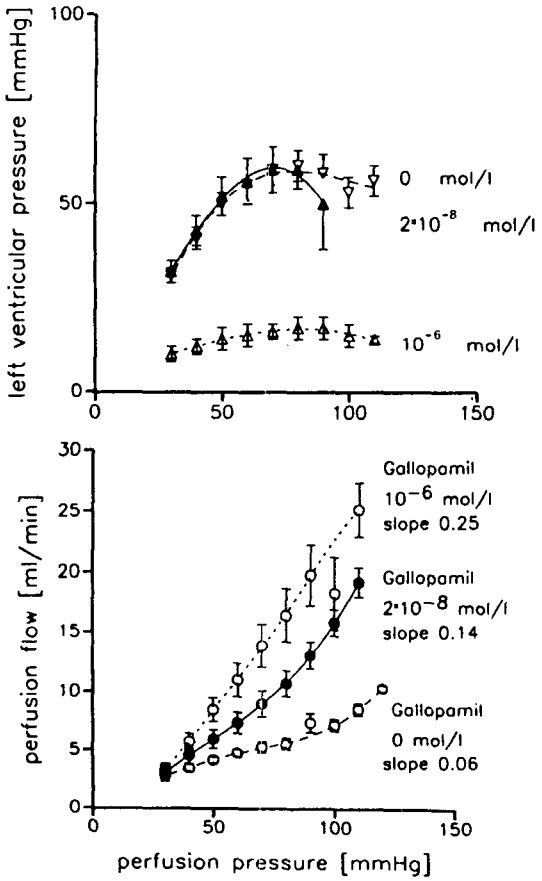

Fig. 10. Ventricular function curves and coronary pressure-flow curves at magnesium concentration 0. Influence of various gallopamil concentrations. *means* +/−S.E.M.

Katusic et al. could not be confirmed. Instead, the hypothesis of Mellander is favoured, namely that stretch-induced activation of ion channels, especially Ca channels, may be the cause of the reactive vasoconstriction. This is consistent with results of Grün and Fleckenstein (1972), Ono et al (1974), Cohen and Fray (1982), Hashimoto and Ono (1983) Döring (1989) who showed that Ca antagonists can abolish the myogenic autoregulation. As an example the effect of the Ca antagonist gallopamil is demonstrated in Fig. 10. Of special interest is the fact that gallopamil obviously can reduce the myogenic autoregulation which was considerably increased by magnesium deficiency as was already shown above: the lower curve demonstrates the pressure-flow relationship without magnesium and without gallopamil. The slope here is 0.04. The upper two curves reflect the situation after application of two different doses of gallopamil.

Heterometric autoregulation

Now the issue of myocardial cell response to rapid stretching caused by an increase of perfusion pressure is to be addressed. According to the sliding filament theory increased prestretch of the muscle and thus increased sarcomere length, results in an

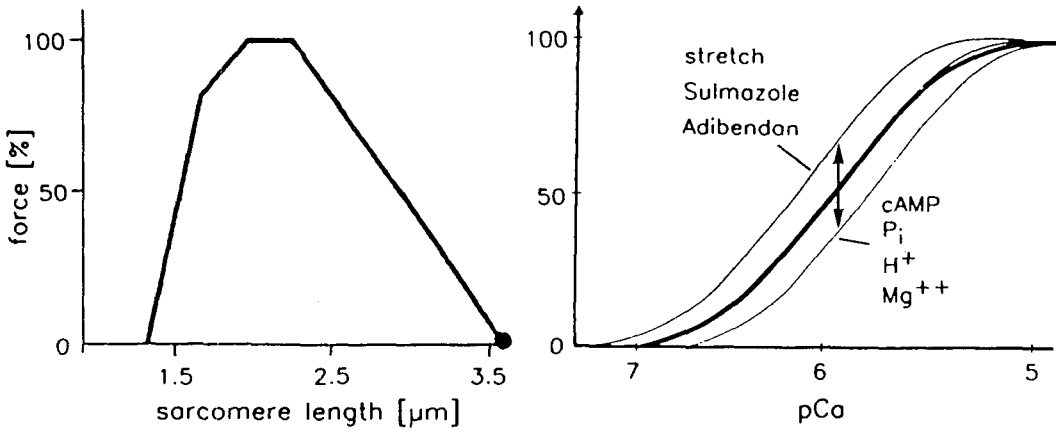

Fig. 11. Dependence of myocardial contractile force upon sarcomere length and intracellular free calcium. Left panel: length-force dependence of a muscle (modified after Gordon et al. 1966). Right panel: pCa-force dependence (according to Kling (1989), modified).

increase of the number of active myosin crossbridges and thus to an increase in contractile force. In the myocardium, however, a second mechanism, namely the sensitization of the myofilaments (Hibberd and Jewell (1982)) as well as of the myofibillar ATPase (Kuhn et al. 1985) for the calcium binding, contributes to the enhancement of contractile force. In the left panel of Fig. 11, this diagram shows the generally known relationship between contractile force and sarcomere length (length-force relationship). In the right panel the calcium-force curve obtained from skinned myocardial fibres is drawn according to Kling (1989). At a pCa of approx. 5 the maximum contractile force taken as 100% is reached. Starting from the control curve (bold line) at a given Ca concentration and following the down-pointing arrow at the same Ca concentration a point with lower force is reached. This point is part of a curve which results if the Ca sensitivity of the myofilaments has decreased. This is the case if the prestretch of the fibres is reduced or under the influence of cAMP, inorganic phosphate, hydrogen or magnesium ions. On the other hand starting from the same Ca concentration the up-pointing arrow marks a curve resulting from increased myofibrillar Ca sensitivity. This is the case after increased prestretch or—at constant prestretch —after the application of some recently developed drugs, termed "Ca sensitizers". Sulmazole (Herzig et al. (1981)) and Adibendan (Müller-Beckmann et al. (1988); Freund et al. (1987)) are two examples (see also Rüegg (1987)).

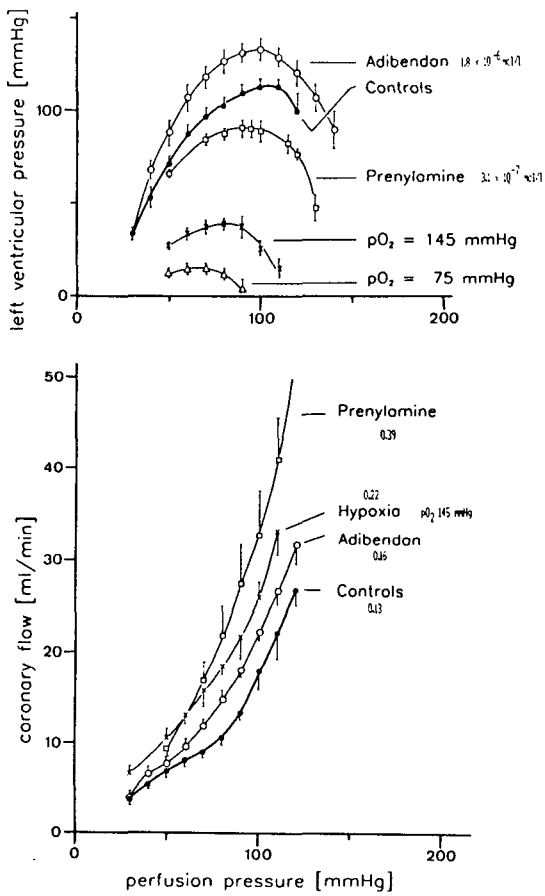

Fig. 12. Left ventricular function curves and coronary pressure flow curves of guinea-pig hearts. Influence of the "Ca sensitizer" Adibendan, the Ca antagonist Prenylamine (group B Ca antagonist after Fleckenstein (1983)) and hypoxia. Hypoxia was achieved by gassing the perfusate with 20% v/v O_2, 5% v/v CO_2, 75% v/v N_2 (PO_2 = 145 mmHg) or 10% v/v O_2 (PO_2 = 75 mmHg).

Results of experiments from our laboratory in which Ca sensitivity was changed by pharmacological means are shown in Fig. 12. At an Adibendan concentration of 1.8×10^{-6} mol/l the maximum left ventricular pressure increased from 112 mmHg (controls) to 132 mmHg at a perfusion pressure of 100 mmHg (= +18%). Taking into consideration the results of Freund et al. (1987), a phosphodiesterase-III-inhibiting effect of Adibendan, which might contribute to the increase of contractile force, has to be postulated. On the other hand contractile force has decreased considerably under the influence of hypoxia and the accompanying intracellular acidosis. According to today's opinion this is the result of a desensitization of the contractile elements for calcium by hydrogen ions.

In the pressure-flow curves in the botton part of the figure the slope is increased by 23% under the

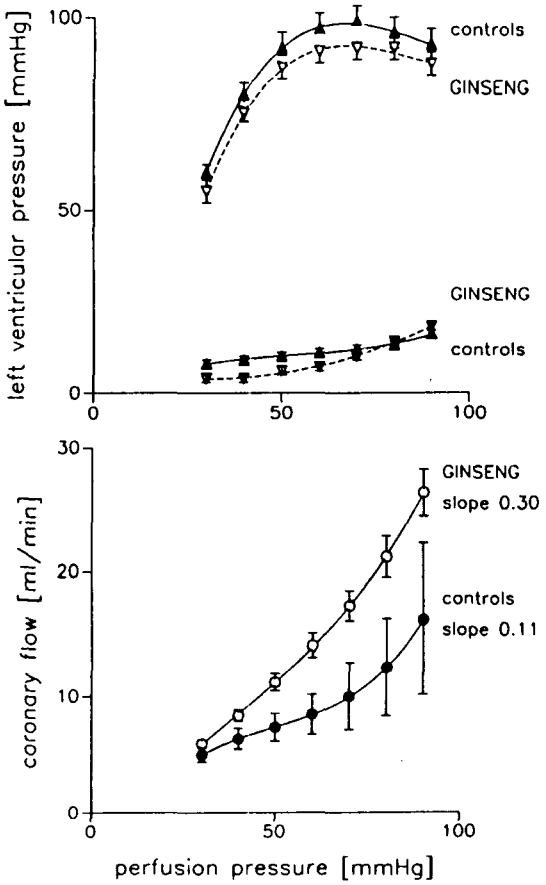

Fig. 13. Effect of ginsenosides (10 mg/l) on left ventricular function curves and coronary pressure-flow curves from guinea-pig hearts (15 min pre-perfusion). mean +/− S.E.M., n=11.

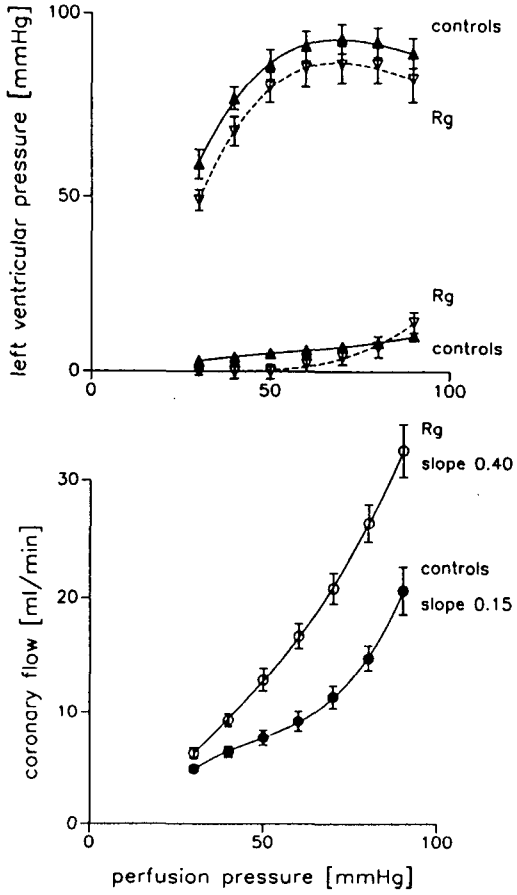

Fig. 14. Effect of Rg saponine from Panax ginseng (2 mg/l) on left ventricular function curves and coronary pressure-flow curves from guinea-pig hearts (10 min pre-perfusion). means +/− S.E.M., n=6.

influence of Adibendan. Since smooth muscle cells do not contain troponin the effect cannot be related to a change of Ca sensitivity. Besides, myogenic autoregulation would have to have increased then. The decrease of myogenic autoregulation seems to be exclusively caused by the inhibition of phosphodiesterase in this case.

With hypoxia the inhibition of myogenic autoregulation by 69% is far more pronounced. However, it is unknown whether this decrease of myogenic autoregulation is due to an inhibition of oxydative metabolism in hypoxia, to liberation of adenosine from ATP splitting or to the accompanying acidosis.

Finally the function curves and the pressure-flow curves resulting from application of the group B Ca antagonist Prenylamine are demonstrated. While contractile force is reduced only by 20%, the slope of the pressure-flow curve has increased by 200%.

As another example of the effectiveness of the method introduced here the function curves and the pressure-flow curves of guinea-pig hearts subjected to ginsenosides of the Panax ginseng root were plotted in Figs. 13 and 14. Even though contractile force was virtually unchanged at a concentration of 10 mg/l of the total ginsenosides, myogenic autor-

egulation is markedly reduced, as can be seen from the increase of slope from 0.11 to 0.30 (Fig. 13). Fig. 14 shows the effects of the ginsenoside Rg in the concentration of 2 mg/l. The saponine Rg is a mixture of Rg_1 and Rg_2. Here, too, no major effect on the contractile force of the heart but a steep increase in the pressure-flow curve (167%) is observed. Thus the ginsenoside Rg is about 5 times as potent as the total ginsenosides. However, based on these preliminary results a statement about the presumable mode of action of ginsenosides can not be given.

DISCUSSION AND SUMMARY

1) At the isolated perfused guinea-pig and rat heart heterometric autoregulation of the myocardium and myogenic autoregulation of the coronary vessels were induced by means of stepwise increases of perfusion pressure.

2) According to this loading test Frank-Starling function curves of the left ventricle and pressure-flow curves of the coronary vessels can be drawn. This graphic evaluation gives more information about the condition of the heart and the coronary vessels than simple evaluation under hemodynamic equilibrium.

3) There are significant differences in both curves between animal species and between different perfusate Mg concentration.

4) Myogenic autoregulation is not affected by the cyclooxygenase inhibitors indometacin and meclofenamate. Thus it appears unlikely that prostanoides are involved in myogenic autoregulation.

5) Ca antagonists (Gallopamil, Prenylamine) depress myogenic autoregulation dose-dependently. Enhanced myogenic autoregulation, induced by low extracellular magnesium, can be reduced effectively by Gallopamil.

6) Ginsenosides from Panax ginseng as well as the ginsenoside "Rg" are effective inhibitors of myogenic autoregulation without major negative inotropic effects.

REFERENCES

Bayliss WM (1902). On the local reactions of the arterial wall to changes of internal pressure. *J Physiol* 28, 220

Cohen AJ & JCS Fray (1982). Calcium ion dependence of myogenic renal plasma flow autoregulation: Evidence from the isolated perfused rat kidney. *J Physiol* 330, 449

Davies PF (1989). How do vascular endothelial cells respond to flow ? *NIPS* 4, 22-25

Döring HJ (1989). Differentiation of various cardiovascular drugs by means of specific myocardial and vascular load tests. Experiments on the isolated perfused heart. *Arzneim Forsch 89/II: in press*

Döring HJ & H Dehnert (1988). The isolated perfused heart according to Langendorff. *Biomesstechnik-Verlag March GmbH March*

Freund P, B Müller-Beckmann, K Strein, L Kling & JC Rüegg (1987). Ca^{2+}-sensitizing effect of BM 14.478 on skinned cardiac muscle fibres of guinea-pig papillary muscle. *Eur J Pharmacol* 136, 243-246

Gordon AM, AF Huxley & FJ Julian (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J Physiol* 184, 170

Govyrin VA (1989). Autoregulation of reactivity and blood vessel to ne. *Proc IUPS* 17, 2317

Grün G & A Fleckenstein (1972). Die elektromechanische Entkoppelung der glatten Gefässmuskulatur als Grundprinzip der Koronardilatation durch 4-(2'-Nitrophenyl)-2, 6-dimethyl-1, 4-dihydropyridin-3, 5-dicarbonsäure-dimethylester (BAY a 1040, Nifedipine). *Arzneimittelforsch* 22, 334-344

Hashimoto KHO (1983). Comparison of spasmolytic effect of Ca antagonists, papaverine and nitroglycerin on the renal autoregulatory vasoconstriction in dogs. in: *A. Fleckenstein, ed.: New calcium antagonists: Fischer*

Herzig JW, K Feile & JC Rüegg (1981). Activating effects of AR-L 115 BS on the Ca^{2+} sensitive force, stiffness and unloaded shortening velocity (V_{max}) in isolated contractile structures from mammalian heart muscle. *Arzneim Forsch/Drug Res* 31, 188-191

Hibberd MG & BR Jewell (1982). Calcium—and length

—dependent force production in rat ventricular muscle. *J Physiol* 329, 527-540

Katusic ZS, JT Shepherd & PM Vanhoutte (1987). Endothelium-dependent contraction to stretch in canine basilar arteries. *Am J Physiol* 252, H671 -H673

Kuhn HJ, K Güth, C Bletz & JC Rüegg (1985). Stretch induced increase in calcium sensitivity of cardiac myofibrillar ATPase. *Pflügers Arch* 405, Suppl 2, R14

Mellander S (1988). Myogenic mechanisms in local vascular control. *Acta Physiol Scand* 133, Suppl 571, 25-41

Müller-Beckmann B, P Freund, P Honerjäger, L Kling & JC Rüegg (1988). In vitro investigations on a new positive inotropic and vasodilating agent (BM 14. 478) that increases myocardial cyclic AMP content and myofibrillar calcium sensitivity. *J Cardiovasc Pharmacol* 11, 8-16

Ono H, H Kokubun & K Hashimoto (1960). Abolition by calcium antagonists of the autoregulation of renal blood flow. Naunyn-Schmiedeberg's Arch Pharmacol 285, 201-207

Sarnoff SJ, JH Mitchell JP Gilmore & JP Remensnyder (1960). Homeometric autoregulation in the heart. *Circ Res* 8, 1077

Vanhoutte PM (1987). Endothelium and control of vascular tissue. *NIPS* 2, 18-22