POSSIBLE MECHANISMS FOR THE TERMINATION OF THE PHYSIOLOGICAL ACTIONS OF CATECHOLAMINES

GEORGE B. KOELLE

Department of Physiology and Pharmacology, Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Most of the present session of the Symposium is devoted to a review of current knowledge concerning the various pathways by which the catecholamines can be metabolized. Prior to the discussion of the individual enzyme systems, it seems appropriate to consider the general problem of the extent to which the metabolic conversion of the catecholamines is responsible for the termination of their physiological or pharmacological actions. This aspect is of importance in elucidating the mechanisms of action of the catecholamines themselves, and of the drugs which simulate, potentiate, or antagonize their effects. The present discussion will be devoted primarily to the catecholamines of endogenous origin, *i.e.*, norepinephrine, epinephrine, dopamine (hydroxytyramine) and possibly isoproterenol (29).

Types of action of catecholamines. The diverse actions of the catecholamines may be placed arbitrarily in four categories: 1) Excitatory actions on autonomic effector cells; 2) Inhibitory actions on autonomic effector cells; 3) Metabolic actions; 4) Actions on the central nervous system. From the now classical studies and calculations of Clark (16), we are accustomed to thinking of the first category, the excitatory sympathomimetic effects, as resulting from the combination of the catecholamine with a receptor site at the cell surface. This concept is strengthened by recent evidence suggesting that these compounds are active only or primarily in the charged, cationic form; as such, they would be expected to penetrate cell membranes slowly (28). The inhibitory sympathomimetic effects likewise have generally been assumed to result from an action at the cell surface. However, the studies of Mohme-Lundholm (30) have provided the hypothesis that the inhibitory actions on smooth muscle are the result of the catecholamine-induced accumulation of lactic acid. If this is actually the case, such effects would be secondary to glycogenolysis, one of the important metabolic actions in the third category. Our knowledge of the mechanism of the glycogenolytic action of epinephrine has been advanced considerably through the recent work of Sutherland and associates (32), which is discussed elsewhere in the Symposium. Both epinephrine and glucagon have been shown to accelerate the phosphorylation, or activation, of glycogen phosphorylase, which apparently constitutes the rate-limiting step in the conversion of glycogen of the liver and muscle to glucose and lactic acid, respectively. This and the other metabolic actions of the catecholamines have been reviewed recently by Ellis (19). From the standpoint of the present discussion, the most important factor to be noted is that these actions may result from effects on enzymes at intracellular sites. Little is known concerning the mechanism of the central actions of the catecholamines. Likewise, there is only indirect evidence for the existence of adrenergic

pathways, other than vasoconstrictor, in the central nervous system (12, 35). In general, the duration of anxiety, nervousness and other central symptoms following the administration of epinephrine and related drugs outlasts that of the peripheral cardiovascular actions. The basis for the central effects may fall within any combination of the other three categories: excitatory, inhibitory, or metabolic.

From the foregoing discussion, it seems likely that the catecholamines act at both extracellular and intracellular sites. The primary mechanisms for termination of effects at these contrasting levels are probably quite different.

Sites of origin or sources of catecholamines. A factor of considerable importance in relation to the problem at hand is the source of the catecholamine which produces a given effect, *i.e.*, whether it arrives at the effector site from a closely adjacent adrenergic nerve or from a more remote source via the circulation. In the early theory of Cannon and Rosenblueth (14), the sympatho-adrenal system was considered to act as a unit, particularly in situations of emergency. More recent studies have required modification of this concept. While the adrenal medulla, with its predominant secretion of epinephrine, probably functions much in the manner that Cannon and Rosenblueth described, the main transmitter of the sympathetic or adrenergic nervous system is now known to be norepinephrine (20). Just as does the parasympathetic system, the sympathetic appears to function continually in a selective, discreet manner to bring about the moment-to-moment adjustments necessary for homeostasis. At the rates of firing encountered physiologically, the effects of adrenergic nerve impulses are probably confined fairly closely to the effector cells in the immediate vicinity of their endings (15). The manner by which such effects are terminated may be quite different from the mechanisms which bring about termination of the actions of circulating catecholamines, whether from the adrenal medulla, other chromaffin tissue, or exogenous sources. Consequently, studies of the disposition of catecholamines from the circulation by metabolic or other means can only suggest possible processes involved in the termination of the effects of adrenergic transmission.

Termination of the actions of catecholamines by processes other than metabolic degradation. In a recent general discussion of the mechanisms available for the termination of actions of drugs, Dr. Thomas C. Butler (13) listed the following mechanisms which do not involve metabolic degradation:

1) Tolerance, tachyphylaxis and related phenomena.
2) Redistribution.
3) Drug antagonisms.
4) Antagonistic reflexes.
5) Excretion.

Although tachyphylaxis, or "acute tolerance," plays an important role in limiting the effects of certain sympathomimetic amines of botanical or synthetic origin (*e.g.*, ephedrine, amphetamine), it does not occur ordinarily with their mammalian catecholamine counterparts. Accordingly it will not be considered here, other than to mention that the mechanisms involved in the production of tolerance are at best poorly understood (34).

Redistribution, on the other hand, may be of great importance in limiting the actions of endogenous catecholamines. In a study of the potentiating effects of some amidines and guanidines on the cardiovascular actions of epinephrine, Dawes (17) concluded that these compounds probably interfere with the passage of the catecholamine into the hepatic cells, and that the latter step is the primary means by which the action of circulating epinephrine is terminated. It has been pointed out by Blaschko (8) that this type of effect, blockade of access of the catecholamine to an intracellular enzyme, could simulate inhibition of the enzyme. In the same general category, redistribution, might be considered the possibilities of storage within fat depots and adsorption to plasma proteins, mechanisms which probably contribute to the termination of the actions of thiopental (11) and neuromuscular blocking agents (10), respectively. Although there is no evidence that the former type of disposition occurs with catecholamines, both epinephrine and norepinephrine are adsorbed to the plasma albumin (2).

The antagonistic actions of the several classes of adrenergic blocking agents to the actions of the catecholamines are well known (31). All such drugs produce adrenolysis (blockade against the actions of circulating sympathomimetic amines) in lower doses than required for sympatholysis (blockade of the effects of adrenergic nerve impulses). This may be dependent partially on a difference in the mechanisms normally involved in the termination of such effects, as discussed above.

The importance of antagonistic reflexes in modifying the actions of catecholamines, particularly on the cardiovascular system, cannot be overemphasized. Several such reflexes have been discovered, or rediscovered, in recent years, and have been reviewed in detail (4, 18). It is quite likely that they play a role also in the production of certain apparently inhibitory effects of the catecholamines on the peripheral circulation (23).

Only very small fractions of circulating norepinephrine and epinephrine are excreted unchanged in the urine (21).

Metabolic degradation. The high concentrations of acetylcholinesterase (AChE) found at cholinergic synaptic and neuroeffector sites, and the extremely high velocity of its hydrolysis of acetylcholine (ACh) indicate that this enzyme plays an important role in limiting the extent and duration of the actions of the cholinergic transmitter. Although many mammalian enzymes can inactivate the catecholamines, none has been demonstrated to do so at a rate approaching that of the ACh-AChE system, and none has been found to be associated selectively with the adrenergic nervous system in all species. Blaschko (7) has raised the question whether the temporal and spatial limitations of adrenergic neuroeffector transmission are such as to require the participation of an enzyme equivalent to AChE in the cholinergic system. It is quite possible that the various mechanisms discussed in the preceding section are sufficient for termination of many of the immediate effects of the catecholamines, and that their metabolism constitutes in certain respects a function related only indirectly. Since the individual enzyme systems will be discussed in detail by the other speakers, they will be considered here only briefly.

Norepinephrine, epinephrine and closely related catecholamines potentially can undergo the following enzymatic conversions:

1) Deamination by monoamine oxidase (MAO).

2) Oxidation to adrenochrome and related products by cytochrome oxidase and tyrosine (DOPA) oxidase.

3) Conjugation to the glucuronide or sulfate.

4) O-methylation by catechol-O-methyl transferase.

Monoamine oxidase (MAO) has been reviewed critically by Blaschko (7). Of the catecholamines under consideration, only dopamine, which lacks the β-OH group, is oxidized by it at a reasonably rapid velocity (9). In the rabbit, MAO is present in higher concentrations in adrenergic neurons than in cholinergic or sensory neurons, whereas in the cat there appears to be no such distinction (26). However, in all species the largest amounts of MAO are found in the liver, kidney and gastrointestinal tract. Phenethylamine derivatives which possess an α-methyl group in the alkyl side-chain (*e.g.*, ephedrine, amphetamine) are not oxidized by MAO, and can act as competitive inhibitors to the oxidation of other amines by the enzyme. Gaddum and Kwiatkowski (22) suggested that this effect might be responsible for some of the pharmacological actions of ephedrine and related drugs. Although the importance of MAO in the oxidation of catecholamines other than dopamine at most sites in the body is controversial (see below), its presence in the gastrointestinal tract and liver may be largely responsible for the failure of epinephrine and norepinephrine to act effectively after oral administration.

The possibility that the catecholamines are oxidized to indole derivatives has been discussed by Bacq (6). Although cytochrome oxidase is present in practically all cells in the body, there is apparently no evidence that it participates in the oxidation of epinephrine to adrenochrome and related compounds *in vivo*. It has been suggested that the DOPA-(tyrosine) oxidase of the uveal tract may promote the oxidation of catecholamines liberated by the adrenergic fibers to the iris, and that this might be the basis for the greater effectiveness of mydriatics in light than in dark eyes (1).

There is ample evidence that both the free catecholamines and their immediate metabolites from other pathways are conjugated in considerable proportions, probably exclusively in the liver (33). The previous speakers have emphasized the importance of accounting for the conjugated products in determinations of the urinary output of these compounds.

The most recent evidence suggests that catechol-O-methyl transferase is the enzyme of major importance in the metabolism of circulating norepinephrine and epinephrine (3, 5, 27). Over 90% of a 1-mg dose of *dl*-epinephrine given to normal human subjects by intravenous infusion was recovered as the free or conjugated form of the primary product, metanephrine (*m*-O-methyl-epinephrine) or its subsequent oxidation product, 3-methoxy-4-hydroxymandelic acid (27). The authors suggested that MAO participates chiefly in the oxidation of metanephrine to the mandelic acid derivative.

The foregoing discussion does not indicate the relative importance of metabo-

lism and the factors discussed above in limiting or terminating the effects of adrenergic transmission at various sites. As an illustration of the complexities involved in this problem, an example from an earlier discussion (25) may be cited. Both iproniazid and isoniazid were found in high doses to potentiate the response of the cat nictitating membrane to stimulation of the sympathetic trunk or the injection of norepinephrine or epinephrine (24). Iproniazid, but not isoniazid, caused marked inhibition of MAO; neither compound inhibited effectively cytochrome oxidase or autoxidation. Both caused some degree of adrenergic blockade. To explain these findings it was proposed that drugs such as the hydrazides, as well as the catecholamines, might react at four or more possible common sites. Combination of the drugs at these sites could prevent access by the catecholamines, with the following results:

1) Receptor sites at the surface of the effector cell: a sympathomimetic effect or adrenergic blockade.

2) "Pores" of the membrane of the effector or other cells: prevention of the intracellular passage of the catecholamine, with resulting potentiation of its actions at the surface or blockade of its intracellular actions.

3) Plasma protein or other "nonspecific receptors": potentiation of the catecholamine.

4) Cellular enzymes: potentiation or altered metabolism of the catecholamine.

Needless to say, it will require studies at the cellular level to resolve most of the questions that have here been raised.

REFERENCES

1. ANGENENT, W. J. AND KOELLE, G. B.: The destruction of epinephrine by the DOPA-oxidase system of ocular tissue. Science **116**: 543–544, 1952.
2. ANTONIADES, H. N., GOLDFIEN, A., ZILELI, S. AND ELMADJIAN, F.: Transport of epinephrine and norepinephrine in human plasma. Proc. Soc. exp. Biol., N.Y. **97**: 11–12, 1958.
3. ARMSTRONG, N. D., MCMILLAN, A. AND SHAW, K. N. F.: 3-Methoxy-4-hydroxy-D-mandelic acid, a urinary metabolite of norepinephrine. Biochim. biophys. Acta **25**: 422–423, 1957.
4. AVIADO, D. M., JR. AND SCHMIDT, C. F.: Reflexes from stretch receptors in blood vessels, heart, and lungs. Physiol. Rev. **35**: 247–300, 1955.
5. AXELROD, J.: O-Methylation of epinephrine and other catechols *in vitro* and *in vivo*. Science **126**: 400–401, 1957.
6. BACQ, Z. M.: The metabolism of adrenaline. Pharmacol. Rev. **1**: 1–26, 1949.
7. BLASCHKO, H.: Amine oxidase and amine metabolism. Pharmacol. Rev. **4**: 415–458, 1952.
8. BLASCHKO, H.: Metabolism of epinephrine and norepinephrine. Pharmacol. Rev. **6**: 23–28, 1954.
9. BLASCHKO, H., RICHTER, D. AND SCHLOSSMAN, H.: The oxidation of adrenaline and other amines. Biochem. J. **31**: 2187–2196, 1937.
10. BOVET, D., BOVET-NITTI, F., BETTSCHART, A. AND SCOGNAMIGLIO, W.: Mécanisme de la potentialisation par le chlorhydrate de diéthylamino-éthyldiphényl-propylacétate des effets de quelques agents curarisants. Helv. physiol. acta **14**: 430–440, 1956.
11. BRODIE, B. B., MARK, L. C., PEPPER, E. M., LIEF, P. A., BERNSTEIN, E. AND ROVENSTINE, E. A.: Fate of thiopental in man and method for its estimation in biological material. J. Pharmacol. **98**: 85–96, 1950.
12. BRODIE, B. B. AND SHORE, P. A.: Concept for role of serotonin and norepinephrine as chemical mediators in brain. Ann. N. Y. Acad. Sci. **66**: 631–642, 1957.
13. BUTLER, T. C.: Introductory remarks: Termination of drug action by elimination of unchanged drug. In: Symposium on processes terminating drug action. Fed. Proc. **17**: 1158–1181, 1958.
14. CANNON, W. B. AND ROSENBLUETH, A.: Autonomic neuro-effector systems. Macmillan, New York, 1937.
15. CELANDER, O. AND FOLKOW, B.: A comparison of the sympathetic vasomotor fiber control of the vessels within the skin and the muscles. Acta physiol. scand. **29**: 241–250, 1953.
16. CLARK, A. J.: The mode of action of drugs on cells. Arnold, London, 1933.
17. DAWES, G. S.: Amidines, guanidines and adrenaline inactivation in the liver. Brit. J. Pharmacol. **1**: 21–37, 1946.
18. DAWES, G. S. AND COMROE, J. H., JR.: Chemoreflexes from the heart and lungs. Physiol. Rev. **34**: 167–201, 1954.
19. ELLIS, S.: The metabolic effects of epinephrine and related amines. Pharmacol. Rev. **8**: 485–562, 1956.
20. EULER, U. S. VON: Identification of the sympathomimetic ergone in adrenergic nerves of cattle (Sympathin N) with laevo-noradrenaline. Acta physiol. scand. **16**: 63–74, 1948.

21. EULER, U. S. VON AND LOFT, R.. Noradrenaline output in urine after infusion in man. Brit. J. Pharmacol. **6:** 286–288, 1951.
22. GADDUM, J. H. AND KWIATKOWSKI, H.. The action of ephedrine. J. Physiol. **94:** 87–100, 1938.
23. GRUHZIT, C. C., FREYBURGER, W. A. AND MOE, G. K.: The nature of the reflex vasodilation induced by epinephrine. J. Pharmacol. **112:** 138–150 1954.
24. KAMIJO, K., KOELLE, G. B. AND WAGNER, H. H.: Modification of the effects of sympathomimetic amines and of adrenergic nerve stimulation by 1-isonicotinyl-2-isopropylhydrazine (IIH) and isonicotinic acid hydrazide (INH). J. Pharmacol. **117:** 213–227, 1955.
25. KOELLE, G. B.: Pharmacologic significance of inhibition of monoamine oxidase. J. clin. exp. Psychopath. and Quart. Rev. Psychiat. **6:** 37–44, 1958.
26. KOELLE, G. B. AND VALK, A. DE T., JR.: Physiological implications of the histochemical localization of monoamine oxidase. J. Physiol. **126:** 434–447, 1954.
27. LABROSSE, E. H., AXELROD, J. AND KETY, S. S.: O-Methylation, the principal route of metabolism of epinephrine in man. Science **128:** 593–594, 1958.
28. LEWIS, G. P.: The importance of ionization in the activity of sympathomimetic amines. Brit. J. Pharmacol. **9:** 488–493, 1954.
29. LOCKETT, M. F.: The transmitter released by stimulation of the bronchial sympathetic nerves of cats. Brit. J. Pharmacol. **12:** 86–96, 1957.
30. MOHME-LUNDHOLM, E.: The mechanism of the relaxing effect of adrenaline on smooth muscle. Acta physiol. scand. **29:** suppl. 108, 1953.
31. NICKERSON, M.: Pharmacology of adrenergic blockade. Pharmacol. Rev. **1:** 27–101, 1949.
32. RALL, T. W., SUTHERLAND, E. W. AND WOSILAIT, W. D.: The relationship of epinephrine and glucagon to liver phosphorylase. III. Reactivation of liver phosphorylase in slices and in extracts. J. biol. Chem. **218:** 483–495, 1956.
33. RICHTER, D.: The inactivation of adrenaline *in vivo* in man. J. Physiol. **98:** 361–374, 1940.
34. SEEVERS, M. H. AND WOODS, L. A.: The phenomena of tolerance. Amer. J. Med. **14:** 546–557. 1953.
35. VOGT, M.: Concentration of sympathin in different parts of central nervous system under normal conditions and after administration of drugs. J. Physiol. **123:** 451–481, 1954.