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THE CORONARY VASCULATURE AND THE MYOCARDIUM

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INTRODUCTION

It has been reported that the activity of bradykinin on vascular endothelial receptors results in vasodilatation (1-3) due to the release of both nitric oxide (NO) and the so called endothelial-derived hyperpolarizing factor (EDHF) (4-7). It has also been suggested that in the rat bradykinin-induced vasodilatation is not mediated by NO but by EDHF only (2). Although a number of compounds can cause hyperpolarization of the smooth muscle fibres of the vascular wall, in the coronary bed of the rat and some other species EDHF has been identified with a non-prostanoid autacoid produced by the activity of cytochrome P-450 on arachidonic acid (3). This autacoid is likely to be one of the epoxiecocystrienoic acids (EETs) (8,9).

It is well known that, depending on the experimental conditions and animal species, bradykinin can induce either an increase or a reduction in myocardial contractility. In particular in the isolated and perfused rat heart preparation it has been observed that, if the experiments are performed at a constant perfusion pressure which allows the flow to increase following vasodilatation, bradykinin causes an increase in contractility (10). On the contrary, if the experiments are performed at constant flow the vasodilatation is revealed by a reduction in perfusion pressure and is accompanied by a reduction in myocardial contractility. The difference in the results depending on the experimental conditions

(i.e. constant perfusion pressure vs constant coronary flow) is attributed to Gregg's phenomen, which relates an increase in coronary perfusion with an improvement of inotropic state.

The present study was planned to investigate whether the activation of Cytochrome P-450 by bradykinin can cause both vasodilatation and reduction in contractility, thus showing that the inotropic myocardium can be affected by the vascular endothelium.

MATERIALS AND METHODS

Experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals, DL 116, January 27, 1992, published in the Gazzetta Ufficiale della Repubblica Italiana (issue 40, Feb. 18, 1992). Five Wistar rats weighing 450-550 g were beheaded after general anaesthesia obtained by intraperitoneal injection of 0.5 g of uretane dissolved in saline solution. Then the hearts were removed and suspended as a Langendorf isolated heart preparation. The coronary vascular bed was thus retrogradely perfused. Along the perfusion line a disposable electromanometer (Monitoring kit mk5-02 DTNVF, Abbott, Milan, Italy) was connected to record perfusion pressure. At the distal end of the same line, the probe of an electromagnetic flowmeter (BL 113, Biotronic Laboratory, Inc., Silver Spring, MD, USA) was placed between the perfusing cannula and the aorta to record the total coronary flow (CF). A catheter connected with another electromanometer was inserted in a small latex balloon and placed in the left ventricle. With this procedure also left ventricular pressure (LVP) could be recorded. The hearts were perfused using a constant flow pump with oxygenated Tyrode solution. Throughout the experiments the ventricles were paced at a constant heart rate of about 300 b.p.m. using a San'ey stimulator.

Experimental protocol - After 20 min were elapsed and the preparation was stable as revealed by the LVP curve, bradykinin was infused for 3 min at the concentration of 100 nM l-1 in the Tyrode solution as a control. Fifteen-twenty min later, i.e. when the effect of bradykinin was over, 1-amino-benzotriazole (ABT), a broad spectrum in inhibitor of cytochrome

P-450, was infused for 10 min at the concentration of 1mM l-1. After ABT, bradykinin was given again at the same dose as in the control. Coronary flow, perfusion pressure and LVP were recorded with a TEAC-71 magnetic tape recorder (TEAC Corporation, Tokyo, Japan).

All the substances were obtained from Sigma Chemical Co. (St. Louis, MO).

Analysis of data - The following data were considered: Heart rate (HR), developed left ventricular pressure (developed LVP), peak rate of pressure development (dP/dTmax) and coronary resistance (CR). Since CF was kept constant by the pump, the changes in coronary perfusion pressure were taken as coronary resistance (CR).

All the above parameters were considered in the control as well as during and after the experimental manoeuvres. Data (means±SD) were compared using Student's "t" test and ANOVA, as appropriated.

RESULTS

The mean values from the results of the various experiments are reported in table 1 and in figs. 1 and 2.

Tab. 1 - Mean values of the experimental results.

	LVP (mmHg)		dP/dt max(mmHg·sec-1)		CR (mmHg·ml-1·min-1)	
	Mean	SD	mean	SD	mean	SD
Control	72.0	13.03840	908.600	174.0669	3.506	0.2693
Bradykinin	55.2	20.47437	696.600	282.4151	2.925	0.2806
Before ABT	63.4	7.66812	854.667	70.3160	3.143	0.2846
ABT	66.4	10.11435	931.333	131.7662	3.868	1.4002
Bradykinin with ABT	66.2	10.03494	936.000	125.2038	3.714	1.3636

LVP = Left ventricular pressure; dP/dt = Peak rate of pressure development; CR = Coronary resistance; SD=Standard deviation; ABT = 1-amino-benzotriazole.

Heart rate - Since the ventricles were paced, heart rate remained unchanged throughout the experiments, its average value being 290±6 b.p.m.

Developed left ventricular pressure - The administration of bradykinin before ABT caused a significant ($p<0.01$) 14% reduction of developed LVP from 72±13 to 62±20 mmHg.

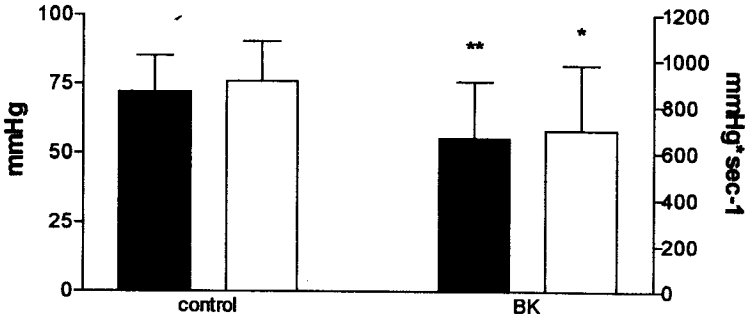

Fig. 1 - Left ventricular pressure (LVP) and peak rate of pressure development (dP/dt max) in the control and after bradikinin (BK) before 1-amino-benzotriazole. With respect to control *p<0.05, **p<0.01.

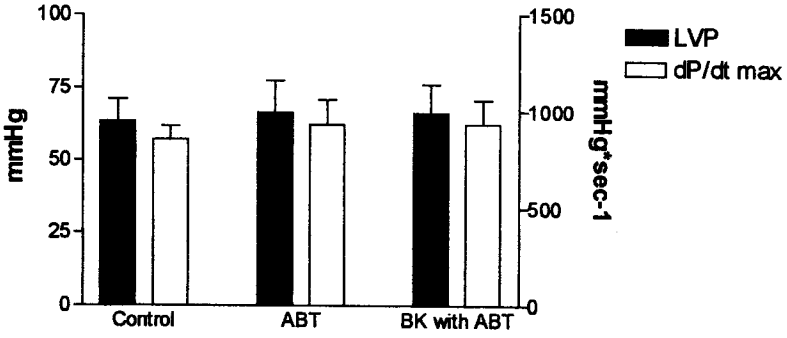

Fig. 2 - Left ventricular pressure (LVP) and peak rate of pressure development (dP/dt max) in the control after the first bradykinin administration, after 1-amino-benzotriazole (ABT) and after the second administration of bradykinin (BK). No significant change is observed.

After 20 min of washout by the oxygenated Tyrode solution, the developed pressure was back to the control (74±16 mmHg). Then ABT was infused as reported in the experimental protocol.

It was observed that Cyt P-450 inhibition did not cause any significant change in developed LVP. No significant change was then observed when bradykinin was given again after ABT infusion.

Peak rate of pressure development (dP/dtmax) - The maximal dP/dt of the increase of LVP in during systole decreased significantly ($p<0.05$) by 23% when bradykinin was given in the absence of ABT. dP/dt was back to the control after the washout following bradykinin and did not change after ABT and the second administration of bradykinin.

Coronary resistance - Since the hearts were perfused at constant flow coronary resistance was obtained directly from the perfusion pressure. Before ABT, the administration of bradykinin caused a significant ($p<0.04$) reduction of CR. No change in coronary resistance was observed when bradykinin was given after ABT. It is also remarkable that no change of coronary resistance was produced by ABT, which was given when the effect of the first administration of bradykinin was completely over.

DISCUSSION

A number of investigators analyzed the possible presence of a paracrine pathway for the regulation of basal cardiac contractile function by coronary vascular and endocardial endothelial cells, but the influence of the compound released by the endothelial cells remains uncertain (11-13). It has also been reported that the simultaneous inhibition of nitric oxide, PGI2 and endothelin-1 in isolated papillary muscle resulted in little effect on baseline contractile performance (11-13). Since in our experiments developed LVP was not significantly affected by the administration of ABT, it may be argued that the basal release of Cyt P-450 results in little, if any, effect on baseline myocardial contractility.

The involvement of the endothelium in mediating the negative inotropic effect of bradykinin has been previously reported by Fort and Lewis (13). It was suggested that a factor different from nitric oxide was responsible for such an effect. Since this factor was presumed to open the ATP dependent potassium channels, hyperpolarization was proposed as the mechanism leading to a reduction in contractility. Since a Cytochrome P-

450-dependent metabolite of arachidonic acid was indentified as one the factors most likely capable to cause hyperpolarization, in the present investigation we wanted to see whether Cyt P-450 inhibition could attenuate the changes in contractility which Fort and Lewis attributed to the hyperpolarization. The results of our experiments clearly showed that, after Cyt P-450 inhibition, bradykinin was no longer able to reduce myocardial contractility which could be attributed to an arachidonic acid-derived hyperpolarizing factor. Furthermore, the fact that the negative inotropic effect of BK was completely abolished and not simply reduced after ABT allows to exclude any role played by NO and other autacoids in our experimental conditions.

Cyt P-450 is an ubiquitous enzyme present not only in the vascular endothelial cells but also in the myocardial fibres. From this characteristic the question arises whether the enzyme involved in the depression of myocardial contractility was of endothelial or myocardial origin. The behaviour of CR throws some light on the problem. The observation that the bradykinin-induced negative inotropic effect was accompanied by coronary vasodilatation and that both effects disappeared after ABT is strongly in favour of the endothelial origin of the compound. In fact the activation of myocardial Cyt P-450 alone would have induced a reduction of contractility without concomitant coronary vasodilatation. It is remarkable that the concentration of Cyt P-450 was found to be much higher in the endothelial cells than in the myocardial fibres (14). In addition it is note-worthy that the inhibition of Cytochrome P-450 blocks L-type Ca2+ sarcolemmal channels and reduces cAMP in the fibres, thus resulting in a decrease in contractility rather than in a protection against the negative inotropic effect exerted by bradykinin. If the inhibition of myocardial Cyt P-450 is expected to be responsible for the depression of myocardial contractility, it may be argued that the depression observed in our experiments after bradykinin was the consequence of the diffusion of an EET released from other cells, i.e. from the endothelial cells of the coronary vessel wall. EETs are non-prostanoid compounds derived in response to the activity of Cyt P-450 on arachidonic acid. Four EET

regioisomers, i.e. 5,6 EET, 8,9 EET, 11,12 EET and 14,15 EET have been identified (9). Usually they are inactive because esterified with phospholipids. Cyt P-450 activation by bradykinin is reported to act not only in inducing their production from arachidonic acid, but also in inducing their release through phospholipid hydrolysis (15). A number of investigations report the effect of Cyt P-450-EETs pathway as depolarizing the smooth muscle fibres thus inducing vasodilatation (8,16). In conclusion our results suggest that the effect of BK in reducing the inotropic state of the heart is mediated by the activation of the endothelial rather than myocardial Cyt P-450, which can induce the production of EETs acting on arachidonic acid. Cytochrome P-450 can also prevent EETs from being inactivated by esterification with phospholipids. In fact, when this activation is prevented by the administration of 1-aminobenzotriazole, bradykinin is completely uneffective in altering myocardial contractility.

The effect of bradykinin (BK) on myocardial inotropic state was tested on 5 isolated rat heart preparations, in which a proper ballon was placed to record left ventricular pressure, whose developed systolic value was taken as an index of contractility. A reduction of developed left ventricular pressure was observed when BK was added to the perfusion oxygenated Tyrode solution. However, when BK was given after 1-aminobenzotriazole, an inhibitor of Cytochrome P-450 (Cyt P-450), developed pressure did not change. Since Cyt P-450 is known to act on arachidonic acid inducing the production of epoxiecocistrienoic acids (EETs) which hyperpolarizes myocardial fibres, it was argued that the reduction in contractility by bradykinin was the result of the hyperpolarizing effect of EETs. The fact that the concentration of Cyt P-450 is higher in the vascular endothelial cells than in the sarcolemma of the myocytes and the observation that the coronary resistance decreases together with the contractility suggest that the endothelium plays a pivotal role in mediating the negative inotropic effect of BK.

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