# NEBIVOLOL INDUCES NITRIC OXIDE PRODUCTIONE IN THE HEART

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Nebivolol is a third generation beta blocker that reduces blood pressure not only by antagonizing beta1-adrenergic receptors, but also by evoking endothelium-dependent vasodilation via induction of NO production. In this study, we show that nebivolol is able to induce NO production also in the heart and we delineate the molecular mechanisms involved.
Hearts were explanted from C57BL/6 mice and incubated in Krebs' buffer at 37°C for 5h in presence of the probe DAF-2, which becomes fluorescent in presence of NO. Hearts were stimulated with increasing doses of nebivolol ($10^{-8}$-$10^{-5}$ M), in presence or absence of the pan-NOS inhibitor L-NAME, of the iNOS inhibitor L-NIL or of the n-NOS inhibitor 7-nitroindazole ($3*10^{-4}$ M); or of the beta2-receptor antagonist ICI118,551, or of the beta3-receptor antagonist SR59230A ($10^{-7}$M).
Nebivolol induces a dose-dependent NO production in the heart. This NO production is statistically significant at $10^{-7}$ M, and increases further at higher nebivolol concentrations. It is not an effect due to the blockade of beta1-adrenergic receptor, since this effect is not shared by another drug of the same class such as atenolol.
Since nebivolol has been reported to act as an agonist on other beta-adrenergic receptors, we tested NO production in presence of receptor antagonists. Nebivolol was not able to induce NO production in presence of the beta3 antagonist SR59230A, while ICI118,551 had no effects. These results indicate that the activation of beta3-adrenergic receptors is fundamental for cardiac NO production by nebivolol.
Moreover, cardiac NO production is completely abolished in presence of L-NAME, as expected. When we blocked single NOS isoforms, we noticed that iNOS inhibition, but not nNOS inhibition, abolishes NO release in the heart stimulated with nebivolol, indicating that the drug induces NO production by acting on the inducible isoform of the enzyme. The action of nebivolol on iNOS has been confirmed by RT-PCR experiments, showing cardiac overexpression of iNOS, but not nNOS or eNOS, after 5h of treatment with nebivolol.
In conclusion, our study demonstrated that nebivolol stimulates NO production also in the heart. This action of nebivolol is exerted via a signalling pathway starting from the activation of beta3-adrenergic receptors and leading to overexpression of iNOS. Cardiac NO production by nebivolol could participate in the protective effects observed after nebivolol treatment in patients affected by hypertension and heart failure.