CATECHOLAMINE METABOLISM IN PATIENTS WITH PHEOCHROMOCYTOMA

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Studies in experimental animals have led to numerous advances in the field of catecholamine metabolism. It is important that such studies be confirmed and extended in man. Human experimentation in this area is difficult to perform because of the small amounts of norepinephrine and epinephrine normally found in the blood and urine and the fact that fresh human tissue is not easily obtained for chemical study. Patients with pheochromocytoma, however, are ideal subjects for investigation of both the biosynthesis and fate of these amines. The tumors release amounts of these substances into the blood sufficient to produce higher concentrations than have ever been achieved with infusions in normal subjects. Furthermore, on surgical removal the tumors provide a fresh and rich source of catecholamine-synthesizing tissue for use in analytical and enzymatic studies. Since the catecholamines are largely metabolized in the body, urine from these patients constitutes an excellent material in which to search for various metabolites.

In the past, attention has been directed chiefly to the diagnostic and therapeutic aspects of this disorder. This report will review only findings in pheochromocytoma which relate to the biochemistry of the catecholamines. It is not surprising that some of these findings in turn have clinical implications.

Biosynthesis

Studies on animal adrenal glands have led to the following concept of the biogenesis of the catecholamines: phenylalanine → tyrosine → 3,4-dihydroxyphenylalanine (dopa) → 3,4-dihydroxyphenylethylamine (dopamine) → norepinephrine → epinephrine. Aside from dopa decarboxylase, the cellular catalysts in the biosynthetic pathway are little known. Studies on pheochromocytoma have led to confirmation of some of these reactions in man. Also, it has been possible to estimate the rate of norepinephrine synthesis by the tumors in precursor experiments using radioactive dopamine.

1. *Catecholamines in blood, urine and tissue.* In patients showing clinical signs, elevated levels of norepinephrine and often epinephrine have been demonstrated by numerous investigators. The normal level of epinephrine plus norepinephrine in plasma is less than 6 μg/l regardless of the method of estimation used, whereas values in the range of 10 to 100 μg/l are found in patients with these chromaffin tumors. It has recently been found by von Euler *et al.* (19) and confirmed by J. R. Crout in our laboratory that selective venous catheterization with blood analysis for catecholamines is of value in localizing the site at which a tumor is discharging catecholamines into the venous system. The urinary excretion of norepinephrine plus epinephrine is usually less than 100 μg/day whereas in pheo-

chromocytoma it is typically in the range of 300 to 3000 μg/day. Most of the tumors contain 500 to 10,000 μg/g of total catecholamines with the ratio of norepinephrine to epinephrine being similar to that found in the urine.

Dopamine is a normal constituent of human urine, its excretion being on the order of 100 to 200 μg/day, some 5 times that of norepinephrine. Although dopamine has recently been demonstrated in a large number of organs and tissues, attempts to show its presence in chromaffin tissues have not been uniformly successful. Shepherd and West (13, 21) studied the adrenal glands of several animal species (including man), 12 human pheochromocytomas and the organs of Zuckerkandl of infants. They were able to confirm Goodall's finding (6) of dopamine in sheep adrenal gland but otherwise found the amine only in the glands of cattle. Apparently dopa has not been found in any tissue except adrenal gland of thyroidectomized sheep (6). Von Euler in 1951 found evidence for an increased urinary excretion of dopamine (approximately 2,000 μg/day) in 1 case of pheochromocytoma. Manger *et al.* (11) reported the presence of dopamine in 1 of 35 tumors analyzed chromatographically. In simultaneous communications in 1956, Weil-Malherbe (20) and McMillan (12) reported the finding of dopamine in each of 3 tumors. The first author examined 2 tumors and found respectively, 494 and 49.8 μg of dopamine/g, 770 and 764 μg of norepinephrine/g and 124 and 107 μg of epinephrine/g. The tumor studied by McMillan was thought to be malignant and contained a very large amount of dopamine (1970 μg/g) with some norepinephrine (150 μg/g). Urinary assays in the first and third cases revealed norepinephrine to be the predominant catecholamine in the urine although moderately elevated amounts of dopamine were also found. Of great interest is the fact that Weil-Malherbe also reported the presence of considerable dopa (147 μg/g) in the first tumor.

The mere findings of dopa and dopamine in some tumors provide connecting links in man to support the concept that these compounds are intermediates in the formation of norepinephrine and epinephrine by chromaffin tissue. Presumably, enzyme relationships in the tumors differed sufficiently from the normal to produce these findings. The interesting possibility exists that some tumors produce only dopamine because of an inability to hydroxylate it to norepinephrine. Since dopamine is a relatively weak pressor agent, such a tumor could erroneously be termed "nonfunctioning."

2. *Studies on biosynthesis in vitro.* Studies with C^{14}-labeled dopa and dopamine have been carried out in fortified incubation mixtures of slices or homogenates of 4 different tumors. Sjoerdsma *et al.* (15) studied 3 tumors and found considerable radioactivity in norepinephrine isolated from the mixture after incubations of 1 1/2 to 3 hours with DL-dopa-3-C^{14} and dopamine-1-C^{14}. Gélinas *et al.* (5) performed similar studies on a tumor homogenate with DL-dopa-2-C^{14}. After 2 hours, 52% of the radioactivity remained in the starting material, 39% of the activity was found in chromatographic zones corresponding to dopamine and smaller amounts were detected in peaks corresponding to norepinephrine and 3,4-dihydroxyphenylacetic acid. A high activity of the enzyme, dopa decarboxylase, has been found in these tumors by Langemann *et al.* (10) and in this laboratory (15).

3. *Studies on biosynthesis in vivo and estimation of norepinephrine turnover.* Additional proof of the precursor relationship of dopa and dopamine to norepinephrine in pheochromocytoma has been obtained by studies on 4 patients (15). Following the intravenous administration of 5 μc of dopa-C^{14} in 1 patient, a significant amount of radioactivity was found in norepinephrine isolated from the urine but quantitative studies were not carried out. After the injection of 5 μc dopamine-C^{14} in 3 patients with norepinephrine-producing tumors, a significant amount of C^{14} was found in norepinephrine isolated from the urine, about 0.5% of the administered counts. The biologic half-life of tumor norepinephrine was estimated from the rate of decline in the specific activity of urinary norepinephrine and found to be on the order of 8 to 12 hours. This relatively rapid turnover rate is in contrast to the slow turnover of catecholamines in animal (and presumably human) adrenal gland which has been shown to be on the order of several days (16). It was felt that in the 3 patients studied the amount of norepinephrine excreted in the urine was consistent with turnover of the entire tumor pool of norepinephrine at the calculated rate. On the other hand, review of tumor assays done in this laboratory and those reported by von Euler and Ström (19) indicates that such a rapid turnover rate could not have existed in several patients with large tumors which contained considerable epinephrine as well as norepinephrine. It is postulated that these larger tumors (generally > 100 g) originate from the adrenal gland and retain its properties of storage, slower turnover and ability to produce epinephrine. This may account for their growth to a larger size before clinical manifestations are sufficient to result in surgical removal. It is desirable that studies of turnover be done in cases where large quantities of both amines are excreted in the urine.

The irreversibility of the conversion of norepinephrine to epinephrine was confirmed by a study in one patient (15) showing that no significant C^{14} could be recovered in urinary norepinephrine after the infusion of *dl*-epinephrine-2-C^{14}.

Metabolism

Little was known of the fate of norepinephrine and epinephrine in the body until recently. Only 1 to 4% of these amines given intravenously has been shown to be excreted in the urine in normal (18) or adrenalectomized (3) human subjects. Due chiefly to the studies of Armstrong, McMillan and Shaw (1) and of Axelrod (2), it is now known that norepinephrine and epinephrine are metabolized by a combination of O-methylation and oxidative deamination to yield the following major metabolites: 3-methoxy-4-hydroxymandelic acid (VMA), the 3-methoxy analogues of the amines (normetanephrine and metanephrine) and 3,4-dihydroxymandelic acid. More recent studies by LaBrosse *et al.* (8) and Kirshner *et al.* (7) indicate that, following the intravenous administration of *dl*-H^3-epinephrine and *dl*-epinephrine-2-C^{14} to human subjects, approximately 50% of the radioactivity appears in the urine as metanephrine (free and conjugated) and 35% as VMA. Similar studies on the fate of labeled norepinephrine have not been reported as yet.

1. *O-Methyl metabolites in the tumors.* These tumors afforded a prompt answer

to the question of whether the O-methyl amines are present in human tissues. Normetanephrine was found in each of four pheochromocytomas (14). One of these tumors contained epinephrine as well as norepinephrine and in this instance two other phenolic amines were found, one of which was tentatively identified as the 3-methoxy analogue of dopamine and the other was not identified. The normetanephrine content of the same tumor was estimated to be about 25 μg/g. It is not known to what extent these tumors secrete the methoxy-amines directly into the blood though they are relatively inactive physiologically (4, 8) in comparison with the parent amines.

2. *Studies on metabolism in vitro.* Two of the enzymes involved in catecholamine metabolism, catechol-O-methyl transferase and amine oxidase, have been demonstrated in the tumors (10, 15). The O-methylation reaction could be demonstrated only when the tissue was presented with the activated form of methionine, S-adenosylmethionine. It is not known whether the tumor can synthesize S-adenosylmethionine *in vivo*.

3. *Studies on metabolism in vivo.* The original report by Armstrong *et al.* (1) included studies on normal adults and 3 patients with pheochromocytoma. The normal level of VMA excretion was about 1.5 to 3.0 mg/day. Values for 3 patients with pheochromocytoma were 90, 23 and 12 mg/day preoperatively and 2.7, 4.4 and 1.5 mg/day postoperatively. The findings of LaBrosse *et al.* (9) leave no doubt that patients with pheochromocytoma also excrete large amounts of normetanephrine. Two patients were reported to be excreting about 20 mg of VMA per day but also 10 to 15 mg of normetanephrine (free plus glucuronide) at a time when the excretion of norepinephrine was about 2 mg/day. Additional studies have shown that a large proportion of the urinary normetanephrine is conjugated not as a glucuronide, but in an acid-hydrolyzable form. Also metanephrine has been shown chromatographically in the urine of a patient whose tumor produced epinephrine as well as norepinephrine. Apparently, attempts to identify and measure the methoxy-amines in normal human urine have thus far been unsuccessful.

It is important that specific and sensitive methods be developed for measuring the methoxy metabolites since they are undoubtedly excreted in much larger amounts than the parent amines. Variations in the production of catecholamines under normal and pathologic conditions could thereby be determined more accurately and the chemical diagnosis of pheochromocytoma be made a simpler routine procedure.

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