Cognac Polyphenolic Compounds Increase Bradykinin-Induced Nitric Oxide Production in Endothelial Cells

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Summary

We recently reported that in vitro Cognac polyphenolic compounds (CPC) induce NO-dependent vasorelaxant effects and stimulate cardiac function. In the present study, we aim to investigate the effect of CPC on both nitric oxide (NO) and superoxide anions (O_2^-) production in cultured human endothelial cells. In addition, its effect on the bradykinin (BK)-induced NO production was also tested. The role and sources of O_2^- in the concomitant effect of BK plus CPC were pharmacologically determined. NO and O_2^- signals were measured using electron paramagnetic resonance technique using specific spin trappings. Both, CPC and BK induced an increase in NO production in human endothelial cells. The combination of both further enhanced NO release. The capacity of CPC plus BK to increase NO signal was blunted by the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester, and was enhanced in the presence either of superoxide dismutase or catalase. Moreover, CPC plus BK response was greater after inhibition of either NADPH oxidase by apocynin or xanthine oxidase by allopurinol but it was not affected by rotenone. CPC did not affect O_2^- level either alone or after its increase upon lipopolysaccharide treatment. Finally, the capacity of BK alone to increase NO was enhanced either by apocynin or allopurinol. Altogether, these data demonstrate that CPC is able to directly increase NO production without affecting O_2^- and enhances the BK-induced NO production in human endothelial cells. The data highlight the ability of BK to stimulate not only NADPH oxidase- but also xanthine oxidase-inhibitor sensitive mechanisms that reduce its efficiency in increasing NO either alone or in the presence of CPC. These results bring pharmacological evidence for vascular protection by CPC via its potentiating effect of BK response in terms of endothelial NO release.

Introduction

Endothelium-derived nitric oxide is a pivotal mediator involved in a variety of biological process, including regulation of smooth muscle tone and endothelium-dependent relaxation, platelet activation and aggregation, neurotransmission and vascular cells signaling (Randriamboavonjy and Fleming 2005, Busse and Fleming 2006, Moncada and Bolanos 2006, Moncada and Higgs 2006, Villalobo 2006). The production of NO from the endothelium can be increased by both pharmacological and physiological agonists (e.g. bradykinin) and this effect is dependent on the interaction of calcium/calmodulin with endothelial NO synthase (eNOS) (Boulanger *et al.* 1990, Schini-Kerth 1999, Bae *et al.* 2003, Schneider *et al.* 2003) – the PKA-dependent phosphorylation of eNOS (Bae *et al.* 2003). Interestingly, the mechanisms by which polyphenols can produce NO in endothelial cells, involve not only an increase in intracellular calcium, an activation of tyrosine kinases (Stoclet *et al.* 1999, Martin *et al.* 2002),

and an up-regulation of eNOS mRNA (Nie *et al.* 2006), but also additional roles of reactive oxygen species (ROS) (Andriantsitohaina 1999, Jaimes *et al.* 2001, Ndiaye *et al.* 2005). However, the interpretation of the studies investigating vascular NO production stimulated by physiological or pharmacological agonists or plant polyphenols need to be considered. With regard to plant polyphenols, Cognac belongs to alcoholic drinks rich in polyphenols and moderate consumption may have potential beneficial effects on health. We recently reported that oral treatment with CPC appears to have no *in vivo* impact on blood pressure, heart rate of the rats or on cardiac contractility *ex vivo*, but it reduces heart work and decreases the infarct size after ischemia-reperfusion (Ralay Ranaivo *et al.* 2004). However, the effects of Cognac polyphenolic compounds (CPC) on production of NO in cultured human aortic endothelial cells, as well as their effect on the capacity of bradykinin, a physiologically active peptide and potent endothelium-dependent vasodilator, to stimulate the release of NO have not been investigated yet. Accordingly, we sought to evaluate the interactions and relationships between bradykinin, and CPC on the production of NO in human endothelial cells. Finally, based on our previous studies on red wine polyphenolic compounds (RWPC) with different polyphenol composition (Andriambeloson *et al.* 1998, Martin *et al.* 2002), it was intended to test the exclusivity and specificity of CPC on human endothelial cells.

Methods

Cell culture

EAhy 926 cells, a cell line derived from human umbilical vein endothelial cells, were cultured in growth medium (DMEM, Ham's F-12, 1:1) supplemented with 1 % l-glutamine, 1 % HAT, 1 % NEAA, 1 % Na-pyruvate, 1 % streptomycin/penicillin (Cambrex, Verviers, Belgium), and 10 % FBS (Invitrogen, Cergy Pontoise, France). All experiments were performed in confluent EAhy cells from passages 8 to 12 serum-deprived for at least 24 h.

For the measurement of NO level, the culture medium was removed and replaced by fresh Hank's Balanced Salt Solution (HBSS), pH 7.4, containing 1 M N-2-hydroxythylpiperazine-N9-2-ethane-sulfonic acid (HEPES), 1 M L-arginine, 1 M $CaCl_2$ and 0.1 % BSA. After 60 min, cells were treated with bradykinin (BK, 40 µM, Sigma-Aldrich, Saint Quentin, Fallavier, France), alone or 15 min prior treatment of CPC (0.03 mg/ml in 0.5

% glucose) for 30 min. CPC was used at a concentration at which it produced maximal relaxation of aortic rings with functional endothelium (Ralay Ranaivo *et al.* 2004).

Parallel sets of experiments were performed in the presence of of either superoxide dismutase, (SOD, 100 UI/ml), catalase (100 UI/ml), the xanthine oxidase inhibitor, allopurinol (50 µM, Sigma-Aldrich), the potent NADPH oxidase inhibitor, apocynin (100 µM, Sigma-Aldrich), the mitochondrial complex I inhibitor, rotenone (5 µM, Sigma-Aldrich) or the NOS inhibitor, nitro-L-arginine (L-NAME, 100 µM, Sigma-Aldrich).

For the measurement of superoxide anions (O_2^-) level, cells were treated with lipopolysaccharide (LPS, 10 µg/ml, Sigma-Aldrich) for 3 h and then with or without CPC (0.03 mg/ml in 0.5 % glucose) for further 60 min.

NO spin trapping and electronic paramagnetic resonance (EPR) studies

Detection of NO production was performed using the technique with Fe^{2+} diethyldithiocarbamate (DETC, Sigma-Aldrich) as spin trap. Briefly, after 30 min of CPC treatment in the absence or presence of different inhibitors, the medium was replaced with 250 µl of Krebs Hepes Buffer (KHB), and then treated with 250 µl of colloid $Fe(DETC)_2$ and incubated for 45 min at 37 °C. NO signal was measured *in situ* by EPR.

NO measurement was performed on a table-top x-band spectrometer Miniscope (Magnettech, MS200, Berlin, Germany). Recordings were made at 77 °K, using a Dewar flask. Instrument settings were 10 mW of microwave power, 1 mT of amplitude modulation, 100 kHz of modulation frequency, 150 s of Sweep time and 5 scans. Signals were quantified by measuring the total amplitude, after the correction of baseline as previously described (Agouni *et al.* 2008). The quantitative measurement of the NO-$Fe(DETC)_2$ signal amplitude was reported to the relative units for protein concentration of cells and results are given as % of control.

Determination of superoxide anion generation

For the detection of superoxide anion production, EAhy 926 cells were incubated for 45 min at 37 °C in KHB (pH 7.4) containing 5 µM DETC, 25 µM deferoxamine and 500 µM 1 hydroxy-3 methoxycarbonyl 2,2,5,5-tetramethylpyrrolidin (CMH, Noxygen, Germany). Briefly, deferroxamin and DETC were dissolved under argon gas bubbling in ice-cold KHB. They were rapidly mixed to the CMH solution, which was used immediately.

Then, cells were detached with trypsin-EDTA (Invitrogen, Cergy Pontoise, France) and frozen in plastic tubes to form rods. Electron spin resonance (ESR) spectra were recorded on a table-top x-band spectrometer Miniscope (Magnettech). The ESR instrument settings were as follows: BO-field, 3327G, microwave power, 1 mW (20 db), modulation amplitude, 5 G, sweep time, 60 s, field sweep, 60 G (BO-sweep) , gain 500 (5x2). Signals were quantified by measuring the total amplitude, after correction of baseline and normalization to the protein concentration of cells.

CPC contents

The enriched and lyophilized CPC powder was provided by the 'Bureau Interprofessionnel du Cognac' and contains, as previously described (Ralay Ranaivo *et al.* 2004) in mg/g of powder: gallic acid, 2.28, ellagic acid, 15.17, 5-hydroxy-methylfurfural, 0.17, syringic acid, 0.74, vanillin, 0.45, syringaldehyde, 2.02, scopoletin, 0.03, coniferaldehyde, 2.35, sinapaldehyde, 8.21. The total polyphenols content was about 48.8 % determined by colorimetric Follin-Ciocalteau method using gallic acid as standard.

Statistical analysis

All values are mean ± S.E.M. n represents the number of experiments as appropriate. Statistical analysis was carried out using one-way ANOVA. A Fisher's PLSD test was used for *post hoc* comparison. P<0.05 was considered to be statistically significant.

Results

Effect of CPC on BK-induced NO production

As illustrated on Figure 1, exposure of EAhy 926 endothelial cells to BK led to an expected increase in NO production (+32±6.8%, P<0.05). CPC also significantly enhanced NO production (+21±10.5 % P<0.05) to the same extent as that triggered by BK. Co-incubation of CPC with BK induced further increase in NO release (+82±8 %, P<0.01). The capacity of CPC plus BK in increasing NO signal was blunted by the NOS inhibitor, L-NAME (–22 ± 7.7 %, P<0.05).

Enhancement of NO production after inhibition of either NADPH oxidase or xanthine oxidase system

As shown on Figure 2A, SOD, which catalyses the reduction of O_2^- to hydrogen peroxide (H_2O_2), significantly enhanced NO production induced by BK plus
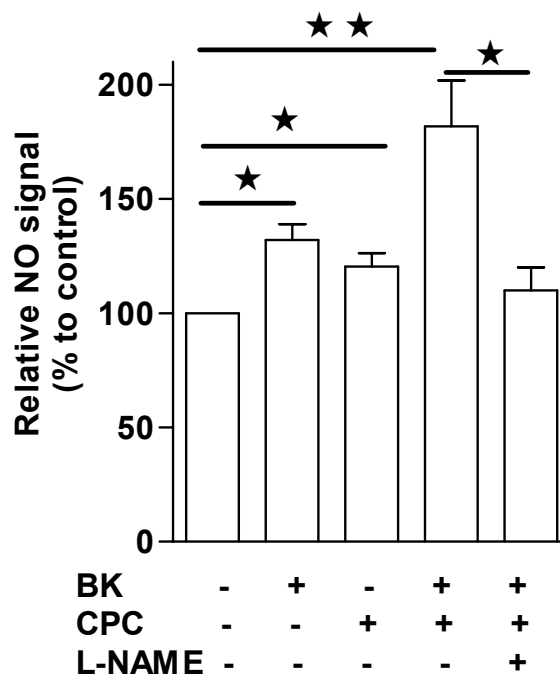

Fig. 1. Enhanced NO production in response to BK and CPC was prevented by the nitric oxide synthase inhibitor, N^w-nitro-L-arginine methyl ester, L-NAME. EAhy cells were pre-incubated with 40µM BK for 15 min before the treatment with CPC (0.03 mg/ml, 0.5 % glucose) for 30 min at 37 °C in the presence of 0.1 mM L-NAME, remained present throughout BK and CPC incubation. After the addition of Fe(II)Diethyldithiocarbamate (DETC)$_2$ complex, EAhy cells were then incubated for 45 min for the detection of the electron paramagnetic resonance [Fe(II)NO(DETC)$_2$] complex by ESR. Results are expressed as the mean ± S.E.M. of experiments performed in duplicate on 4 separate occasions. * p<0.05; ** p<0.01 (one-way ANOVA).

CPC (+41±8 %, P<0.01). Moreover, catalase, which is known as an efficient enzyme for the conversion of H_2O_2 into water and oxygen, significantly increased NO production elicited by BK plus CPC (+21±3.4 %, P<0.05). Altogether, these data strongly suggest the involvement of ROS in response to either BK and/or CPC that blunted the production of NO.

The sources of endogenous ROS were investigated by testing the effect of different pharmacological inhibitors (Fig. 2B). Blockade of either NADPH oxidase by apocynin (+38±15.7 %, P<0.05) or the cellular xanthine oxidase by allopurinol (+64±5.7 %, P<0.01) significantly enhanced NO production elicited by CPC plus BK. In contrast, the inhibitor mitochondrial respiratory chain at site I, rotenone, did not affect the response to both agents.

These results show that the additive effect of CPC and BK on NO production was blunted by the production of ROS sensitive to inhibitors of both NAPDH oxidase and xanthine oxidase.
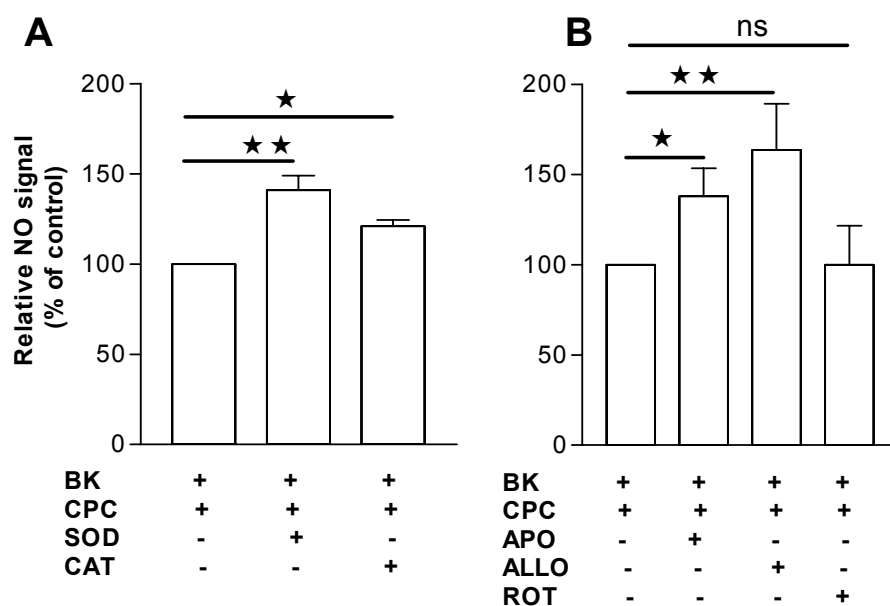
Fig. 2. (**A**) The potentiate NO production following BK and CPC exposure was enhanced by SOD and catalase. EAhy cells were preincubated with 40 µM BK for 15 min before the treatment with CPC (0.03 mg/ml, 0.5 % glucose) for 30 min at 37 °C in the presence of either 100 UI/ml SOD or catalase, which remained present throughout BK and CPC incubation. (**B**) The enhanced NO production following BK and CPC exposure was modulated by inhibitors of endogenous sources of ROS. EAhy cells were pre-incubated with 40 µM BK for 15 min before the treatment with CPC (0.03 mg/ml, 0.5 % glucose) for 30 min at 37 °C in the presence of either 50 µM allopurinol (ALLO), 2 mM apocynin (APO) or 5 µM rotenone (ROT), which remained present throughout BK and CPC incubation. NO level was quantified after ESR measurement. Results are expressed as the mean ± S.E.M. of experiments performed in duplicate on 4 separate occasions. ns, not significant; * p<0.05; ** p<0.01 (one-way ANOVA).
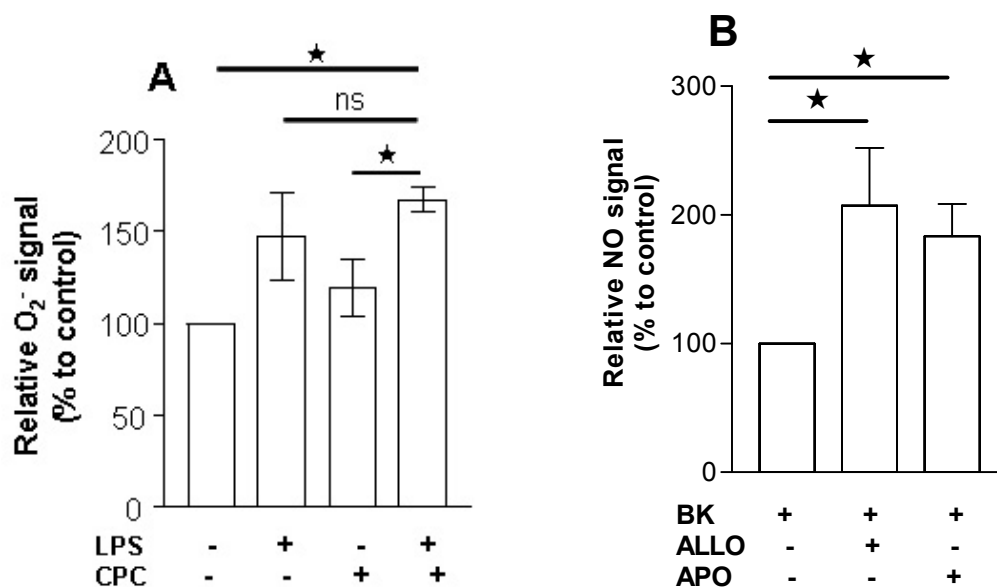
Fig. 3. (**A**) Enhanced O_2^- production in response to LPS and CPC. EAhy cells were preincubated with 40 µM BK for 15 min before the treatment with CPC (0.03 mg/ml, 0.5 % glucose) for 30 min at 37 °C. After the addition of CMH solution, EAhy cells were then incubated for 45 min for the detection of the electron paramagnetic resonance CM-nitroxide complex by ESR. (**B**) The NO production following BK exposure was enhanced after inhibition of either NADPH oxidase or xanthine oxidase system. EAhy cells were incubated with 40 µM BK for 15 min before the treatment with 50 µM allopurinol (ALLO) or 2 mM apocynin (APO) for 30 min at 37 °C. NO level was quantified after ESR measurment. Results are expressed as the mean ± S.E.M. of experiments performed in duplicate on 4 separate occasions. ns, not significant; * p<0.05 (one-way ANOVA).

Superoxide anions production originate from BK but not from CPC stimulation of endothelial cells

To test which compound used was able to promote O_2^- production that blunted the increase of NO production elicited by addition of BK plus CPC, we directly measured either the capacity of CPC to increase O_2^- or the effect of pharmacological inhibitors on BK-induced NO response in endothelial cells.

For the former (Fig. 3A), CPC alone was not able to significantly increase O_2^- production under the experimental conditions used. This was not linked to the incapability of EAhy cells to produce O_2^- because the treatment of these endothelial cells with LPS resulted an increase of O_2^- generation ($+47\pm23.4$ %, $P<0.05$). Moreover, the ability of LPS to increase O_2^- was not significantly enhanced by concomitant treatment of the cells with CPC ($+67\pm6.5$ %). The increase in O_2^- was abolished either by the superoxide mimetic MnTMPyP or by the cell-membrane permeable radical scavenger, tempol (data not shown).

Interestingly, the capacity of BK to increase NO production was significantly augmented in the presence of either apocynin ($+83\pm38.9$ %, $P<0.05$) or allopurinol ($+107\pm45.1$ %, $P<0.05$, Fig. 3B).

Discussion

This present study provides new findings on the interaction between BK, a physiologically active peptide of the kinin group, and polyphenols from a non-alcoholic extract of cognac. Altogether, our data demonstrate that BK was able to stimulate NADPH oxidase- and xanthine oxidase-inhibitor sensitive mechanisms that reduced its efficiency in increasing NO either alone or in the presence of CPC. They also highlight the fact that CPC enhanced NO production without an effect on ROS production.

In human endothelial cell line, NO release is significantly modulated by interaction of BK and CPC, following activation of NO synthase and enzymes that generate intracellular O_2^-. Indeed, the data demonstrate that CPC is able to directly increase NO production without affecting O_2^- and enhances the capacity of BK to produce NO in human endothelial cells. Our data elucidate the ability of BK to stimulate not only NADPH oxidase- but also xanthine oxidase-inhibitor sensitive mechanisms that reduce its efficiency in increasing NO either alone or in the presence of CPC.

The mechanisms by which BK and other endogenous vasoactive substances such as acetylcholine or adenosine, stimulate NO production in vascular tissues have been extensively described. They include a transient phosphorylation of the B_2 receptor on tyrosine residues in cultured endothelial cells (Marrero *et al.* 1999), a calcium-store-dependent calcium influx as well as a PI3-kinase activation (Kitayama *et al.* 2000, Schneider *et al.* 2003) (Tran *et al.* 2000) and a protein kinase A-dependent phosphorylation of eNOS-Ser(1179) (Bae *et al.* 2003).

The role of ROS in BK-induced NO release is of particular interest. In bradykinin-stimulated coronary arteries, antisense oligonucleotides against CYP 2C (a cytochrome P450 isozyme homologous) potentiated NO-mediated relaxation (Fleming *et al.* 2001). Folates, O_2^-, and peroxynitrite scavengers restore the NO-generating activity of eNOS, collectively suggesting that extracellular cellular redox state plays an important role in NO-generating function of this enzyme (Zhang *et al.* 2000). In porcine aortic endothelial cells, Jaimes et *al.* (2001) have reported that H_2O_2 impairs NO production in response to both receptor-dependent and receptor-independent agonists and that these effects are due, at least in part, to inactivation of eNOS cofactors, whereas HOCl inhibits NO production by interfering with receptor-operated mechanisms at the level of the cell membrane. Furthermore, endogenous H_2O_2 derived from NAD(P)H oxidase, mediates endothelial NO production in response to another agonist such as angiotensin II in bovine aortic endothelial cells (Cai *et al.* 2002). In the present study, we demonstrated in human endothelial cells that the capacity of BK in increasing NO production was blunted by its ability to activate both NADPH oxidase- and xanthine oxidase-inhibitor sensitive mechanisms. Although, the two enzymes are known to enhance O_2^- production in endothelial cells, especially after angiotensin II stimulation of NADPH oxidase. We report for the first time that BK can activate not only NADPH but also xanthine oxidase. It might be possible that O_2^--induced changes in the disturbance of cytosolic calcium homeostasis participate on the blunting effect of either NADPH or xanthine oxidase inhibitor on NO production elicited by BK. However, it is likely that O_2^- produced by such enzymes interacts with NO to produce peroxynitrite and thus reduced NO bioavailability upon BK treatment.

With regard to CPC, we reported that CPC induce a vasorelaxant response of rat aortic rings in the presence but not in the absence of functional endothelium

suggesting the obligatory role of the endothelium (Ralay Ranaivo *et al.* 2004). The involvement of prostacyclin was unlikely as the vasorelaxant response to CPC was not altered in the presence of the cyclo-oxygenase inhibitor, indomethacin. However, the relaxant effect of CPC was totally abolished in the presence of the NO synthase inhibitor, L-NAME. These data suggest that endothelial relaxation of rat aorta by CPC involved NO-dependent pathway. The implication of NO could result from an increased production of NO or from a diminution of its breakdown. In the present study, we demonstrated that CPC was able to directly stimulate NO production from endothelial cells without affecting O_2^- production. Thus, the ability of CPC to enhance endothelial NO production was not linked to its antioxidant properties. These results also contrast with our data with RWPC showing the involvement of O_2^- in the cytosolic calcium increase evoked by these compounds leading to the activation of enzymes involved in the release of endothelial relaxant factors including eNOS (Martin *et al.* 2002, Duarte *et al.* 2004). Moreover, our results are not in accordance with the study showing that RWPC promotes the release of endothelial NO through the redox sensitive PI3/Akt pathway (Ndiaye *et al.* 2005). It is likely that the nature of polyphenols involved in the response to CPC and RWPC might explain the different mechanisms of these two compounds to promote NO release. For RWPC, the molecular identity of polyphenols responsible for this effect may include oligomeric condensed tannins and anthocyanins (Andriambeloson *et al.* 1998). Among the identified components of CPC, phenolic acids (gallic acid and vanillic acid) are unlikely to be implicated because they fail to induce relaxant response in the aorta. We cannot exclude that ellagic acid might participate in the endothelial-dependent relaxation of CPC by potentiating the action of other compounds through its antioxidant capacity. Finally, we could not exclude that unidentified compounds, present in CPC, mediated the capacity of CPC to increase endothelial NO production.

Here we demonstrate that BK and CPC acted synergistically to enhance endothelial NO production. Moreover, both compounds probably mediate their effect *via* distinct pathways at least with regard to the activation of enzymes that promote O_2^- production such as NADPH oxidase and xanthine oxidase. Although, the mechanism by which BK promote NO release within endothelial cells is well described (see above), that of CPC requires further investigations. It is interesting to note that the mechanism by which CPC mediates its effect does not involve ROS system. Nevertheless, we report here that the potentiating effect of CPC on BK-induced NO release probably plays a role in the vascular protection induced by compounds in Cognac.

In conclusion, the present study elucidates on the synergistic effect of CPC and BK in inducing NO production in human endothelial cells. The data underscore the ability of BK to stimulate not only NADPH oxidase- but also xanthine oxidase-inhibitor sensitive mechanisms that reduce its efficiency in increasing NO either alone or in the presence of CPC as well.

Conflict of Interest
There is no conflict of interest.

Acknowledgements

References

AGOUNI A, LAGRUE-LAK-HAL AH, DUCLUZEAU PH, MOSTEFAI HA, DRAUNET-BUSSON C, LEFTHERIOTIS G, HEYMES C, MARTINEZ MC, ANDRIANTSITOHAINA R: Endothelial dysfunction caused by circulating microparticles from patients with metabolic syndrome. *Am J Pathol* **173**: 1210-1219, 2008.

ANDRIAMBELOSON E, MAGNIER C, HAAN-ARCHIPOFF G, LOBSTEIN A, ANTON R, BERETZ A, STOCLET JC, ANDRIANTSITOHAINA R: Natural dietary polyphenolic compounds cause endothelium-dependent vasorelaxation in rat thoracic aorta. *J Nutr* **128:** 2324-2333, 1998.

ANDRIANTSITOHAINA R: Regulation of vascular tone by plant polyphenols: role of nitric oxide. *Gen Physiol Biophys* **18 (**Suppl 1)**:** 3-5, 1999.

BAE SW, KIM HS, CHA YN, PARK YS, JO SA, JO I: Rapid increase in endothelial nitric oxide production by bradykinin is mediated by protein kinase A signaling pathway. *Biochem Biophys Res Commun* **306:** 981-987, 2003.

BOULANGER C, SCHINI VB, MONCADA S, VANHOUTTE PM: Stimulation of cyclic GMP production in cultured endothelial cells of the pig by bradykinin, adenosine diphosphate, calcium ionophore A23187 and nitric oxide. *Br J Pharmacol* **101:** 152-156, 1990.

BUSSE R, FLEMING I: Vascular endothelium and blood flow. *Handb Exp Pharmacol* **176** (Pt2): 43-78, 2006.

CAI H, LI Z, DIKALOV S, HOLLAND SM, HWANG J, JO H, DUDLEY SC, JR, HARRISON DG: NAD(P)H oxidase-derived hydrogen peroxide mediates endothelial nitric oxide production in response to angiotensin II. *J Biol Chem* **277:** 48311-48317, 2002.

DUARTE J, ANDRIAMBELOSON E, DIEBOLT M, ANDRIANTSITOHAINA R: Wine polyphenols stimulate superoxide anion production to promote calcium signaling and endothelial-dependent vasodilatation. *Physiol Res* **53:** 595-602, 2004.

FLEMING I, MICHAELIS UR, BREDENKOTTER D, FISSLTHALER B, DEHGHANI F, BRANDES RP, BUSSE R: Endothelium-derived hyperpolarizing factor synthase (cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries. *Circ Res* **88:** 44-51, 2001.

JAIMES EA, SWEENEY C, RAIJ L: Effects of the reactive oxygen species hydrogen peroxide and hypochlorite on endothelial nitric oxide production. *Hypertension* **38:** 877-883, 2001.

KITAYAMA J, KITAZONO T, IBAYASHI S, WAKISAKA M, WATANABE Y, KAMOUCHI M, NAGAO T, FUJISHIMA M: Role of phosphatidylinositol 3-kinase in acetylcholine-induced dilatation of rat basilar artery. *Stroke* **31:** 2487-2493, 2000.

MARRERO MB, VENEMA VJ, JU H, HE H, LIANG H, CALDWELL RB,VENEMA R: Endothelial nitric oxide synthase interactions with G-protein-coupled receptors. *Biochem J* **343**: 335-340, 1999.

MARTIN S, ANDRIAMBELOSON E, TAKEDA K, ANDRIANTSITOHAINA R: Red wine polyphenols increase calcium in bovine aortic endothelial cells: a basis to elucidate signalling pathways leading to nitric oxide production. *Br J Pharmacol* **135:** 1579-1587, 2002.

MONCADA S, BOLANOS JP: Nitric oxide, cell bioenergetics and neurodegeneration. *J Neurochem* **97:** 1676-1689, 2006.

MONCADA S, HIGGS EA: Nitric oxide and the vascular endothelium. *Handb Exp Pharmacol* **176** (Pt 1): 213-254, 2006.

NDIAYE M, CHATAIGNEAU M, LOBYSHEVA I, CHATAIGNEAU T, SCHINI-KERTH VB: Red wine polyphenol-induced, endothelium-dependent NO-mediated relaxation is due to the redox-sensitive PI3-kinase/Akt-dependent phosphorylation of endothelial NO-synthase in the isolated porcine coronary artery. *FASEB J* **19:** 455-457, 2005.

NIE L, WISE ML, PETERSON DM, MEYDANI M: Avenanthramide, a polyphenol from oats, inhibits vascular smooth muscle cell proliferation and enhances nitric oxide production. *Atherosclerosis* **186:** 260-266, 2006.

RALAY RANAIVO H, DIEBOLT M, SCHOTT C, ANDRIANTSITOHAINA R: Polyphenolic compounds from Cognac induce vasorelaxation in vitro and decrease post-ischaemic cardiac infarction after an oral administration. *Fundam Clin Pharmacol* **18:** 331-338, 2004.

RANDRIAMBOAVONJY V, FLEMING I: Endothelial nitric oxide synthase (eNOS) in platelets: how is it regulated and what is it doing there? *Pharmacol Rep* **57 (**Suppl): 59-65, 2005.

SCHINI-KERTH VB: Dual effects of insulin-like growth factor-I on the constitutive and inducible nitric oxide (NO) synthase-dependent formation of NO in vascular cells. *J Endocrinol Invest* **22:** 82-88, 1999.

SCHNEIDER JC, EL KEBIR D, CHEREAU C, LANONE S, HUANG XL, DE BUYS ROESSINGH AS, MERCIER JC, DALL'AVA-SANTUCCI J, DINH-XUAN AT: Involvement of Ca^{2+}/calmodulin-dependent protein kinase II in endothelial NO production and endothelium-dependent relaxation. *Am J Physiol Heart Circ Physiol* **284:** H2311-2319, 2003.

STOCLET JC, KLESCHYOV A, ANDRIAMBELOSON E, DIEBOLT M, ANDRIANTSITOHAINA R: Endothelial no release caused by red wine polyphenols. *J Physiol Pharmacol* **50:** 535-540, 1999.

TRAN QK, WATANABE H, LE HY, YANG J, TAKEUCHI K, KADOMATSU K, MURAMATSU T, OHASHI K: Midkine inhibits bradykinin-stimulated Ca^{2+} signaling and nitric oxide production in endothelial cells. *Biochem Biophys Res Commun* **276:** 830-836, 2000.

VILLALOBO A: Nitric oxide and cell proliferation. *FEBS J* **273:** 2329-2344, 2006.

ZHANG X, LI H, JIN H, EBIN Z, BRODSKY S, GOLIGORSKY MS: Effects of homocysteine on endothelial nitric oxide production. *Am J Physiol* **279:** F671-F678, 2000.