Kinetic studies on the inhibition of dopami-nebeta-hydroxylase by BIA 5-453

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BIA 5-453 ((R)-5-(2-aminoethyl)-1-(6,8-difluorochroman-3-yl)-1H-imidazole-2(3H)-thione hydrochloride) is a novel inhibitor of the enzyme dopamine-beta hydroxylase (DβH, EC 1.14.17.1) designed not to cross the blood brain barrier (1). DβH is a copper II ascorbate-dependent monooxygenase that catalyses the conversion of dopamine into noradrenaline in the catecholamine biosynthetic pathway and has been suggested to be a potential target for the therapy of chronic heart failure and hypertension.

The aim of the current study was to characterize the kinetics of DβH inhibition by BIA 5-453 using SK-N-SH cells, a human neuroblastoma cell line. DβH activity was evaluated by a modification of the method of Matsui (2), using dopamine as substrate and measuring noradrenaline formed. In brief, the reaction mixture contained sodium acetate pH 5.0 (200 mM), N-ethylmaleimide (30 mM), CuSO4 (5 µM), catalase aqueous solution (0.5 mg/ml), pargyline-HCl (1 mM), sodium fumarate (10 mM) and ascorbic acid (10 mM). The reaction was initiated with the substrate and was terminated with perchloric acid (2 M). Noradrenaline formed was quantified by high pressure liquid chromatography with electrochemical detection. Fifteen minutes of reaction time and protein amounts up to 150 µg per reaction were the experimental conditions chosen for the measurement of initial velocities in the subsequent kinetic studies. Results are presented with 95% confidence intervals or, in the case of Vmax, as mean ± SEM.

Noradrenaline was formed by the SK-N-SH homogenates with a Km value of 21 (16; 26) mM for dopamine and a Vmax of 623 ± 33 nmol(mg prot)-1h-1. The Km value determined for ascorbate was determined to be 0.75 (0.58; 0.93) mM. BIA 5-453 inhibited DβH with an IC50 value of 196 (169; 227) nM and the inhibition was partially reversed by dilution. Using a two-fold higher amount of protein did not change the IC50 value obtained, 212 (171; 252) nM, indicating that DβH inhibition by BIA 5-453 was independent of protein concentration. The behaviour of BIA 5-453 as a classical type inhibitor was further corroborated when the plot of initial velocities at different enzyme amounts in the presence or absence of inhibitor (Ackermann-Potter plot) was shown to be linear.

Enzyme activity was also evaluated at increasing dopamine concentrations and with different inhibitor concentrations. The Km values obtained by nonlinear regression analysis for 20 nM and 40 nM BIA 5-453 were respectively 26 (21; 31) nM and 23 (13; 32) mM. Concerning Vmax values obtained, they were respectively 527 ± 25 nmol (mg prot)-1h-1 and 394 ± 34 nmol(mg prot)-1h-1 for 20 nM and 40 nM BIA 5-453.

In conclusion, the data obtained suggests that BIA 5-453 is a classical type reversible DβH inhibitor, displaying mixed (non-competitive) type inhibition with respect to dopamine with a Ki value of 64 (51; 77) nM.