INHIBITION OF DOPAMINE-β-HYDROXYLASE BY A METABOLITE OF PYRAZOLE, 4-HYDROXYPYRAZOLE, IN VITRO

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1. Introduction

Pyrazole and its derivatives have been widely used in the studies on alcohol metabolism due to their ability to inhibit alcohol dehydrogenase. It was also observed that pyrazole treatment interfered with catecholamine metabolism in rat and mouse brain [1,2]. The decrease in concentration of brain noradrenaline (NA) was suggested to be caused by the inhibition of dopamine-β-hydroxylase (DβH, EC 1.14.2.1) by pyrazole in vivo. This suggestion was later supported by the studies showing that subacute pyrazole treatment did, indeed, inhibit rat hypothalamic DβH, in vivo, but not in vitro [3]. These results were partly confirmed by a report that single doses of pyrazole decreased [NA] in rat brain [4]. However, chronic treatment with lower doses increased [NA] in rat brain. No change in brain DβH activity was found.

It has been reported that 4-hydroxypyrazole (4-HP) is a major metabolite of pyrazole in the rat [5]. The effect of 4-HP on the activity of DβH was studied [6]. They found that DβH activity was strongly inhibited by 4-HP in vitro though the inhibition was reversible by dialysis. 4-HP inhibited DβH activity in plasma and adrenals also in vivo but DβH activity in brain remained unchanged. Since our preliminary studies with 4-HP gave quite different results in vitro we decided to study this subject further [7]. We present results suggesting that the effect of 4-HP on DβH activity is mediated through the inhibition of stimulatory effect of catalase used in the assays in vitro.

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2. Experimental

DβH activity was determined by the method in [8]. Two different types of enzyme preparations were used in this study:

(1) Bovine adrenals were homogenized in 1% Triton–H_2O, centrifuged at $6 \times 10^6 \times g$ min. DβH activity was precipitated from the supernatant by solid ammonium sulphate, and the precipitate was dialyzed against 50 mM Tris–HCl (pH 7.4). The clear dialysate was fractionated in a Sephacryl S-200 column (equilibrated with the same buffer). The active fractions were concentrated on an Amicon ultrafiltration apparatus. This preparation still needed extra Cu^{2+} in order to give maximal activity.

(2) Fresh bovine adrenal medullae were homogenized in 0.3 M sucrose and chromaffin granules were isolated as in [9]. Chromaffin granules were broken by hypotonic shock and DβH activity was further purified using con A–Sepharose column chromatography. We thus obtained an enzyme preparation which was 95% pure as estimated by polyacrylamide gel electrophoresis. This preparation did not need addition of any exogenous Cu^{2+} when the proper quality of catalase was used.

3. Results and discussion

Preliminary experiments showed that 4-HP did not decrease DβH activity in vitro at 0.7 mM final conc. This seemed to be contradicted by the report [6] that DβH could be inhibited by 4-HP in vitro and that the

inhibition seemed to be competitive with respect to tyramine [6]. Catalase could be inhibited in vitro by 4-HP, with ~3 mM needed for 50% inhibition [10]. We have confirmed those results and have shown that a 50% inhibition can be obtained at ⩽0.1 mM depending on the preincubation time [11]. Based on these results and on the fact that catalase is an essential component in the assay of DβH in vitro we have examined the effect of concentration of 4-HP on DβH activity in vitro in the presence of catalase. The results are presented in table 1. As can be seen there is a clear reciprocal correlation between the [4-HP] and activity of DβH. However, the [catalase] used had to be suboptimal (20% of optimal) to demonstrate the inhibition. This inhibition was similar using either enzyme preparation. Cu^{2+} did not effect the inhibition. Preincubation of enzyme with inhibitor, from 0–60 min, in the absence or in the presence catalase did not have any significant effect on the inhibition by 4-HP. We know that preincubation of catalase with 4-HP for 60 minutes will irreversibly inactivate catalase in the catalase assay [11]. At the much higher [catalase] used in the assay of DβH, the inactivation is not complete after 60 min. Only a small amount of catalase has to be active in order to get maximal DβH activity in vitro [12]. Therefore it is understandable that the preincubation for 60 min did not affect DβH inhibition by 4-HP.

This effect of catalase concentration on the DβH inhibition by 4-HP is clearly seen in table 2. In the absence of catalase there was no inhibition. Instead, there was a small but consistent increase in DβH activity in the presence of 3.7 mM 4-HP. This is in agreement with the report that many nitrogen-containing heterocyclics stimulate DβH [12]. In the presence of catalase, especially in suboptimal concentrations, there is a clear inhibition of DβH activity. This inhibition could be antagonised by increasing the concentrations of catalase. This phenomenon could be repeated using either enzyme preparation. An excess of catalase seemed to inhibit DβH activity even though it still protected DβH activity against the inhibition by 4-HP.

Based on the above results we conclude that the reported inhibition of DβH by 4-HP is due to its ability to abolish the activation caused by catalase in the assay system used in vitro. It has been proposed that catalase in the assay in vitro stimulates DβH in two different ways: (1) it destroys peroxides; and (2) it stabilizes the enzyme by an unknown mechanism (even when denatured) [12,13]. Our results (not shown) suggest that 4-HP inhibits DβH activity in vitro mainly by competing with catalase activity.

Table 1
Effect of 4-hydroxypyrazole on the activity of dopamine-β-hydroxylase in vitro

Addition of 4-HP	Activity	% of control
–	5500	–
0.74 mM	3100	57%
1.50 mM	2250	41%
3.70 mM	1480	26%

Duplicate assays were done as in the text. Catalase was 300 units/assay. The [4-HP] given in the table are the final concentrations in the incubation mixture. The activities are expressed as relative activities

Table 2
Effect of the concentration of catalase on the inhibition of dopamine-β-hydroxylase by 4-hydroxypyrazole in vitro

Amount of catalase[a]	Activity		% of control	% of control[b] *n* (mean ± SEM)
	Control	4-HP		
0	280	300	108	(2) 108; 112
150 U	2670	1040	38	(6) 46.8 ± 6.5
300 U	6460	1460	22	(5) 31.2 ± 4.0
1500 U	8010	5880	67	(10) 70.0 ± 3.7
6000 U	2700	2900	107	(5) 112.0 ± 4.2

[a] Duplicate assays were done as in the text. The amounts of catalase are expressed as units/300 μl final vol. The activities are expressed as relative activities. 4-hydroxypyrazole was 3.7 mM final conc.

[b] Means and SEM are calculated from similar repeated experiments

Hypothalamic regions are regions of high-permeability in the brain [14]. Therefore we can not exclude the possibility that the effects of pyrazole on brain biogenic amine levels and DβH activity in hypothalamus are due to the formation of 4-HP. 4-HP could locally inactivate catalase and thus locally increased levels of peroxides could be a cause for inactivation of DβH. However, we have found that other pyrazole derivatives which are incapable of being metabolised to 4-HP possess similar brain noradrenaline lowering effects as pyrazole thus suggesting another mechanism [2].

The assay of DβH is full of pitfalls, as documented in [15]. Therefore one has to be very careful in interpreting sometimes conflicting results.

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