Effect of Endogenous Metabolites on Autoregulation and the Dilatatory Capacity of Coronary Vessels

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Along with myogenic mechanisms of tone regulation of the coronary vessels an important role is played by metabolites produced in the vascular wall and myocardium [3]. Endothelium is known to be a source of contractility modulators of the adjacent vascular smooth muscle cells. Such substances are eicosanoids [4], the endothelium-derived relaxing factor nitric oxide [2,8,9], endothelin [11], and angiotensin II, formed on the surface of endotheliocytes [10]. The autoregulation mechanism of the coronary blood flow is traditionally considered to be of major importance [1,5], whereas the role of metabolites in this process has been far less studied, even though humoral factors fulfill a very important role in the regulation of the coronary blood flow.

In the light of the above, the effect of some metabolites (eicosanoids, angiotensin II, and nitric oxide) on autoregulation, the maximal hyperemic coronary flow (MHCF), and the dilatory reserve of the coronary vessels of the isolated rat heart was studied in the present work. For this purpose blockers of the above-mentioned metabolites were used: indomethacin, captopril, and N^G-monomethyl-L-arginine (N^GMMLA), an NO-synthase blocker, as well as the Ca^{2+} channel blocker verapamil.

MATERIALS AND METHODS

The experiments were carried out on 48 isolated (after Langendorff) hearts of female rats weighing 170-230 g for a stepwise increase of perfusion pressure (PP) in the range of 40-120 mm Hg. The hearts were perfused with carbogen-saturated (95% O_2 + 5% CO_2) Krebs-Henseleit solution (pH 7.3-7.4, t=27° C). The hearts were divided into 5 groups: 1) control (n=8), 2) verapamil-treated hearts (n=7), 3) hearts of rats which received indomethacin (n=7), 4) hearts of rats which received captopril (n=7), 5) N^GMMLA-treated hearts. Verapamil (10^{-7} M) was added to the perfusion fluid; indomethacin in a dose of 10 mg/kg (per os) was administered to the animals over 3 days; a single dose of captopril (10 mg/kg) was introduced per os before the experiment and it was also added to the perfusion fluid in a final concentration of 36 mg/liter. N^GMMLA was administered via a cannula in the aorta (PP=40 mm Hg, 10 min) in a final dose of 100 μM in a volume of 1/40 coronary volume flow rate (CVFR). The preparation was kindly supplied by Dr. S. Moncada (Wellcome Research Laboratories). The hearts contracted at an electrostimulator-controlled frequency of 240 beats per min. CVFR was determined by measuring the 10 sec perfusion volume passing through the right (free) and left (drained) ventricle. The coronary dilatory reserve was determined as the ratio between the maximal hyperemic coronary flow (MHCF) developing after cessation of perfusion at the 60th sec and the initial flow for PP of 40, 80, and 120 mm Hg. The autoregulation index, reflecting the vascular contractile response to an increase of the PP, was determined as described by Novikova [1]. For analysis of the results the intraventricular pressure was also measured by means of a latex balloon placed in the left ventricular cavity and connected to an EMT-35

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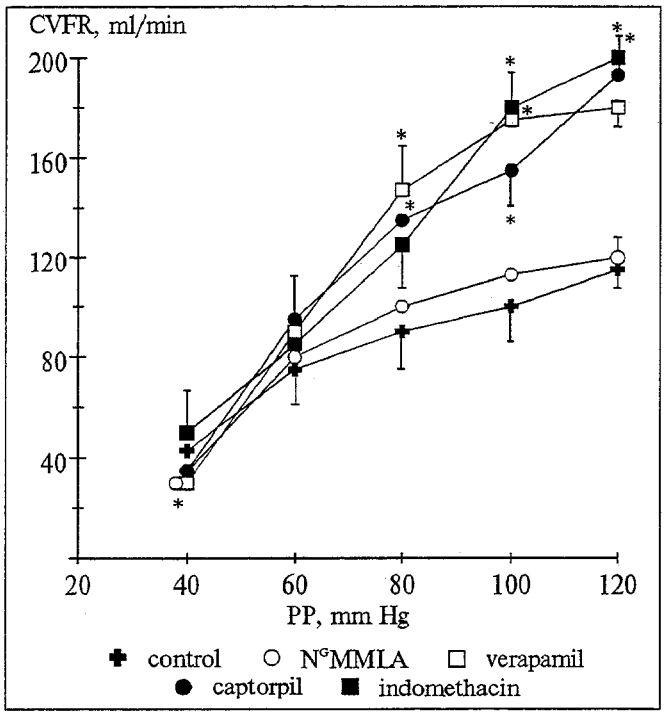

Fig. 1. Effect of verapamil, captopril, indomethacin, and N^GMMLA on the coronary volume flow rate (CVFR) at different levels of perfusion pressure (PP). Asterisk: $p<0.05$ as against the control.

electromanometer (Mingograf 81). The effectiveness of NO-synthase blockade was measured as the decrease of the coronary PP altered after bolus acetylcholine injection (100 μl, 3×10^{-4} M, intracoronary). These experiments were carried out on 12 KCl-arrested (34 mM) isolated hearts perfused at a constant flow rate (4 ml/min). The results were statistically processed using the Wilcoxson-Mann-Whitney nonparametric *U* test.

RESULTS

The level of CVFR response to captopril, indomethacin, and verapamil administration did not differ from the control at the moment of PP increase, although during autoregulatory adjustments the coronary blood flow at a PP of 80-120 mm Hg was maintained at a reliably higher level (Fig. 1). After captopril, indomethacin, and verapamil administration the mean autoregulation index dropped 64, 77, and 90%, respectively. The coronary dilatatory reserve at a PP of 80 and 120 mm Hg was reliably (28-39%) lower than the control (Table 1). MHCF did not alter after administration of the above-mentioned blockers.

After intracoronary injection of N^GMMLA in KCl-arrested hearts the acetylcholine-induced decrease of the coronary PP was 3.3±1.4% vs 22.6±5.4% in the control. Therefore, N^GMMLA in a dose of 100 μM was effective in blocking liberation of nitric oxide (a known dilator of the coronary vessels) from the endothelial cells. NO-synthase blocking was attended by a 25% decrease of CVFR at a PP of 40 mm Hg, but at the other PP levels CVFR was unchanged (Fig. 1).

The autoregulation index fell by 79% on average and the coronary dilatory reserve by 29% due to an MHCF decrease (Table 1). Effective autoregulation of the coronary blood flow was observed after N^GMMLA injection as well as in the case of captopril, verapamil, and indomethacin administration beginning with a PP of 80 mm Hg, whereas in the control this was noted beginning with a PP of 60 mm Hg (Fig. 2). A PP increase from 40 to 80 mm Hg in all groups of isolated hearts was accompanied by an increase of the intraventricular pressure according to the Anrep and Gregg effect and no augmentation of the intraventricular pressure was observed for a further PP increase. Captopril and N^GMMLA did not alter the intraventricular pressure, whereas verapamil and indomethacin reduced its value by 60 and 30%, respectively (Table 1). Hence, the CVFR decrease observed after N^GMMLA injection and its increase after the administration of other blockers were obviously not determined by functional changes in the myocardium.

Thus, first, the most pronounced changes both of coronary blood flow autoregulation and contractility

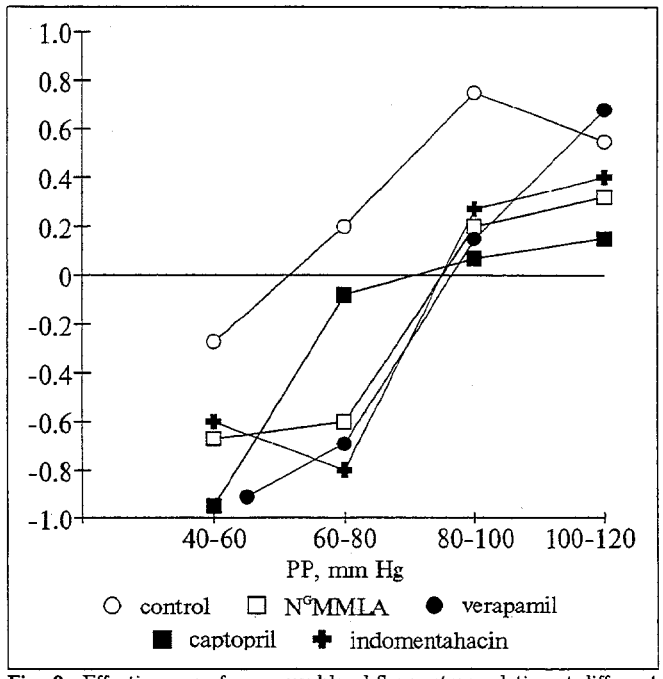

Fig. 2. Effectiveness of coronary blood flow autoregulation at different levels of perfusion pressure (PP). Ordinate: effectiveness of autoregulation calculated according to formula [7]: $1-(\Delta Q/Q_1:\Delta P/P_1)$, where ΔQ is the change of the coronary blood flow when PP is increased by ΔP, P_1 and Q_1 are initial PP and coronary blood flow values. "Plus" indicates presence of autoregulation, "minus" indicates its absence, and zero shows that the degree of flow changes is similar to that of PP.

TABLE 1. Effect of Verapamil, Indomethacin, Captopril, and N^{G}MMLA on Autoregulation Index, Coronary Dilatory Reserve, Maximal Hyperemic Coronary Flow, and Myocardial Contractility

Group	Perfusion pressure, mm Hg			
	60	80	100	120
	Autoregulation index			
Control	0.28±0.07	0.41±0.07	0.68±0.09	0.60±0.07
Verapamil	0	0	0.20±0.10*	0.44±0.17
Captopril	0.10±0.02*	0.24±0.15*	0.24±0.09*	0.24±0.09*
Indomethacin	0.14±0.10	0	0.29±0.06*	0
N^{G}MMLA	0	0	0.27±0.07*	0.31±0.13*
	Coronary dilatory capacity			
Control	–	1.67±0.04	–	1.63±0.06
Verapamil	–	1.02±0.01*	–	1.17±0.06*
Captopril	–	1.13±0.05*	–	1.16±0.06*
Indomethacin	–	1.18±0.08*	–	1.14±0.07*
N^{G}MMLA	–	1.18±0.07*	–	1.12±0.06*
	Maximal hyperemic coronary blood flow, ml/min			
Control	–	162.3±26.2	–	200.0±28.6
Verapamil	–	154.1±10.6	–	214.5±24.9
Captopril	–	147.1±20.9	–	228.6±19.8
Indomethacin	–	157.4±17.8	–	243.6±46.5
N^{G}MMLA	–	121.2±6.7*	–	149.1±8.8*
	Developed intraventricular pressure, mm Hg			
Control	85.0±11.8	108.8±13.3	109.2±9.3	103.0±11.2
Verapamil	23.2±4.2*	38.8±6.5*	40.8±7.8*	38.0±0.7*
Captopril	63.5±7.4	82.0±8.1	86.0±6.2	82.0±10.8
Indomethacin	55.3±5.8*	80.7±7.0	81.3±6.6*	93.3±5.7
N^{G}MMLA	72.0±9.1	101.0±10.2	100.0±9.9	103.6±6.3

Note. Asterisk: $p<0.05$ vs. control.

of the myocardium are observed under the influence of verapamil. This once again proves that coronary vessel autoregulation as well as myocardial contractility are Ca^{2+}-dependent phenomena [12]. Second, eicosanoids and angiotensin II play an important role in the maintenance of the tone of the coronary vessels, and inhibition of their synthesis therefore results in a PP increase, possibly determined by the prevalent effect of vasodilatory substances. Eicosanoids also participate in the autoregulation of myocardial contractility, this obviously potentiating transmembrane Ca^{2+}-transport and activating intracellular Ca^{2+}-binding structures [6]. Third, the CVFR decrease during N^{G}MMLA injection provides evidence of NO release from the basal endothelium of the coronary vessels, while its absence leads to their increased tone, weakened autoregulation, a drop of MHCF, and, thereby, a decrease of the dilatory reserve.

Hence, coronary vascular smooth muscle tone is affected by the substances continuously produced not only in the myocardium but in the vascular wall itself. On the one hand, eicosanoids, angiotensin II, etc., and, on the other hand, nitric oxide and other endothelium-derived relaxing factors make up a system modulating the coronary blood flow autoregulation independently of the functional shifts of myocardial activity.

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