Enzymatic Formation of Adrenaline and Other Catechols from Monophenols

Abstract. *An enzyme that forms adrenaline from* p- *and* m-*sympathol and dopamine from* p- *and* m-*tyramine is localized in the microsomes of rabbit liver and requires the reduced form of nicotinamide adenine dinucleotide phosphate. The enzyme is nonspecific and can form catechols from the following normally occurring and foreign phenols:* p- *and* m-*octopamine,* p-*hydroxyephedrine, phenol, stilbestrol,* N-*acetyl*-p-*aminophenol, estradiol and* N-*acetylserotonin.*

The main pathway for the formation of catecholamine hormones is

tyrosine → dopa → dopamine → noradrenaline → adrenaline.

Most of the enzymes involved in the biosynthesis of these catecholamines have been described, but little is known about the hydroxylation of tyrosine to dopa. Recently monophenolic amines, such as *p*- and *m*-tyramine, octopamine (*p*-hydroxyphenylethanolamine), and *p*-sympathol (synephrine), have been found to be excreted normally in the urine (*1*). The administration of C^{14}-tyramine and C^{14}-octopamine resulted in the excretion of trace amounts of noradrenaline and larger amounts of normetanephrine and metanephrine (*2*). Thus there may be many alternate pathways for the formation of catecholamines, and monophenolic amines may serve as precursors for catecholamines. We now describe an enzyme system that can convert *p*-sympathol to adrenaline and tyramine to dopamine. The enzyme also hydroxylates a number of normally occurring and foreign monophenols to catecholamines and catechols.

Microsomes and the soluble supernatant fraction from rabbit liver were prepared in isotonic postassium chloride (*3*) and incubated with *p*-sympathol and nicotinamide adenine dinucleotide phosphate (NADP). After 1 hour, the incubation mixture was extracted with *n*-butanol and assayed fluorometrically for adrenaline (*4*). The enzyme preparation formed a compound that had the same activation and fluorescence spectra as adrenaline after oxidation with I_2. When NADP or the soluble supernatant fraction was omitted, the formation of adrenaline was markedly diminished (Table 1). The requirement for NADP and the soluble supernatant fraction suggested that the adrenaline-forming enzyme is in the microsomes; the soluble supernatant fraction serves to reduce the NADP (*3*). *p*-Sympathol was then incubated with the rabbit liver microsomes and the reduced form of NADP ($NADPH_2$), and this also resulted in the formation of adrenaline. When *m*-sympathol was used as a substrate, there was an even greater formation of adrenaline (Table 1).

Further evidence for the identity of adrenaline synthesized enzymatically from *p*- and *m*-sympathol was obtained by forming an *O*-methylated derivative, metanephrine. Adrenaline can be converted to radioactive metanephrine with catechol-*O*-methyl transferase (an enzyme present in the soluble supernatant fraction) and *S*-adenosylmethionine-$C^{14}H_3$ (*5*). It has been shown that this enzyme *O*-methylates catechols but not monophenols. Thus, if the sympathols are incubated with the catecholamine-forming enzyme in the microsomes and catechol-*O*-methyl transferase in the soluble supernatant fraction and *S*-adenosylmethionine-$C^{14}H_3$, C^{14}-methoxymetanephrine should be generated as adrenaline is formed from sympathol. The sympathols were incubated together with the hydroxylating and *O*-methylating enzyme. After 1 hour of incubation, 0.5 ml of 0.5*M* borate buffer (at *p*H 10) was added to the incubation mixture and the C^{14}-methoxymetanephrine was extracted into 6 ml of a mixture of toluene and isoamyl alcohol (3:2). A 4-ml sample of the extract was transferred to a vial containing 1 ml of ethanol and 10 ml of phosphor (*6*), and the radioactivity was measured in a scintillation spectrometer. The isolated C^{14}-methoxymetanephrine was subjected to paper chromatography with solvent systems B and C previously described (*7*). After chromatography, there was a single radioactive peak in both solvent systems that had the same R_F values as authentic metanephrine.

The ability of the microsomal enzyme system to form dopamine from *p*- and *m*-tyramine was examined in the same way (Table 1). After incuba-

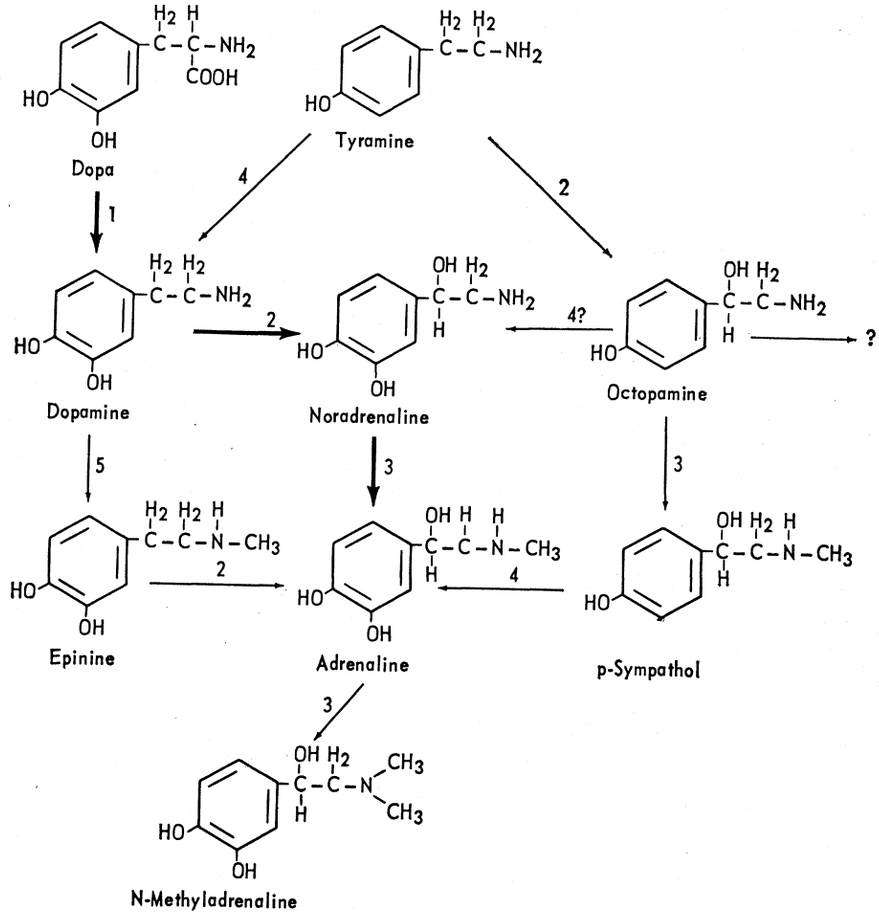

Fig. 1. Pathways in the formation of catecholamines. Heavy arrows show the main routes. *1*, Dopa decarboxylase. *2*, Dopamine-β-oxidase. *3*, Phenylethanolamine-*N*-methyl transferase. *4*, Catechol-forming enzyme. *5*, Nonspecific *N*-methyl transferase.

Table 1. Enzymatic formation of catecholamines from monophenolic amines. Dialyzed microsomes and soluble supernatant fraction obtained from 30 mg of rabbit liver were incubated in air at 37°C with 100 μl of 0.5*M* phosphate buffer at *p*H 7.4, 25 μl of 0.5*M* $MgCl_2$, 0.5 μmole of NADP, 1.0 μmole of glucose-6-phosphate, and 0.5 μmole of substrate in a final volume of 250 μl. After 1 hour of incubation, the reaction mixture was assayed fluorometrically for catecholamines. These results are typical of five similar experiments.

System condition	Substrate	Catecholamine formed per gram of liver (mμmole)
Complete	*p*-Sympathol	158
NADP omitted	*p*-Sympathol	46
Microsomes + NADP	*p*-Sympathol	90
Microsomes + $NADPH_2$	*p*-Sympathol	230
Complete	*m*-Sympathol	730
Complete	*p*-Tyramine	500
Complete	*m*-Tyramine	200
Complete	*p*-Octopamine	37
Complete	*m*-Octopamine	30

tion with tyramine, the mixture was extracted with *n*-butanol and assayed for dopamine (*8*). Both tyramines formed a compound that had the same activation and fluorescence spectra as authentic dopamine after oxidation. The tyramines were incubated with the microsomes, soluble fraction, and *S*-adenosylmethionine-$C^{14}H_3$; the apparent C^{14}-methoxytyramine formed was isolated in the same manner as metanephrine had been isolated. Considerable quantities of radioactive methoxytyramine were formed from *p*- and *m*-tyramine which had the same R_F values as synthetic methoxytyramine when chromatographed as described above.

When *p*-octopamine was incubated with the catechol-forming enzyme, a small quantity of material resembling noradrenaline was formed (Table 1). When *p*-octopamine was incubated with the microsomal enzyme system, soluble supernatant fraction, and radioactive *S*-adenosylmethionine, a relatively large amount of a C^{14}-methoxy derivative was formed. The latter compound, however, did not have the same R_F values as expected for synthetic normetanephrine in the two solvent systems. In all probability *p*-octopamine or a transformation product formed a catechol; otherwise, a radioactive *O*-methylated derivative would not have been formed. The unknown *O*-methylated product had the solubility characteristics of a phenolic amine.

When *m*-octopamine was incubated with the microsomal enzyme, a small amount of noradrenaline was formed (Table 1). Incubation with catechol-*O*-methyl transferase and *S*-adenosylmethionine-$C^{14}H_3$ resulted in the generation of a radioactive compound having the same R_F values as synthetic normetanephrine.

The enzymes involved in the biosynthesis of catecholamines are relatively nonspecific. Dopa decarboxylase can decarboxylate tyramine as well as other amino acids (*9*); dopamine-β-oxidase not only oxidizes dopamine to noradrenaline but can form octopamine from tyramine and adrenaline from epinine (*10*); phenylethanolamine-*N*-methyl transferase *N*-methylates octopamine, noradrenaline, and a wide variety of phenylethanolamine derivatives (*11*); a nonspecific *N*-methyl transferase can *N*-methylate dopamine to epinine (*12*) and the enzyme system described here can transform a number of monophenolic amines to catecholamines. Hence the alternate pathways shown in Fig. 1 are suggested for the formation of catecholamines.

The ability of an enzyme in the rabbit liver microsomes to hydroxylate other phenolic compounds was examined by incubating the phenol with microsomes (Table 1). The catechol formed was trapped as a radioactive *O*-methylated derivative by incubating the microsomal preparation, *S*-adenosylmethionine-$C^{14}H_3$, and the soluble supernatant fraction of rabbit liver which contained catechol-*O*-methyl transferase. The radioactive metabolites were extracted and measured as described above. The following phenolic compounds formed catechols as trapped *O*-methylated derivatives: *p*-hydroxyephedrine, *N*-acetyl-*p*-aminophenol, estradiol, stilbestrol, and *N*-acetylserotonin. The relative nonspecificity of this reaction suggests that more than a single enzyme is involved. The enzymatic formation of a dihydroxytryptamine is of particular interest since it has been suggested that such compounds regulate the heart beat of crustaceans (*13*).

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References and Notes

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Menstrual Cycle Influences Grooming Behavior and Sexual Activity in the Rhesus Monkey

Abstract. *The time spent by the female rhesus monkey in grooming the male fluctuates rhythmically and reaches a minimum at mid-cycle. At this time the male's grooming activity reaches a maximum. The rhythmic changes in male mounting behavior, together with the males' and females' grooming cycles, are abolished by ovariectomy and have a hormonal basis.*

Although it can be argued that any behavioral interchange between oppositely sexed members of the same species may possess a sexual component, nevertheless, that part of the behavioral interaction not directly concerned with copulation can also be regarded as part of the general social behavior of the species. Heterosexual grooming (picking through a partner's fur) in many monkeys, including the rhesus (*Macaca mulatta*), is without doubt an integral part of the total pattern of sexual behavior, but this same grooming activity in a different behavioral setting also serves to define an individual's position within the highly organized primate society (*1*). Thus, grooming behavior occupies a position intermediate between a specifically sexual and a more generally social type of activity. In lower mammals, the female's sexual behavior depends upon the secretory activity of the ovaries, but this dependence is less clear-cut in female primates, some of which accept males throughout their cycle (*2*). The role of ovarian hormones as a determining factor in nonsexual forms of behavioral interaction in primates has received little attention (*3, 4*).

To study this problem, mature, intact male and female rhesus monkeys (7 to 14 kg) were studied in glass-