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Inhibition of dopamine β-hydroxylase by captopril

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The exact mechanism of action of captopril is still unclear. The original supposition that its antihypertensive effect is the result of the sole inhibition of angiotensin converting enzyme has been questioned by several authors (see Refs. 1–3 for reviews). Convincing evidence has accumulated that captopril acts also through a reduction of the sympathetic tone, an effect in part unrelated to converting enzyme inhibition [3]. For example, Antoniaccio and coworkers showed that captopril, but not saralasin or other converting enzyme inhibitors, such as tetprotide and enalapril, causes prejunctional inhibition of noradrenaline release [4] and inhibition of the pressor responses to sympathetic nerve stimulation [5] in pithed spontaneously hypertensive rats. These results were confirmed *in vitro* by Collis and Keddie [6] and Clough *et al.* [7] who, using the model of isolated rat mesenteric arteries, showed that captopril, at concentrations of 100–300 μM, markedly attenuates, by an angiotensin-independent mechanism, the vasoconstriction induced by sympathetic nerve stimulation. It was suggested that the sulfhydryl moiety of captopril is responsible for such effects, since enalapril, that contains no sulfhydryl groups, was found devoid of any effect on sympathetic function [5]. Sulfhydryl compounds have long been known to inhibit, both *in vitro* and *in vivo*, dopamine β-hydroxylase (DBH; 3,4-dihydroxy-phenylethylamine, ascorbate: oxygen oxidoreductase (β-hydroxylating), EC 1.14.17.1) the enzyme that converts dopamine to noradrenaline [8–10]. Inhibition of DBH has been shown to result in decreased sympathetic activity and marked hypotensive effects (see Ref. 10 and references therein). We, therefore, tested the effect of captopril on this enzyme and investigated its mechanism of inhibition. Enalalprilic acid, the pharmacologically active species of enalapril [1], was also tested in order to assess the relevance of the sulfhydryl group to the observed effects.

Materials and methods

Bovine adrenal dopamine β-hydroxylase and all components of the reaction mixtures were obtained from Sigma. Enzyme activity was determined with tyramine as substrate by a modification [9] of the spectrophotometric method of Pisano *et al.* [11]. The kinetic properties of the enzyme preparation used proved to be quite similar to those of the other currently used preparations (pH optimum 5.2; K_m values for tyramine and ascorbate 1.4 and 0.9 mM, respectively). Except when otherwise specified, reaction mixtures contained, in a final volume of 0.5 ml, 100 mM phosphate buffer pH 5.2, 3 mM ascorbate, 4 mM tyramine and sufficient catalase (1000–1500 units) to give maximal stimulation of enzyme activity. After thermal equilibration of the incubation medium at 37°, the reaction was started by the addition of 1–1.5 μg of enzyme and stopped after 10 min by the addition of 0.1 ml of 50% trichloroacetic acid. It was verified that product formation was linear with time also in conditions of maximum substrate depletion, which was never allowed to exceed 5%. Initial enzyme velocity at saturating substrate concentrations was about 3 μmol per min per mg of protein. Protein concentration was determined according to Lowry *et al.* [12]. All data presented are the mean of three to four experiments performed in duplicate. The interassay error was less than 8% coefficient of variation. Regression lines were determined by a weighed least square method.

Results and discussion

From a dose–response curve, the captopril concentration giving 50% inhibition of DBH activity (IC_{50}) was determined to be 140 μM. Enalaprilic acid had no effect on the enzyme activity up to 2 mM. The inhibitory effect of captopril was found fully reversible upon dilution. In addition, the inhibition was found to be independent of time when the time of incubation was varied from 2 to 20 min. These results are indicative of a truly reversible interaction between enzyme and inhibitor. The effect of captopril on DBH kinetics is shown in Fig. 1 in the form of a reciprocal velocity plot vs the reciprocal of the concentration of ascorbate. This plot indicates noncompetitive inhibition with respect to ascorbate, according to the definition of Cleland [13]. The double-reciprocal plot of Fig. 2 shows that captopril behaved as a noncompetitive inhibitor also

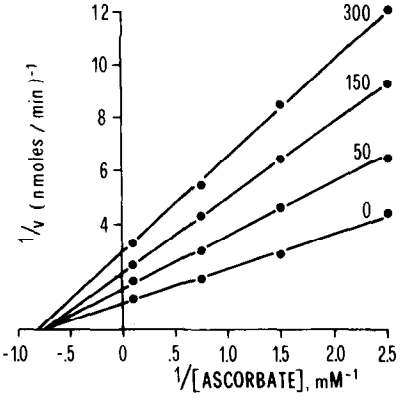

Fig. 1. Plots of reciprocal of initial velocity vs reciprocal of ascorbate concentration in the absence and presence of captopril. Ascorbate concentration was varied from 0.4 to 10 mM, whereas tyramine concentration was held constant at 4 mM. The numbers above the lines indicate captopril concentrations in micromolar units.

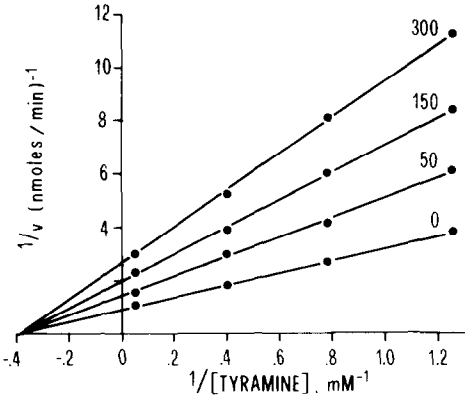

Fig. 2. Plots of reciprocal of initial velocity vs reciprocal of tyramine concentration in the absence and presence of captopril. Tyramine was varied from 0.8 to 20 mM, whereas ascorbate was held constant at 3 mM. The numbers above the lines indicate captopril concentrations in micromolar units.

with respect to tyramine. Slope and intercept replots of the double-reciprocal plots of Figs 1 and 2 were linear ($r > 0.987$ for each line), indicating that no more than one molecule of inhibitor interacted with the same enzyme species [13]. The values of K_i-slope and K_i-intercept, determined from the abscissal intercepts of these replots, were both found quite similar to the IC_{50} value (140 μM), as was to be expected for a noncompetitive inhibitor [14]. Since DBH is activated by various anions, the most effective of which is fumarate [15], the inhibitory effect of captopril was studied also in the presence of this anion. Fumarate, at concentrations up to 10 mM, had no effect on the IC_{50} value, neither did it modify the inhibition patterns with respect to ascorbate and tyramine.

The inhibitory effect was reversed in a dose-dependent manner by cupric ions. Reversal of the inhibition by 140 μM captopril was virtually complete at 1.5 μM Cu^{2+}, as $CuSO_4$.

On the basis of their copper chelating ability in solution and the reversal of their inhibitory effect by cupric ions, sulfhydryl compounds have been proposed to interact with the copper atoms at the active sites of DBH [8]. A noncompetitive inhibition vs ascorbate, such as that observed with captopril, is consistent with this interpretation if it is assumed that the mechanism of DBH is ping-pong and the inhibitor interacts with both the copper (II) and copper (I) forms of the enzyme [15]. However, if a sequential mechanism applies, as proposed by other authors [9, 16, 17], an inhibitor interacting reversibly with the enzyme-bound copper should give competitive inhibition vs ascorbate, since this substrate has been shown to interact directly with the copper atoms of DBH [16]. These uncertainties about the mechanism of DBH do not consent at present time a definitive conclusion about the binding site of captopril.

The most important finding of this study is that captopril inhibits DBH *in vitro* at concentrations similar to those causing angiotensin-independent inhibition of the sympathetic function in experimental animals and isolated vessels [4–7]. Since captopril readily penetrates across biological membranes, as testified by its rapid intestinal absorption [2] and its ability to cross the brain–blood barrier [3, 18], this drug is expected to reach also the interior of the catecholamine storage vesicles, where DBH is localized, and to inhibit this enzyme also *in vivo*. This biochemical effect of captopril may thus constitute a plausible explanation for its angiotensin-unrelated effects on sympathetic function. Consistent with this hypothesis are the observations that, a) inhibitors of DBH cause a marked reduction of noradrenaline release [10], b) enalaprilic acid, which does not inhibit DBH, has no effect on sympathetic nerve activity [5], and c) captopril, but not enalapril, lowers the blood pressure of rats with DOCA (desoxycorticosterone)-salt hypertension, a form of hypertension associated with increased activity of the peripheral sympathetic nervous system and adrenal medulla [19]. DBH inhibition has been shown to result in blood-pressure-lowering effects also in clinical trials [20]. In addition, it has been recently shown that congenital DBH deficiency is accompanied by severe hypotension [21]. The possibility can, therefore, be considered that reduction of DBH activity may contribute to the antihypertensive effect of captopril also in humans. Evidence for decreased plasma levels of noradrenaline in captopril-treated hypertensive patients is controversial, since extremely different dosages have been used (from 37.5 to 600 mg/day; [22, 23]). When high doses have been administered (300–600, mean 510 mg/day) a significant correlation has been observed between fall in blood pressure and reduction of plasma noradrenaline [23]. This suggests a participation of DBH inhibition in the blood-pressure-lowering effect of captopril also in human hypertension at the highest dosages employed.

In summary, the present study has shown that captopril effectively inhibits DBH *in vitro*, an effect that may account for its inhibitory action on sympathetic function and may also contribute to its antihypertensive effect.

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Phenol UDP-glucuronosyltransferase deficiency in Gunn rats: mRNA levels are considerably reduced

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Humans with the hereditary disease Crigler–Najjar syndrome type I suffer severe, non-haemolytic unconjugated hyperbilirubinaemia, usually resulting in infant death, due to a deficiency of UDP-glucuronosyltransferase (UDPGT) [EC 2.4.17] activity towards bilirubin [1]. The congenitally hyperbilirubinaemic Gunn rat has been used as a model for human Crigler–Najjar syndrome type 1 as this rodent strain is also incapable of glucuronidating bilirubin [1]. The genetic lesion has been shown to be due to the absence of the bilirubin UDPGT enzyme protein in this mutant rat [2].

The glucuronidation of planar phenols such as 2-aminophenol, 1-naphthol and 4-nitrophenol has been shown to be impaired in the Gunn rat [2, 3] and also in some human Crigler–Najjar patients [4, 5]. Nagai *et al.* have reported that the defects in bilirubin and phenol glucuronidation in Gunn rats always co-segregate and suggested that these genes may be linked [6]. Therefore, the genetic deficiency of glucuronidation in the Gunn rat has been investigated using a rat phenol UDPGT cDNA as a molecular probe.

Materials and methods

Male Wistar rats and Gunn rats were from the colonies maintained in the Institute's Animal Unit. Congenic Wistar and Gunn rats [7] were obtained from the Catholic University of Leuven, Belgium. A full length rat phenol UDPGT cDNA (RKUG39) was isolated by low stringency cross-hybridisation to a radiolabelled human UDPGT [8] cDNA probe. RKUG39 was found to contain an identical coding region to that of the 4-NP UDPGT cDNA isolated by Iyanagi *et al.* [9] and was used as a hybridisation probe to analyse the phenol glucuronidation deficiency. The 3′ non-coding region of rat testosterone UDPGT cDNA [10] was used as a control probe. The cDNAs were labelled with [α-^{32}P]dCTP as described by Feinberg and Vogelstein [11]. Dot blots of a number of UDPGT cDNA clones were prepared as described by Jackson and Burchell [12] and hybridised with the phenol and testosterone UDPGT probes to demonstrate the specificity of the cDNA probes used. Total RNA was prepared from rat livers and transfer of total RNA from Wistar and Gunn rats to the nylon Hybond-N membrane was as described previously [13]. Slot blotting of total RNA from congenic Gunn rats was performed using a Hybri Slot™ manifold purchased from Bethesda Research Laboratories. Southern blotting of total genomic DNA prepared from Wistar and Gunn rats was achieved as described by Corser *et al.* [13]. Genomic DNA (20 μg) was digested with *Eco* RI or *Hin* dIII before Southern transfer. All blots were washed in 0.1 × SSPE at 68° for 1 hr before autoradiography at −70° using Fuji RX X-ray film. The Fuji RX X-ray film was preflashed prior to autoradiography to allow quantitation by laser densitometry scanning.

Results and discussion

Areas of strong nucleotide sequence identity exist between some UDPGT cDNAs which could cause cross-