

STRUCTURAL REQUIREMENTS OF *N*-BENZYL TOCAINIDE ANALOGS FOR STEREOSELECTIVE AND POTENT BLOCK OF SKELETAL MUSCLE SODIUM CHANNELS

¹De Bellis Michela, ¹De Luca Annamaria, ²Corbo Filomena, ²Muraglia Marilena, ²Franchini Carlo, ²Tortorella Paolo and ¹Conte Camerino Diana.

1. Unit of Pharmacology, Department of Pharmacobiology, Faculty of Pharmacy, University of Bari and 2. Department of Medical Chemistry, Faculty of Pharmacy University of Bari, Italy

Previous structure-activity relationship studies with Tocainide (Toc) analogues defined some critical molecular determinants for high affinity and use-dependent block of skeletal muscle Na⁺ channels. We showed that the constraint of the stereogenic centre of Toc in a rigid proline-like cycle, as in To5 [*N*-(2,6-dimethylphenyl)pyrrolidine-2-carboxamide], increases both potency and stereoselectivity, while the introduction of a benzyl moiety on the pharmacophore amino group strongly enhances potency and use-dependent behaviour, probably for the establishment of specific hydrophobic interactions with the binding site (1). On the basis of these data, we have evaluated whether the combination of the above mentioned structural requirements could modify the drug stereoselectivity for blocking Na⁺ channels. Newly synthesized *N*-benzyl-Toc analogs were tested as pure enantiomers on sodium currents (*I*_{Na}) of frog skeletal muscle fibres using the vaseline-gap voltage-clamp method. Depolarizing steps to -20 mV from the holding potential of -100mV at different stimulation frequencies (0.25, 0.5, 1, 2, 5, 10Hz) were applied in order to evaluate tonic and use-dependent blocks by drugs. The presence of the benzyl moiety on the amino terminal group, as in *N*-benzyl-Toc, did not introduce a stereoselective behaviour in blocking *I*_{Na}; however, as expected, both enantiomers were 10 and 30 fold more potent than Toc ones in producing tonic and 10Hz use-dependent block, respectively. Interestingly, the introduction of a benzyl group on the α -proline derivative, as in *N*-benzyl-To5, maintained the stereoselectivity of parent compound, the *R*-isomer being more potent than *S*-one. In particular, the eudismic ratio [IC₅₀ distomer/IC₅₀ eutomer] increased with the stimulation frequency, being 1.3 and 3.1 for tonic and 10Hz use-dependent block, respectively. A higher increase in potency for use dependent block was observed with the *N*-benzyl analog of β -proline derivative, To10 [1-benzyl-*N*-(2,6-dimethylphenyl)pyrrolidine-3-carboxamide], with a ratio (IC₅₀ tonic block/ IC₅₀ 10Hz use-dependent block) of 12. Surprisingly, To10 was almost devoid of stereoselectivity, suggesting that this property might be strictly determined by the position of the amino terminal group with respect to the chiral centre. We can conclude that To10 is the most potent Tocainide analogue described and that it may be considered as a potential therapeutic agent in the treatment of myotonic syndromes or other hyperexcitability disorders.

(1) De Luca A., Talon S., De Bellis M., Desaphy J.F., Lentini G., Corbo F., Scilimati A., Franchini C., Tortorella V. and Conte Camerino D. (2003) *Mol. Pharmacol.* 64:932-945.