Autoregulation of Coronary Blood Flow in Isolated Rat Heart after NO-Synthase Blockade

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Endothelial cells have been shown to be involved in local regulation of the tonus of heart vessels, in particular through production of the endothelial relaxation factor nitric oxide [3,7]. The use of a blocker of NO-synthase made it possible to evaluate its role in agonist-induced vasomotions [3] caused by a change in the shear stress [5], as well as in the mechanism of reactive hyperemia [4]. However, the role of nitric oxide in autoregulation of the coronary blood flow has not yet been clarified.

The aim of the present study was to investigate the effect of a blocker of nitric oxide synthase (N^G-monomethyl-L-arginine) on autoregulation, the maximal hyperemic coronary flow, and the coronary dilatory reserve of isolated rat heart.

MATERIALS AND METHODS

Experiments were performed on 67 female rats weighing 180-200 g. Isolated hearts were perfused with Krebs-Henseleit solution (pH 7.3-7.4; t=37°C; carbogen aeration) after Langendorf.

Two series of experiments were performed: the first was the control series (n=22); in the second series (n=21) N^G-monomethyl-L-arginine (N^G-MMLA) was introduced into the perfusate in a volume of 1/40 of the coronary flow during 10 min to a final concentration of 100 μM. The preparation was kindly supplied by Dr. S. Moncada (Wellcome Research Laboratories, UK).

Each series included the following groups: in the first group, the hearts contracted in an isotonic regime under a constant pressure; in the second group a latex balloon of a constant volume was

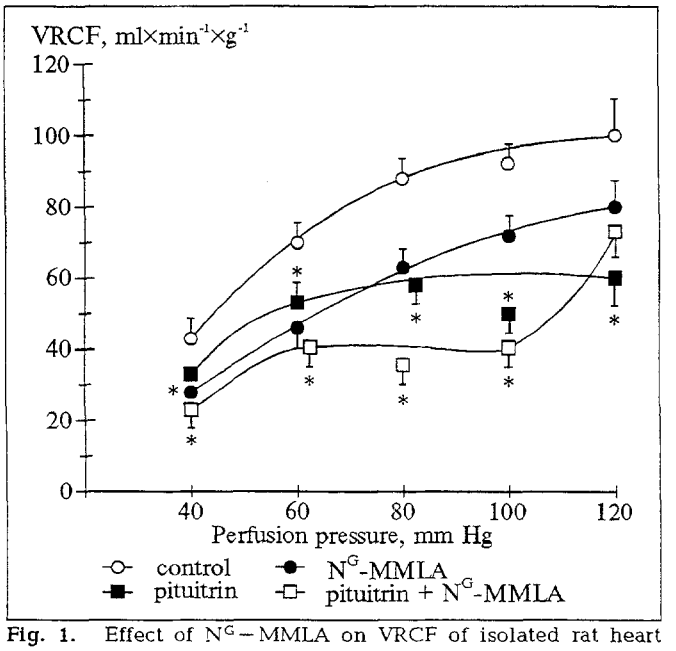

Fig. 1. Effect of N^G-MMLA on VRCF of isolated rat heart at different PP levels. Here and in Fig. 2: *: changes reliable in comparison with the control (p<0.05).

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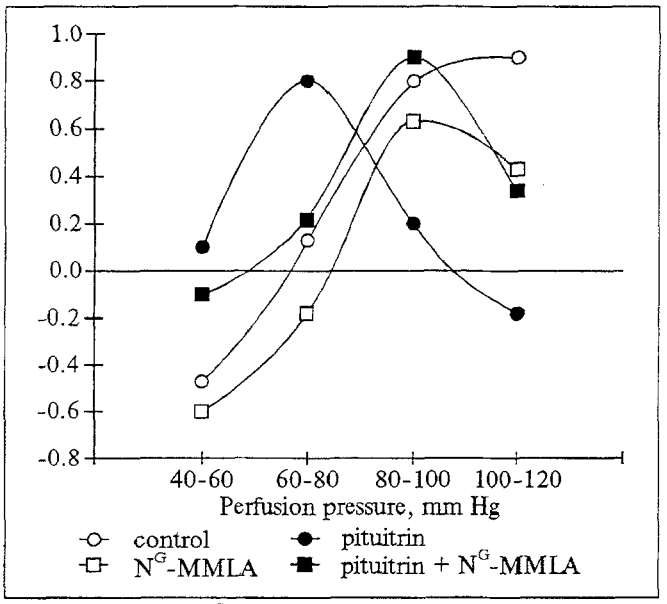

Fig. 2. Effect of N^G-MMLA, pituitrin and their combination on efficacy of autoregulation of coronary flow. Ordinate: efficacy of autoregulation, calculated by the formula [6]: $1-(\Delta Q/Q_1:\Delta P/P_1)$, where ΔQ is the change of the coronary flow with a PP rise by ΔP, and P_1 and Q_1 are the initial values of the PP and coronary flow; + and −: presence or absence of autoregulation; 0: the flow changes to the same degree as the PP.

inserted into the left ventricle to record the intraventricular pressure along with the parameters of the coronary blood flow; in the third group the hearts were perfused under conditions of a constant flow (15 ml/min).

In the 1st and 2nd groups of experiments the perfusion pressure (PP) was raised stepwise from 40 to 120 mm Hg (each step was 20 mm Hg). The volumetric rate of the coronary flow (VRCF) was determined every minute by measuring the volume of perfusate flowing out during 10 sec through the right (free) and the left (drained) ventricle. The relative coronary flow was calculated per gram dry weight of the left ventricle. In all groups the hearts contracted in a constant rhythm of 4 Hz (the stimulation mode was 3-7 V, 3-5 msec).

The ability of the vessels to contract under tension was estimated by the index of autoregulation (IA) [1].

The coronary dilatory reserve (CDR) was determined at perfusion pressures of 40, 80, and 120 mm Hg as the ratio of the flow at the peak of reactive hyperemia after a 60-sec cessation of perfusion to the initial flow.

The degree of blocking of NO-synthase was tested by bolus (100 μl) intracoronary infusion of acetylcholine (3×10^{-4} M) and sodium nitroprusside (10^{-3} M) by the decrease in PP in 12 KCL-arrested (34 mM) isolated hearts, perfused under a constant flow of 4 ml/min.

In the case of high coronary tonus, the perfusion was accomplished by adding 1.1 mIU/ml pituitrin to the perfusate (n=12). The results were processed statistically using the nonparametric Wilcoxon-Mann-Whitney U test.

RESULTS

The intracoronary infusion of N^G-MMLA almost completely blocked the acetylcholine-induced drop of the coronary PP (3.2±1.4% vs. 22.6±5.4% in the control), whereas the reaction to sodium nitroprusside remained unchanged (17.4±2.0% vs.

TABLE 1. Effect of N^G-MMLA, Pituitrin and Their Combination on Index of Autoregulation, VRCF, Measured in the First 10 sec of PP Rise, and Effect of NO-Synthase Blocking on MHCF and CDR in Isotonically Contracting Isolated Heart

Group	Perfusion pressure, mm Hg			
	60	80	100	120
VRCF during first 10 sec after PP rise, ml×min^{-1}×g^{-1}				
Control	83.7±4.2	115.6±11.2	114.5±22.2	113.5±24.3
N^G-MMLA	55.4±7.8	77.8±8.1	83.2±10.7	85.1±8.8
Pituitrin	59.7±10.2	80.6±10.6*	71.2±8.4	76.4±8.4
Pituitrin + N^G-MMLA	36.5±11.2*	51.8±17.3*	45.7±12.9*	56.0±15.3*
Index of autoregulation				
Control	0.27±0.04	0.54±0.10	0.83±0.09	0.76±0.17
N^G-MMLA	0.28±0.05	0.18±0.08*	0.45±0.08*	0.36±0.05*
Pituitrin	0.41±0.03*	0.50±0.05	0.89±0.10	0.49±0.09
Pituitrin + N^G-MMLA	0.16±0.06	0.80±0.18	0.20±0.09*	0
Maximal Hyperemic Coronary Flow (MHCF), ml×min^{-1}×g^{-1}				
Control	–	149.5±12.7	–	171.8±8.5
N^G-MMLA	–	85.5±11.3*	–	92.5±10.8*
Coronary Dilatory Reserve (CDR)				
Control	–	1.59±0.04	–	1.60±0.05
N^G-MMLA	–	1.18±0.04*	–	1.11±0.03*

Note. * – $p<0.05$ in comparison with the control.

24.0± 9.7% in the control). Hence, N^G-MMLA in a dose of 100 μM, quite effectively blocked the release of nitric oxide from endotheliocytes. In hearts isotonically contracting at 40 and 60 mm Hg, N^G-MMLA reduced the VRCF by 36 and 31%, respectively, while at 80-120 mm Hg the VRCF did not differ from the control (Fig. 1). Blocking of NO-synthase evidently increases the coronary tonus by reducing the VRCF during the first 10 sec of the pressure rise to 60 and 80 mm Hg by 32.5% on average in comparison with the control (Table 1), the index of autoregulation being lowered by 54.9% on average. The blocking of NO-synthase in our case shifted the onset of effective autoregulation to the right, i. e., to 80 mm Hg vs. 60 mm Hg in the control (Fig. 2).

The primary pituitrin-induced rise of the coronary tonus was not accompanied by a drop of the IA or by a diminished efficacy of this process (Fig. 1, Table 1). N^G-MMLA in combination with pituitrin reduced the VRCF to the same extent as pituitrin alone, however, the IA dropped 54% on average, and the region of effective autoregulation was shifted to lower PP values, the PP range being markedly narrowed. In other words, the changes were similar to those caused by N^G-MMLA alone. Consequently, the N^G-MMLA-induced changes in autoregulation parameters could not have resulted from the high tonus of the coronary vessels or their reduced dilatability.

In the isometrically contracting isolated heart, blocking of NO-synthase led to a reduction of the VRCF by 28.5% at 40 mm Hg, but at other values of PP neither the VRCF nor the developed intraventricular pressure changed significantly (Fig. 3). The index of autoregulation, however, dropped by 79% on average. Thus, the drop of the VRCF and the IA following the blocking of NO-synthase in the isometrically contracting heart was similar to that observed in the isotonically contracting heart, and, apparently, was not determined by a change in the contractile function of the myocardium.

The blocking of NO-synthase produced the most significant changes in the mechanisms of reactive hyperemia. Under constant perfusion pressures of 80 and 120 mm Hg N^G-MMLA lowered the peak value of the hyperemic coronary flow by 30.3 and 57.2%, respectively (Table 1). This resulted in an average 28.3% reduction of the CDR. For perfusion under a constant flow the duration of reactive hyperemia was found to be reduced to 28.2±2.3 vs. 45.6±7.7 sec in the control, which is in conformity with data reported previously [4].

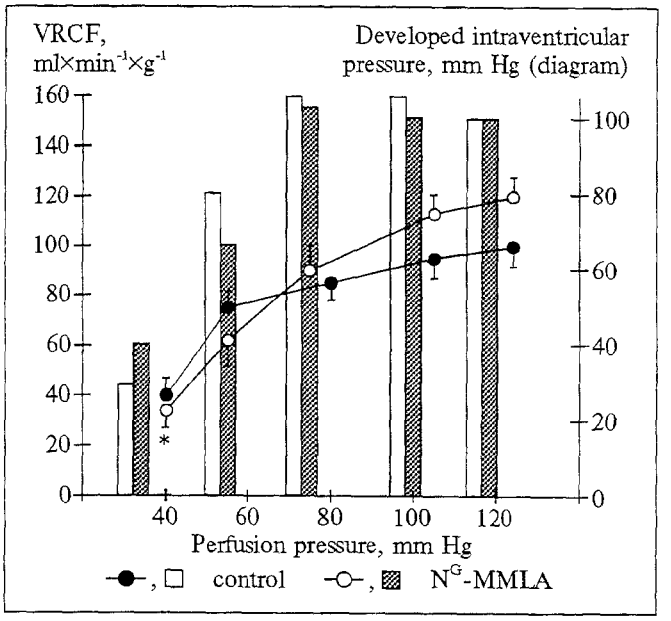

Fig. 3. Effect of N^G-MMLA on VRCF and developed intraventricular pressure of isolated rat heart (isometric regime of contraction).

The findings show that, first, N^G-MMLA causes the most pronounced changes in the coronary flow during the early period of the vascular response to PP changes: the absolute value of VRCF, measured during the first 10 sec of the PP rise, fell, the autoregulation response was weakened, and the magnitude and duration of the reactive hyperemic coronary flow were reduced. Second, since the mechanisms of autoregulation and reactive hyperemia, which are essentially similar, are governed by the function of microvessels [2,8], it may be assumed that the observed changes were related to a blocking of NO-synthase in the endotheliocytes and to the reactivity state of these vessels. Thus, it may be concluded that nitric oxide, released from endotheliocytes of the coronary vessels, plays an essential role in the mechanism of autoregulation of the heart vessels.

REFERENCES

1. E. B. Novikova, *Fiziol. Zh. SSSR*, **58**, № 1, 61-72 (1972).
2. P. Johanson, *Circulat. Res.*, **59**, № 5, 483-495 (1989).
3. M. Kelim and J. Schrader, *Ibid.*, **66**, № 6, 1561-1575 (1990).
4. M. Kostic and J. Schrader, *Ibid.*, **70**, № 1, 208-212 (1992).
5. D. Lamontagne, U. Pohl, and R. Busse, *Ibid.*, pp. 123-130.
6. C. P. Norris, C. E. Barner, S. Smith, *et al.*, *Amer. J. Physiol.*, **237**, H174-H177 (1979).
7. R. M. Palmer, D. S. Ashton, and S. Moncada, *Nature*, **333**, 646-666 (1988).
8. F. Sato, Sh. Isdoyma, and T. Iakishima, *Hypertension*, **16**, 26-34 (1990).