Nebivolol pharmacological characterization under noradrenaline-induced $\beta_1$ receptor downregulation and $\beta_3$ receptor upregulation in neonatal rat cardiomyocytes

Francesca Bellucci, Stefania Meini, Paolo Santicioli, Paola Cucchi, Claudio Catalani, Sandro Giuliani, Carlo Alberto Maggi

Menarini Ricerche S.p.A., Florence, Italy

Nebivolol, a highly selective $\beta_1$-adrenergic receptor antagonist, has been reported to have beneficial effects in patients with chronic heart failure, improving ventricular function and survival (Bristow, 2000, Dessy et al., 2005). The mechanism that mediates these actions remains controversial, however the $\beta_1$ and $\beta_3$ adrenergic receptors have been shown to be involved (Brixius et al., 2001; Maffei et al., 2007). We aimed at investigating the pharmacological profile of nebivolol, and its d- and l- enantiomers at the $\beta$ adrenergic receptors in primary cultures of neonatal rat cardiomyocytes (Wistar, male and female, four days old) under normal physiological conditions or following chronic exposure to noradrenaline (NOR, 100 $\mu$M for 24 h). This treatment was demonstrated to upregulate the $\beta_3$ adrenergic receptor and downregulate the $\beta_1$ and $\beta_2$ subtypes (Germack et al., 2006).

Radioligand ($[^{125}\text{I}]$(-)-cyanopindolol) binding experiments were performed with membranes prepared from control cells: nebivolol and its d-enantiomer inhibited the specific binding with comparable affinity (pIC$_{50}$ 7.5 and 7.7, respectively, n = 3), whereas the l-enantiomer was about 10-fold less active (pIC$_{50}$ 6.2, n = 3). No detectable $[^{125}\text{I}]$(-)-cyanopindolol binding was observed with membranes prepared from NOR-treated cells.

In functional experiments (cAMP production) with control cells, nebivolol (100 nM, PA$_2$ 7.9, n = 4) antagonized the isoprenaline responses (pEC$_{50}$ 6.5, n = 4), but not that induced by the $\alpha_1$ adrenergic receptor agonist phenylephrine (pEC$_{50}$ 4.5, n = 3). Nebivolol did not affect per se the basal cAMP production.

On the contrary, nebivolol and its enantiomers when tested in NOR-treated cells concentration-dependently inhibited both the basal or the forskolin-induced cAMP accumulation. The racemate (pIC$_{50}$ 6.7 and 8.6, respectively, n = 5) and d-enantiomer (pIC$_{50}$ 8.4 and 8.2, respectively, n = 5) were significantly more active than l-enantiomer (pIC$_{50}$ < 5, n = 5). The inhibitory effect of nebivolol on forskolin-induced cAMP accumulation was completely abolished by pretreatment with the selective $\beta_1$ or $\beta_3$ antagonists CGP20712A (1 $\mu$M) and SR59230A (1 $\mu$M), but not by the selective $\beta_2$ antagonist ICI118551 (1 $\mu$M). Experiments using pertussis toxin (250 ng/ml for 18 h or 100 ng/ml for 18 h or 30 min of incubation) failed to revert nebivolol-induced inhibition, thus ruling out a possible G$\text{i}$ mediated signalling.

Altogether obtained data highlight the possibility that nebivolol, and its enantiomers, may have a dual mechanism of action, mediated by $\beta_1$ and $\beta_3$ adrenergic receptors, depending on cell physiological status and receptors expression pattern


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