Acceleration of the Rate Limiting Step in Norepinephrine Biosynthesis by Potassium

A number of recent experiments have indicated that norepinephrine (NE) biosynthesis is accelerated by increased nerve stimulation in vitro[1-4] or by factors which cause an increase in sympathetic activity in vivo[5-9]. The enzymatic step in the biosynthetic pathway for NE formation which is accelerated appears to be the rate limiting hydroxylation of tyrosine[3,6,8]. The actual mechanism(s) involved in this acceleration of synthesis produced by increased nerve activity remains obscure: It has been suggested that tyrosine hydroxylase activity may be increased as a result of depletion of a small 'strategic' pool of NE within the neuron closely associated with the enzyme (tyrosine hydroxylase) and acting as a feedback inhibitor of this rate limiting enzyme[2,9,10].

Recently, we have found that angiotensin amide II (Ciba) also causes an acceleration of NE biosynthesis in sympathetically innervated tissue at concentrations as low as $10^{-9} M$[11]. Since nerve stimulation and also possibly angiotensin produce an alteration of ion movements within sympathetically innervated tissue[12-14], we decided to investigate the effects of various inorganic ions on NE synthesis from ^{14}C-tyrosine in sympathetically innervated tissue. During this study (BOADLE, HUGHES and ROTH, in preparation) we made the interesting observation that NE biosynthesis in the vas deferens was markedly accelerated when the concentration of potassium ion in the Krebs-Henseleit medium was increased. The following report describes the effect of potassium on catecholamine biosynthesis in the isolated guinea-pig vas deferens preparation.

Methods. Vasa deferentia were removed from 350–400 g guinea-pigs and incubated at 37 °C for various time intervals in normal Krebs-Henseleit medium, and Krebs-Henseleit medium in which a portion of the sodium chloride was replaced by equimolar amounts of potassium chloride thus maintaining the solutions isoosmotic and isotonic. The concentration of ^{14}C-tyrosine (L-tyrosine-^{14}C-uniformly labeled; final specific activity after purification and dilution with unlabeled L-tyrosine = 10 mc/mm, New England Nuclear Corp.) in the medium was $5 \times 10^{-5} M$. After incubation for 1 h the tissues were removed, blotted, frozen on dry ice, weighed, homogenized and the endogenous and newly formed (^{14}C-labeled) catecholamines assayed as described previously[15].

Results and discussion. Increasing the potassium concentration produced a marked acceleration of catecholamine biosynthesis (Table). This increase in synthesis is manifested both by a significant increase in the amount of labeled catecholamine found in the vas deferens and by a marked increase in the specific activity of the NE isolated from this tissue. The maximal stimulatory effect was observed when 40% of the sodium chloride was replaced by potassium. This stimulatory effect appeared to be localized at the tyrosine hydroxylase step since incubation of the vasa deferentia with ^{14}C-dihydroxyphenylalanine (D,L-dihydroxyphenylalanine, specific activity = 3.94 mc/mmole, New England Nuclear Corp.) in the presence of high potassium (40%) produced no observable increase in the formation of labeled NE when compared with controls incubated in normal Krebs. In fact, there was a slight but significant decrease in the amount of labeled catecholamine formed in the presence of high potassium (40%). A ganglionic site of action for potassium in the production of this effect is unlikely since potent ganglionic stimulants such as nicotine (10–100 µg/ml) and McN-A-343 (4-(*m*-chlorophenylcarbamoyloxy)-2-butynyl-trimethylammonium chloride) 10–100 µg/ml) did not accelerate catecholamine biosynthesis in this tissue. In addition, if the vasa differentia were stripped of their ganglia[16] prior to incubation, potassium still produced an acceleration of NE formation from C^{14}-tyrosine. This increase in cate-

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The effect of potassium on the conversion ^{14}C-tyrosine to ^{14}C-norepinephrine in guinea-pig vasa deferentia

% NaCl replacement with KCl	*n*[c]	C^{14}-tyrosine[a] ± SEM	NE[b] synthesis nmoles/g/h ± SEM	% increase synthesis	Specific activity DPM/µg NE ± SEM
0[d]	8	100	1.54 ± 0.18	–	(4)[c] 3538 ± 96
10	4	102 ± 14	1.80 ± 0.15	17	–
20	8	90 ± 7	2.16 ± 0.11	40	(4) 3958 ± 133
30	4	97 ± 10	3.84 ± 0.60	149	–
40	12	95 ± 7	4.17 ± 0.27	171	(4) 7173 ± 180
50	3	97 ± 4	2.86 ± 0.13	86	–
60	4	67 ± 3	2.38 ± 0.16	54	(4) 6552 ± 444
80	4	66 ± 4	1.90 ± 0.16	23	(4) 5782 ± 232
100	4	67 ± 8	1.62 ± 0.27	5	–

[a] C^{14}-tyrosine content/g of vas deferens expressed as % of control value ($1.95 \pm 0.016 \times 10^6$ dpm/g). [b] Identified as > 80% intact norepinephrine by Amberlite chromatography. [c] Number of individual vasa deferentia analyzed. [d] Normal Krebs-Henseleit medium.

cholamine biosynthesis induced by potassium appears to be a more potent effect than that induced by nerve stimulation. Thus, nerve stimulation for 1 h periods produces variable increases in NE formation which seldom exceed 100%[2,4]. On the other hand, the increase induced by potassium (40%) is always greater than 100%. However, preliminary experiments do indicate that like the acceleration of NE biosynthesis induced by nerve stimulation, the acceleration induced by potassium can also be blocked by the presence of NE ($10^{-6}M$) in the medium. We are currently investigating the mechanism of this potassium-induced acceleration of catecholamine biosynthesis.

Zusammenfassung. Kalium erhöht die Bildung von markiertem Noradrenalin aus markiertem Tyrosin im isolierten Vas deferens. Es wird angenommen, dass dieser Effekt auf eine Steigerung der Tyrosin-Hydroxylase zurückzuführen ist.

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Studies on the Biochemical Characterization of Human Ceruloplasmin

The pathological picture of Wilson's disease has been attributed to the abnormal deposition of copper in liver, kidney and brain, along with a decrease in serum ceruloplasmin[1]. That no direct relationship, however, has been observed between serum ceruloplasmin concentration and the severity of the disease[2-4], has led us to a hypothesis for the independent role of copper and ceruloplasmin in the possible pathogenesis of Wilson's disease.

Materials and methods. The ceruloplasmin was obtained from serum by adsorption onto DEAE-Sephadex and by ammonium sulfate precipitation. $^1/_{10}$% ceruloplasmin in sodium acetate buffer (0.1 *M*, pH 5.5) was used for the analytical ultracentrifuge at 59,780 rpm. Disc gel electrophoresis was performed at pH 8.9 for 45 min to 1 h at 4 mamp/sample with a 7.5% cross-linked polyacrylamide gel.

Oxidase activity was detected essentially by the method of Owen and Smith[5]. Subunits were determined after treatment of the protein in 9 *M* urea with 2-mercaptoethanol and iodoacetamide[6] followed by chromatography on DEAE-Sephadex. Peptide maps were prepared of tryptic digests by electrophoresis (70 V/cm; pyridine acetate, pH 5.5; 45 min) in one dimension and chromatography (butanol:acetic acid:water – 200:30:75; 16–18 h) at right-angles. Mitochondrial respiration in the presence of ceruloplasmin was measured with a YSI Oxygen Monitor (Yellow Springs Instrument Co., Ohio) using a rat-liver preparation as noted in the footnote to the Table.

Results and discussion. Preliminary chromatographic separation of the subunit reaction mixture of bovine ceruloplasmin revealed a single major peak while that of the human protein showed 2 principal peaks. In disc gel electrophoresis as well as with the cellulose acetate procedure, normal ceruloplasmin migrates further towards the anode than does the Wilson protein. The basic nature of

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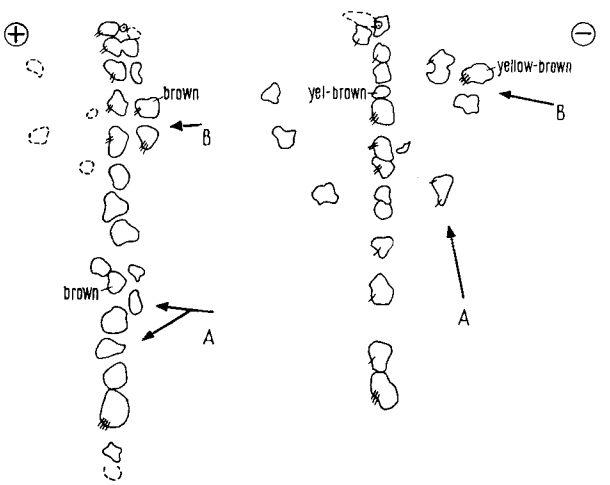
Effect of ceruloplasmin on mitochondrial oxygen consumption

Incubation medium[a]	O_2 consumption, µl/h/mg protein	Inhibition (%)
No Cp	190	–
200γ Bovine Cp	170	10.5
200γ Normal Cp	168	11.6
200γ Wilson's Cp	27	85.8
80γ Wilson's Cp	27	85.8
60γ Wilson's Cp	67	64.7
40γ Wilson's Cp	80	57.9
20γ Wilson's Cp	150	21.1
0.2 m*M* Cu^{+1}	190	0
0.2 m*M* Cu^{+2}	57	70.0

[a] Mitochondria were prepared from rat liver and suspended in buffer so that 0.2 ml of preparation had 0.672 mg protein. The incubation medium in addition contained a final concentration of 40 m*M* K_2HPO_4, 40 m*M* KCl, 24 m*M* ADP and 20 m*M* succinate. Incubation was for 15 min at 30 °C and pH 7.4.



Peptide maps of a tryptic digest of normal human ceruloplasmin (left) and Wilson's ceruloplasmin (right). The conditions are given in the text. The arrows at positions A and B indicate the major peptide changes between the two.