BBA 61146

The effect of hyperbaric oxygen on dopamine-β-hydroxylase activity

Dopamine-β-hydroxylase is a mixed function oxidase that catalyzes the conversion of 3,4-dihydroxyphenylethylamine to norepinephrine[1].

Fumarate[1] and acetate[2] stimulate the hydroxylation reaction by a mechanism which is still obscure. The hydroxylating enzyme is a Cu-protein, and the Cu^{2+} of the enzyme is reduced by ascorbate or other reducing agents and partially reoxidized during the enzymatic β-hydroxylation[3–6]. In the present study we have shown that hyperbaric O_2 enhances the enzymatic activity and can partially replace fumarate in the stimulation of the enzymatic β-hydroxylation. The results obtained with kinetic studies provide a clue to the mechanism by which O_2 and carboxylic acids stimulate the enzymatic activity.

Dopamine-β-hydroxylase was prepared as previously described[1,3]. The enzyme activity was assayed fluorimetrically[7] or by the periodate method[8]. Incubation under O_2 was carried out in Thunberg tubes and the delivery of gas was regulated by a reducing valve.

The effect of oxygen on the activity of the reduced enzyme intermediate. It is evident from the results presented in Table I that exposure to O_2 tension of the reduced enzyme intermediate in absence of fumarate results in approx. a 2-fold increase in [^{3}H]tyramine β-hydroxylation. In presence of fumarate exposure of the reduced enzyme intermediate to O_2 tension has no effect on enzymatic activity.

The effect of oxygen on the enzymatic β-hydroxylation at different concentrations of fumarate and substrate. The enzymatic dopamine-β-hydroxylation can be increased in absence of fumarate by carrying out the reaction in O_2 rather than in air.

TABLE I

THE EFFECT OF INCREASED OXYGEN TENSION ON THE ACTIVITY OF THE REDUCED ENZYME INTERMEDIATE

The enzyme (0.1 mg protein) was reduced either aerobically with 0.02 μmole ascorbate and 1 min later the excess of ascorbate was removed by addition of ascorbate acid oxidase[5] or anaerobically by addition of 0.02 μmole cysteine and 1 min later *p*-chloromercuribenzoate (0.05 μmole) were added to react with the remaining sulphydryl groups[6]. The incubations were carried out in a mixture that contained the following components: reduced enzyme intermediate (0.1 mg of protein); potassium phosphate buffer, pH 6.4, 100 μmoles; catalase, 500 units (Sigma); uniformly ^{3}H-labeled tyramine, 0.8 mμmole (500 000 counts/min). The additions were made in the order shown in the table and the above time intervals were followed after each addition. After the addition of enzyme and phosphate buffer, O_2 gas was delivered for 3 min prior to the addition of substrate and the incubations were carried out under O_2 for 5 min at room temperature. Fumarate (10 μmoles) was added to the reduced enzyme intermediate where indicated in the table prior to the O_2 exposure.

Order of additions			*[^{3}H]Octopamine formed (mμmoles/mg protein)*
Reduced enzyme	Air	Tyramine	1.20
Reduced enzyme	O_2	Tyramine	2.35
Reduced enzyme + fumarate	Air	Tyramine	3.00
Reduced enzyme + fumarate	O_2	Tyramine	3.10

In presence of high fumarate concentrations (10 μmoles), increased O_2 tension does not stimulate the enzymatic activity. A double reciprocal plot of velocity against substrate concentrations in presence of 10 μmoles of fumarate and in absence of fumarate carried out in incubation mixtures under hyperbaric O_2 or under air is shown in Fig. 1. It is evident that fumarate and O_2 do not change the V but both lower the K_m. Fumarate, as well as O_2, stimulate the enzymatic reaction at low substrate concentrations, but have no effect at high substrate concentrations.

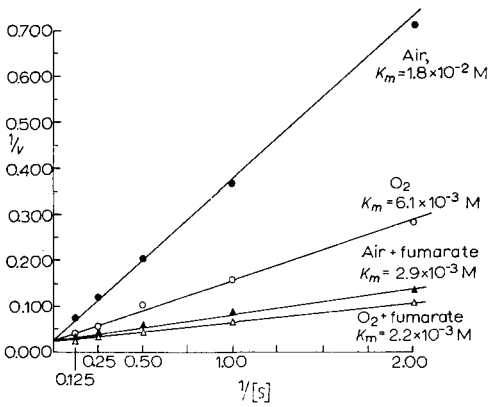
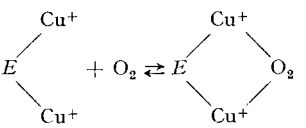


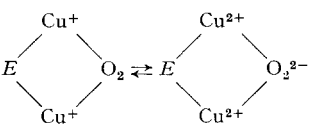
Fig. 1. Double reciprocal plot of velocity against substrate concentration in the presence and absence of fumarate (10 μmoles). Experiments were performed under air or under hyperbaric O_2 in the presence of the following components: phosphate buffer, pH 6.4, 100 μmoles; ascorbic acid, 6 μmoles; catalase, 500 units (Sigma) and enzyme (0.1 mg of protein) in a total volume of 1 ml. The reaction mixture was incubated for 5 min at 37°. Velocity in arbitrary units; dopamine concentration in mM.

The finding that O_2 can replace fumarate for the stimulation of the enzymatic β-hydroxylation suggests that both act at the same active site of the enzyme. The stimulation by O_2 is most likely due to a more rapid formation of the reduced enzyme O_2 complex under O_2 than under air according to the following reaction.



(a)

Some of the effects of O_2 can be explained by a mechanism which has been previously postulated for the stimulation of the enzymatic β-hydroxylation by fumarate[9]. It was suggested that fumarate favors the oxidation of the enzyme Cu^+ to Cu^{2+} according to the following reaction.

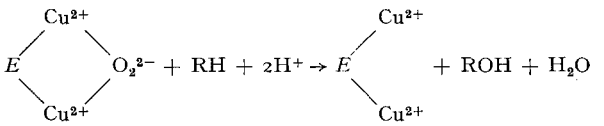


(b)

It is possible that fumarate by displacing the equilibrium of reaction (b) causes also a shift in the equilibrium of the reaction (a) to the right. Such shifts in

the equilibrium of the reactions (a) and (b) to the right will explain why the stimulation of the enzymatic activity by fumarate and O_2 is synergistic.

The finding that fumarate or O_2 does not stimulate the enzymatic β-hydroxylation at high substrate concentration could be explained if it is assumed that the product of the reaction (b) is rapidly utilized in presence of high substrate concentration according to the following reaction.



(c)

(RH = substrate; ROH = product.)

A rapid utilization of the product of reaction (b) in presence of high substrate concentration will shift the equilibrium of reaction (b) to the right even in absence of fumarate or O_2. If the product of the reaction (a) or (b) is a true intermediate in the overall β-hydroxylation reaction then the "apparent" V should increase with increasing O_2 pressure. However, the variation in the V might have been of such small magnitude that it was not possible to determine when the reciprocal velocity was plotted against the reciprocal substrate concentration. When double reciprocal velocities against substrate concentrations were plotted at different concentrations of O_2 a series of straight lines were obtained which intercept on the ordinate at one point. The data were calculated using the kinetic equations for multiple substrates[10,11] and it was found that the variation of the O_2 concentration changed only the slopes of the lines but not the intercepts.

It is aimed in future experiments to determine whether the stimulation of dopamine-β-hydroxylase by O_2 is of physiological importance.

One of us (M.G.) is a Research Career Development Awardee of the U.S. Public Health Service (grant No. 2-K3-MH-14,918-06). This work was supported by the National Institutes of Health (grant No. MH-02717-09) and the National Science Foundation (grant No. GB 5941).

New York University School of Medicine,
Department of Psychiatry and Neurology,
Neurochemistry Laboratory,
New York, N.Y. (U.S.A.)

MENEK GOLDSTEIN
TONG HYUB JOH

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Received August 24th, 1967