J. Biol. Res. 2003 n. 1 - Vol. LXXIX - Rubbettino - Soveria Mannelli

The Endothelial Cytochrome P-450 Pathway and Cardiac Contractility

P. Pagliaro

Dipartimento di Scienze Cliniche e Biologiche, Università degli Studi di Torino

There is evidence that the activation of a Cytochrome P-450 (CYP) epoxygenase in endothelial cells is an essential step in nitric oxide (NO)- and prostacyclin-independent vasodilatation of several vascular beds, particularly in the heart and kidney. Specific CYPs localized in the vascular smooth muscle and endothelium contribute to the regulation of coronary vasomotor tone and cardiac function. CYP enzymes can metabolize arachidonic acid to substances which affect arterial tone. Moreover, CYP-epoxygenase and CYP-hydroxylase products are intracellular signal transduction molecules involved in several signalling cascades affecting numerous cellular processes, including vascular cell proliferation and angiogenesis. One of the epoxyeicosatrienoic acids (EETs), which are CYP-derived metabolites of arachidonic acid, generated within endothelial cells when stimulated by bradykinin (BK), as well as by acetylcholine (ACh), induces in isolated rat hearts a negative inotropic effect as revealed by a 20-25% reduction in developed left ventricular pressure. The cardio-depressive effects of BK can be reproduced by this particular EET, namely the 14,15-EET. The BK-effect did not occur during CYP inhibition by 1-aminobenzotriazole (ABT) or proadifen (two CYP inhibitors) as well as after the endothelium has made dysfunctional. The BK-induced negative inotropic effect is still present after NO+cyclooxygenase inhibition. Soon after recovery from cardio-depression induced by 14,15-EET, a BK infusion reduces myocardial contractility in a greater extent than before 14,15-EET. ACh-induced cardiodepression is only partially inhibited by ABT, whereas it is unaffected by NO+cyclooxygenase inhibition. In conclusion, the only mechanisms by which BK induces cardio-depression is the activation of endothelial CYP-pathway with the release of 14,15-EET. It is also likely that 14,15-EET incorporated in plasmalemmal membrane may be released by the hydrolyzing activity of BK. This hypothesis explains why the BK-induced cardio-depression is enhanced by prior administration of this EET. ACh-induced cardiodepression depends in part by endothelial CYP-pathway and in part by the direct action on myocardial muscarinic receptors.

CYP-enzyme system has been found in the cardiovascular system of several mammalian species including rat and man [1]. Bradykinin is a well-known endothelium-dependent vasodilator that affects coronary resistance and myocardial contractility. Its production can increase during myocardial ischemia and angiotensin-converting enzyme inhibition, which protects BK from inactivation. While vasodilatation depends on the release of endothelial factors such as NO, prostacyclin, and endothelial derived hyperpolarizing factor (EDHF), the mechanisms leading to changes in myocardial contractility was not fully investigated. Compounds different from NO are involved in the endothelium-mediated cardiodepression induced by BK. A CYP monoxygenase metabolite of arachidonic acid, namely, one of EETs, has been reported to be responsible for BK-induced vasodilatation in the rat coronary circulation and has been proposed as the most likely candidate for the role of EDHF in the coronary bed of many species. In porcine coronary arteries, CYP 2C fulfils the property of EDHF synthase. Indeed, BK can activate both CYP and a membrane phospholipase, which causes the release of arachidonic acid from phospholipids, thus providing the substrate to CYP for the formation of various EETs. These are incorporated into the membrane phospholipids of endothelial and smooth muscle cells, from which they are released by the hydrolizing activity of BK. Thus BK could be responsible for both the production and release of various CYP products from the endothelium. Yet while only one EET is likely to be EDHF, data on the role of the other CYP products remain scant, many of them concerning solely the vasomotor effects [2].

Because the relative contribution of EDHF/EET to vasodilatation is greater in microvessels, which lie in juxtaposition to cardiomyocytes, than in conduit arteries and because EETs are diffusible compounds that act on a number of membrane channels [e.g. Na^+, Ca^{2+}-dependent potassium, and L-type calcium channels], it can be inferred that one or more EET, released by the endothelium, influence heart contractility. We investigated whether the CYP pathway is involved in the negative inotropic response induced by BK and whether EETs mediate this response. In isolated rat hearts perfused at constant flow, we assessed the effects of BK infusion on left ventricular pressure and coronary perfusion pressure before and after the administration of ABT or proadifen, two structurally different inhibitors of CYP activity, with and without inhibition of NO synthase and cyclooxygenase. We also investigated whether perfusion with solutions containing EETs induces cardiodepression and/or vasodilatation. Finally, we examined whether the responses of the

myocardium and vasculature to BK were affected by the preliminary administration of EETs.

In this study [3] the hearts were allowed to stabilize for 20–30 min before baseline values were recorded and the following six groups were used: Group 1: BK before and during ABT. Group 2: BK before and during proadifen. Group 3: BK before and after N^G-nitro-L-arginine methyl ester + indomethacin and after ABT. Group 4: BK before and after Triton X-100. Group 5: BK before and after EETs. Group 6: role of BK tachyphylaxis.

In our investigation [3] we have shown that the administration of BK causes a reduction in myocardial contractility. This cardiodepression was fully dependent on the release of EETs from arachidonic acid of the endothelial cells and consequent diffusion to the myocardium in response to the BK-induced activity of CYP.

It is well known that the heart has a dense distribution of muscarinic receptors and that their activation by ACh can profoundly decrease its inotropic state. ACh of vagal origin also acts on endothelium [4]. Whether or not the endothelial release of nitric oxide (NO) contributes to cardiodepression by ACh is still a matter of debate. Since the endothelial effect of ACh is also attributed to the release of an EDHF, in response to *endothelial* muscarinic M_1 or M_3 receptor activation [5], in a second study (unpublished observations) we aimed at investigating whether or not part of the negative inotropic effect of ACh can be attributed to the formation of EETs/EDHF induced by the activation of endothelial CYP.

In this study the hearts were allowed to stabilize for 20–30 min before baseline values were recorded and the following three groups were used: Group 1: ACh before and during ABT. Group 2: ACh before and after N^G-nitro-L-arginine methyl ester + indomethacin. Group 3: ACh before and after Triton X-100.

This last study indicates that CYP system, although not fully responsible for, is involved in the negative inotropic effect induced by ACh. In fact, in baseline conditions ACh exerted a negative inotropic activity, which was only partially blunted by CYP inhibitor ABT. Endothelial denudation obtained with Triton X-100 also limited, but did not suppress, the negative inotropic effect of ACh. It follows that the CYP involved in the cardiodepression by ACh is of endothelial origin.

In conclusion, our studies provide a novel explanation for the negative inotropic effect of BK and ACh, which persists after NO inhibition, suggesting that, in the rat, the CYP pathway is the major physiological mechanism of BK-induced cardiodepressive response and that the relevant cytochrome is of vascular endothelial origin. The main product of CYP involved in the response may be 14,15-EET, which, on the other hand, is not likely to be responsible for vasodilatation. Finally, we cannot exclude that other CYP products, different from 14,15-EET, could be involved in the cardiodepressive response induced by BK. Yet the negative inotropic effect of ACh is in part mediated by an endothelial CYP as this effect is reduced either by endothelial denudation or by CYP inhibition. Nitric oxide and prostaglandins do not play any role, suggesting that the residual ACh-induced cardiodepression depends on the well known direct effect on myocardial muscarinic M_2 receptors.

References

[1] Wu S., Moomaw C.R., Tomer K.B., Falck J.R. & Zeldin D.C. 1996. J Biol Chem, 271: 3460-3468.
[2] Campbell W.B., Harder D.R., 2001. Am. J. Physiol., 280: H2413-H2416.
[3] Rastaldo R., Paolocci N., Chiribiri A., Penna C., Gattullo D., Pagliaro P., 2001. Am. J. Physiol., 280: H2823-H2832.
[4] Broten T.P., Miyashiro J.K., Moncada S., Feigl E.O., 1992. Am. J. Physiol., 262: H1579-H1584.
[5] Feletou M., Vanhoutte P.M., 1999. J Mol Cell Cardiol, 31: 15-22.