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BIA 5-1058 is a new noncompetitive dopamine-β-hydroxylase inhibitor

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Dopamine-β-hydroxylase (DβH; EC 1.14.17.1) is expressed in the chromaffin cells of adrenal medulla and in noradrenergic nerve terminals of the central and peripheral sympathetic nervous system and catalyses the conversion of dopamine to noradrenaline in the catecholamine biosynthetic pathway. It has been considered an important therapeutic target for hypertension and chronic heart failure. BIA 5-1058 ((R)-5-(2-(benzylamino)ethyl)-1-(6,8-difluorochroman-3-yl)-1H-imidazole-2(3H)-thione) is a novel DβH inhibitor shown to decrease noradrenaline levels after oral administration to rats and mice while increasing dopamine levels.

This study was intended to not only dissect the interaction mechanism of BIA 5-1058 with the human DβH (from SK-N-SH cells) using a kinetic approach, but also to evaluate the potential capacity of BIA 5-1058 to induce DβH expression in mice.

DβH activity was measured by a modification of the Nagatsu and Udenfriend (1) spectrophotometric method based on the conversion of tyramine into octopamine. RNA expression was measured by quantitative real time PCR. All data analyses were performed using GraphPad Prism software. Results are presented with 95 % confidence intervals, or as mean ± sem. Statistical analysis was performed using Student´s t test.

Under optimized experimental conditions octopamine was formed by the SK-N-SH homogenates with a Km value of 9 (6; 13) mM for tyramine and a Vmax of 1747 ± 128 nmol/mg prot/h. The Km value determined for ascorbate was determined to 3 (2; 4) mM. BIA 5-1058 inhibited DβH with an IC50 value of 90 (72; 114) nM and the inhibition was reversed by dilution. To study the inhibition mechanism, velocities were determined for various concentrations of the substrate tyramine and co-substrate ascorbic acid and at several concentrations of BIA 5-1058. Velocities were then globally fitted to the appropriate models (competitive, noncompetitive and uncompetitive) by nonlinear regression analysis using GraphPad Prism software. BIA 5-1058 was shown to be noncompetitive regarding the tyramine binding sites with a Ki value obtained of 43 (20; 65) nM. Regarding the ascorbic acid binding site BIA 5-1058 appears also to exhibit noncompetitive inhibition modality.

To evaluate the effect of BIA 5-1058 on DβH in vivo, NMRI mice were administered 30 mg/kg BIA 5-1058 and, at various time intervals, enzyme activity was determined in the adrenals. BIA 5-1058 led to maximal enzyme inhibition (65 ± 3% inhibition) at 1-3 h post administration with full activity being recovered at 15 h post-administration. The expression of the enzyme was evaluated in adrenals of mice daily administered 100 mg/kg of BIA 5-1058 for 5 consecutive days. No significant changes were observed in DβH mRNA levels.

In conclusion, BIA 5-1058 is a reversible DbH inhibitor that displays binding affinity for both the free enzyme and the complex enzyme-substrate and does not affect the expression of the enzyme in vivo.

(1) Nagatsu, T. and S. Udenfriend (1972) Clin Chem 18(9): 980-3.