The Activation and Inhibition of Phenylamine-β-Hydroxylase

The enzyme phenylamine-β-hydroxylase catalyzes the β-hydroxylation of dopamine[1] and other phenylethylamines and phenylpropylamines[2-4]. Many compounds were tested as possible inhibitors or activators of phenylamine-β-hydroxylase. The degree of inhibition or activation of phenylamine-β-hydroxylase by the tested compounds was determined by a comparison of the amount of norepinephrine formed in an incubation mixture which contained only dopamine as a substrate and an incubation mixture which contained dopamine and the test compound. The amount of formed norepinephrine was determined either by the fluorometric or the periodate method[3]. The results are presented in Table I where the activity was set at 100% in the absence of the test compound.

Serotonin, tryptamine, and histamine are effective inhibitors at a concentration of $10^{-3}M$. The inhibition of phenylamine-β-hydroxylase by such physiological agents may be of significance. Based on spectrophotometric changes in the quinone test[3] it was established that these compounds are not substrates of phenylamine-β-hydroxylase.

Tab. I. Inhibitors and activators of phenylamine-β-hydroxylase

Compound tested	Concentration of the tested compounds	% activity
None	0.00	100
Serotonin	$2\times10^{-3}M$	60
Tryptamine	$2\times10^{-3}M$	70
Histamine	$2\times10^{-3}M$	80
Imipramine[a]	$2\times10^{-3}M$	80
Desmethyl imipramine[a]	$2\times10^{-3}M$	55
Guanethidine	$2\times10^{-3}M$	100
Bretylium	$2\times10^{-3}M$	100
TM 10	$2\times10^{-3}M$	100
Dibenamine[a]	$2\times10^{-3}M$	150
EDTA	$2\times10^{-4}M$	32
Mg^{++}	$2\times10^{-4}M$	105
Mn^{++}	$2\times10^{-4}M$	110
Zn^{++}	$2\times10^{-4}M$	110
Co^{++}	$2\times10^{-4}M$	110

[a] These compounds were pre-incubated with the enzyme for 30 min.

Tab. II. Activation and inhibition of Phenylamine-β-hydroxylase by metal ions after treatment with EDTA[a]

Metal ion[b]	% activation	% inhibition
Ca^{++}	0	
Mg^{++}	0	
Zn^{++}	40	
Mn^{++}	75	
Co^{++}	75	
Cu^{++}		70

[a] The concentration of EDTA was 0.2 μmoles per ml and the enzyme activity was reduced 50%.

[b] The concentration of the tested metal ions was 0.2 μmoles per ml.

Recently it was found that imipramine exerts an antidepressant action through desmethyl imipramine, a metabolite derived from imipramine by removal of one methyl group. From the data in Table I it is evident that desmethyl imipramine is a more effective inhibitor than imipramine which was previously described as an inhibitor of phenylamine-β-hydroxylase[5]. Whether the antidepressant action of these drugs is somewhat related to their inhibitory effects will have to be investigated.

Sympathetic blocking agents such as quanethidine, bretylium and TM 10 have no effect on the phenylamine-β-hydroxylase activity. However, dibenamine stimulates the activity of phenylamine-β-hydroxylase. Dibenamine seems to prevent the destruction of the enzyme which occurs during the pre-incubation period. The protective action of dibenamine may be due to the structural isosterism of dibenamine ethyleniminium ion with adrenergic phenylethyl-β-amines[6].

The inhibition of phenylamine-β-hydroxylase by EDTA suggests as previously reported[5] that a metal is necessary for the enzyme activity. Various metals were tested for their ability to stimulate the activity of the enzyme. A slight stimulation was obtained with Mg^{++}, Mn^{++}, Zn^{++}, and Co^{++} ions. In another experiment the stimulation of enzyme activity by metals was further investigated. To 1 ml of enzyme preparation 0.2 μmoles/ml of EDTA were added and the mixture was pre-incubated for 15 min. A pre-incubation without EDTA served as a control. Both preparations were dialysed 24 h against $0.02M$ phosphate buffer, pH 6.4. After dialysis the enzyme was further incubated in a standard incubation mixture with and without the addition of metals. Table II shows that a stimulation of enzyme activity was obtained by the addition of Zn^{++}, Mn^{++}, and Co^{++} ions. This finding demonstrates that Mn^{++} or Co^{++} or Zn^{++} ions are necessary for the enzyme activity and may provide an indication of the nature of the enzyme. A more purified enzyme fraction is now being prepared in order to establish the specific binding of the metal to the enzyme site[7].

Zusammenfassung. Serotonin, Tryptamin, Histamin, Imipramin und Desmethyl-Imipramin in $10^{-3}M$ Lösung hemmen die Aktivität der Phenylamin-β-hydroxylase. Eine Erhöhung der Aktivität durch Dibenamine wurde beobachtet. Die Hemmung durch EDTA und die Erhöhung der Aktivität durch Mn^{++}, Co^{++} und Zn^{++} macht wahrscheinlich, dass für die enzymatische Aktivität ein Metall nötig ist.

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