

2nde réunion jointe franco-allemande d'Hambourg

La 2nde réunion jointe franco-allemande d'Hambourg a été positive dans l'ensemble, mais avec une faible participation des jeunes chercheurs français malgré notre soutien. Organisée par le Dr Rainer Böger, elle s'est déroulée sur deux jours ½ bien remplis pendant lesquels plusieurs thématiques ont été abordées. Une centaine de chercheurs, dont une vingtaine de français y participaient. La parole a largement été donnée aux jeunes; les 2 plus méritants ont d'ailleurs été récompensés par des prix, le 1^{er} étant revenu à un français, Xavier Loyer (Inserm Lariboisière, Paris), pour son travail sur la NOS neuronale dans l'insuffisance cardiaque. Le poster de Kiryl Turpaev (ICSN, Gif sur Yvette) a également été primé (rôle du NO dans l'expression des gènes).

Bourses voyages :

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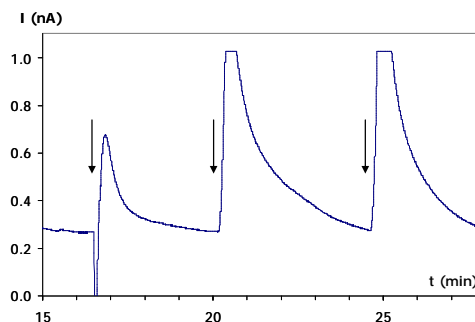
Electroanalytical methodology for the detection of NO in tumour-bearing mice.

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Since it has been shown that the vasculature of tumours plays an essential role in their development, research on "tumour angiogenesis" has become an active field of investigation¹. The concept of antivasular therapy is related to that of the anti angiogenesis therapy, but it implies a singularly different approach: rather than targeting the formation of new vessels (angiogenesis), the antivasular molecules target the existing tumoral vasculature in order to cause the death of the tumoral cells². A fundamental prerequisite of the antivasular approach is based on the postulate that the tumoral vasculature is different from the normal one, at both morphological and molecular levels. One of the principal actors in this is nitric oxide (NO)³. Indeed, several anticancer agents induce the release of NO (e.g. flavones, xanthone acetic acid)^{2,4}. Thus, it is believed that this "induced NO-release" could be considered, at least partly, as a marker of the anticancer activity of these classes of chemicals *in vivo*. To explore the mechanism of action of standard anticancer agents of various classes, a well-designed electroanalytical concept is needed in order to locally detect and quantify NO release.

The study described here reports on preliminary experiments aimed at assessing NO in tumours (*in vivo*). This has been achieved by performing, for the first time, the electrochemical detection of NO in tumour-bearing mice. In a first approach, we performed the insertion of the needle-type electrochemical NO sensor⁵, by placing it into the tumour. Then a simple electroactive probe, namely $\text{Fe}(\text{CN})_6^{4-}$, was injected locally in the tumour and electrochemically detected. This allowed standardizing the analytical approach in terms of sensor setting and measurements. In a second step, aliquots of NO stock solution (prepared from NO donor) were injected in the tumour to assess its amperometric intra-tumoral detection.



Experimental device and typical amperogram obtained at 0.8 V in 3LL tumour upon addition of NO saturated buffered solution prepared from DEA-NONOate (pH 7.4). Each arrow indicates addition of 4×10^{-8} mol of NO.

The obtained results are encouraging and allow anticipating the possibility of electrochemically detecting endogenous NO release in tumours. Thus, the use of this approach should help in assaying a particular anticancer drug-induced NO release, and at screening purposes to detect new anticancer agents acting *in vivo*.

¹ J. Folkman, *Semin. Oncol.*, 29 (2002) 15.

² G. M. Tozer, C. Kanthou & B. C. Baguley, *Nat. Rev. Cancer*, 5 (2005) 423.

³ S. Kashiwagi, Y. Izumi & T. Gohongi, *J. Clin. Invest.*, 115 (2005) 1816.

⁴ B. C. Baguley, *Lancet Oncol.*, 4 (2003) 141.

⁵ F. Bedioui, N. Villeneuve, *Electroanalysis*, 15 (2003) 5.

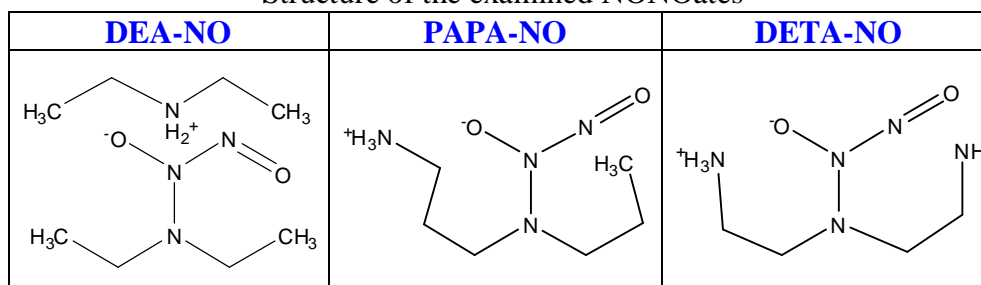
UV-visible and Electrochemical analysis of the decomposition of NO donors (diazoniumdiolates) in phosphate buffer solution.

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The study of the spontaneous generation of nitric oxide (NO) upon the decomposition of diazeniumdiolate anions ($R_2N[N(O)NO]^-$) is of particular interest since this class of molecules is now being widely used as pharmacological compounds to generate the natural bioregulatory NO. However, there are no systematic studies performed with these prodrugs in order explain the previously reported variability in their dissociation rates. In this study, we report on the UV-visible and electrochemical analysis of the decomposition of three diazeniumdiolates (so-called "NONOates"), namely : diethylammonium (Z)-1-(N,N-diethylamino)-diazene-1-ium-1,2-diolate (DEA-NO); (Z)-1-[N-(2-aminoethyl)-N-(2-ammonioethyl)amino]-diazene-1-ium-1,2-diolate (DETA-NO) and (Z)-1-[N-(2-aminoethyl)-N-(2-ammonioethyl)amino]-diazene-1-ium-1,2-diolate (PAPA-NO).

Structure of the examined NONOates



The decomposition of the NO donors was followed by UV-visible spectrophotometry at 250 nm, while the determination of the released NO was performed electrochemically by a home made nickel-porphyrin and Nafion® coated platinum electrode at 0.80 V. Besides DETA-NO which showed interfering electrochemical signals, the obtained results for DEA-NO and PAPA-NO were numerically fitted to a theoretical curve modelling NO production and degradation. This allows obtaining the following indications for DEA-NO and PAPA-NO.

Theoretical maximal concentration of NO released from NONOates at 26°C in aerobic phosphate buffer solution (pH 7.4) versus initial NONOate concentration

[NONOate] _{t=0} (μM)	0.125	0.5	1	5	10	20	50	100
Maximal NO concentration from PAPA-NO (μM)	0.05	0.11	0.17	0.38	0.56	0.80	1.30	1.80
t _{max} (s)	3200	1900	1400	800	520	400	300	200
Maximal NO concentration from DEA-NO (μM)	0.08	0.19	0.29	0.70	1.00	1.50	2.35	3.40
t _{max} (s)	1400	800	600	310	260	200	140	90

Gender differences of the red wine polyphenols- and green tea polyphenols-induced endothelium-dependent NO-mediated relaxation in the rat aorta

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Introduction: Epidemiological studies have indicated that regular consumption of beverages rich in polyphenols such as red wine and green tea is associated with a reduced risk of coronary artery disease. The vascular protective effect has been attributable at least in part to an enhanced endothelial formation of vasoprotective factors such as nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). Since polyphenols have been shown to have phytoestrogen properties, endothelium-dependent relaxations to polyphenols were studied in male and female rats.

Materials and Methods: 12 to 14 week-old male and female Wistar rats were used. Aortic rings with or without endothelium were suspended in organ chambers for the measurement of changes in isometric tension.

Results: Red wine polyphenols (RWPs) and the major green tea polyphenol epigallocatechin-3-gallate (EGCG) caused concentration-dependent relaxations in rings with endothelium but not in those without endothelium, which were more pronounced in aortic rings from female than male rats. In contrast, relaxations to sodium nitroprusside were similar in male and female aortic rings. Relaxations to RWPs were abolished by nitro L-arginine and the superoxide dismutase mimetic MnTMPyP, markedly reduced by polyethyleneglycol-catalase, and not affected by the estrogen antagonist ICI 182,780 in both male and female aortic rings. Relaxations to EGCG were also abolished by nitro-L-arginine and MnTMPyP but not affected by PEG-catalase and ICI 182,780 in both male and female aortic rings.

Conclusion: Both red wine and green tea polyphenols cause redox-sensitive endothelium-dependent NO-mediated relaxations in the aorta, which are more pronounced in female than male rats. In addition, activation of estrogen receptors does not play an important role in the endothelial formation of NO by red wine and green tea polyphenols.

Progestins prevent the potentiating effect of 17 β -estradiol on the NO-mediated anti-aggregatory effect of endothelial cells by inhibiting the expression of endothelial NO synthase and GTP cyclohydrolase

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Objectives: Epidemiological studies have indicated that estro-progestin treatments are associated with an increased risk of venous and arterial thromboembolic events in postmenopausal women. Estrogens have been shown to increase the expression of endothelial nitric oxide synthase (eNOS), a potent anti-thrombotic factor. This study examined whether progestins affect the beneficial effect of estrogens on the endothelial formation of NO.

Methods: Experiments were performed with human umbilical vein endothelial cells. eNOS and GTP cyclohydrolase (GTPCH) mRNA expression was assessed by quantitative real-time PCR, eNOS protein by Western blotting, NO formation by electron spin resonance spectroscopy, and platelet aggregation by an aggregometer.

Results: 17 β -estradiol (17 β -E) increased the expression of eNOS mRNA and protein and caused an increased formation of NO. Medroxyprogesterone acetate (MPA), progesterone, levonorgestrel (LNG) and nomegestrol acetate (NOMAC) markedly inhibited the stimulatory effect of 17 β -E on eNOS mRNA and protein level. This effect was associated with a reduced 17 β -E-induced formation of NO in the case of MPA and progesterone whereas LNG and NOMAC did not have such an effect. In addition, 17 β -E increased the expression of GTPCH mRNA, this effect was prevented by MPA and progesterone but not by LNG and NOMAC. In addition, 17 β -E potentiated the NO-mediated inhibitory effect of endothelial cells on thrombin-induced platelet aggregation. The potentiating effect of 17 β -E was prevented by MPA and progesterone whereas LNG and NOMAC had no or only small effects. Mifepristone, a glucocorticoid and progesterone receptor antagonist, prevented the inhibitory effect of MPA and progesterone.

Conclusion: The present findings indicate that certain progestins, including MPA, blunt the ability of 17 β -E to enhance the NO-mediated anti-aggregatory effect of endothelial cells. The inhibitory effect of progestins is due, at least in part, to the prevention of the stimulatory effect of 17 β -E on eNOS and GTPCH expression most likely via activation of the glucocorticoid receptor.

Cardiomyocyte overexpression of neuronal nitric oxide synthase protects from heart failure in response to pressure overload

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The presence of a neuronal NOS isoform (NOS1) in the cardiac sarcoplasmic reticulum has been recently discovered, leading to the hypothesis that NOS1 may alter calcium cycling and cardiac hypertrophy. However, an *in vivo* role for NOS1 in the development of cardiac hypertrophy has not been defined.

We evaluate the *in vivo* role of NOS1 in response to chronic pressure overload-induced cardiac hypertrophy, using mice overexpressing NOS1 in cardiomyocytes (NOS1-Tg). Four weeks of thoracic aorta constriction (TAC), WT mice showed a 50% increase in heart weight-to tibia length ratio (HW/TL). Surprisingly NOS1-Tg underwent a further 20% increased HW/TL ratio (P = 0.0005). Morphometric analysis of cardiomyocyte cross-sectional areas revealed that the observed difference between WT and NOS1-Tg mice resulted from an increased cardiomyocyte enlargement in NOS1-Tg. Echocardiographic analysis demonstrated that WT mice exhibited an increased wall thickness, increased left ventricular end-diastolic and -systolic dimensions (LVEDD, LVESD, P<0.0001 vs WT-Sham), associated with a significant decreased fractional shortening (FS), in response to TAC. Conversely, NOS1-Tg developed a further increased wall thickness, showed reduced LV dimensions compared with WT-TAC (P<0.0001) and had a preserved FS (P<0.001 vs WT-TAC). Analysis of intracellular signaling events indicated that NOS1 overexpression induced an increase in both calcineurin expression and phosphorylation of GSK3b (P<0.05 compared to WT-TAC).

To prove the NOS1 requirement in cardiomyocyte hypertrophy process, we investigated *in vitro* its involvement, using a specific NOS1 siRNA. NOS1 siRNA blunted phenylephrine (PE) induced neonatal cardiomyocyte hypertrophy, assessed by *de novo*

protein synthesis, measurement of myocyte surface area. We demonstrated that NOS1 siRNA blunted PE-stimulated calcineurin protein expression, translocation of NFAT-c4 to the nucleus and phosphorylation of GSK3-b.

Thus, NOS1 critically controls the transitional step between adaptative hypertrophy and cardiac dysfunction in response to pressure overload and could be a suitable target to prevent the occurrence of heart failure.