Central and Peripheral Mechanisms of Cardiovascular Regulation

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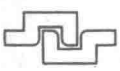

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PREFACE

There is increasing awareness that the autonomic nervous system, through its central and peripheral pathways, plays a critical role in the regulation of the circulation. Peripherally, the autonomic representation, largely that of sympathetic nerves, innervate virtually all segments of the vascular tree as well as the adrenal medulla. Through the interaction of nerve terminals, their transmitters, receptors and intracellular mediators in smooth muscle, sympathetic neurons control vascular tone as well as the basal performance of the heart.

In turn, the performance of the autonomic nervous system is highly controlled by the brain. Once viewed as a black box with only a vague influence on cardiovascular performance, the introduction of concepts and techniques of neuroscience into the field of cardiovascular medicine has led to the realization of the critical role of this organ in cardiovascular control. It is now well recognized that within the brain, the represenation of cardiovascular function is highly restricted anatomically, engages a number of specific transmitters for its actions, and has highly selective and topographically restricted functions to influence circulatory performance.

This volume represents a selected overview of some mechanisms through which the autonomic nervous system contributes to overall cardiovascular control. The contributors touch upon the most critical control points now recognized to be of relevance in control of the circulation. These range through studies of: vascular reactivity, cellular mechanisms mediating smooth muscle contraction, the action of drugs upon blood vessels and nerve terminals and, finally, central neural pathway transmitters and their actions. In selecting the contributors, the editors have not only sought expertise but also for representation of the major areas presently at the frontier in understanding the role of the autonomic nervous system in circulatory control. It is hoped that the reader will confirm the editors' belief that this monograph will be of interest not only to basic scientists but also to cardiologists and other practitioners.

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CONTENTS

INACTIVATION OF CATECHOLAMINES IN THE BLOOD VESSEL WALL

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INTRODUCTION

It is well known that effector organ responses to catecholamines are evanescent in character, disappearing in a few minutes after bolus administration of these substances or short electrical stimulation of the adrenergic innervation of the effector organ. Since experiments on isolated vessels uniformly have shown that after termination of electric stimulation (leading to release of endogenous transmitter) or washout of the added exogenous catecholamine the motor effects quickly disappear, the conclusion is inescapable that the catecholamines are rapidly removed from the biophase and/or metabolized to inactive or at least much less active substances. Thus, the blood vessel wall itself must be endowed with mechanisms which are able to inactivate the catecholamines. In fact, a large amount of evidence has accumulated, showing that the mechanisms presently known to dispose of catecholamines in nonvascular structures are all present and operant in the blood vessel wall. An appreciable number of comprehensive reviews has dealt with these vascular mechanisms (Somlyo and Somlyo, 1970; Iversen, 1971; Spector, Tarver and Berkowitz, 1972; Trendelenburg, 1972, 1980; Bevan and Su, 1973; Langer, 1974; Osswald, 1976, 1978, 1979, 1984; Kalsner, 1979; Bevan, Bevan and Duckles, 1980; Bevan et al, 1980; De la Lande, 1981; Vanhoutte, Verbeuren and Webb, 1981; Osswald and Guimarães, 1983).

Elucidation of the processes leading to inactivation of catecholamines in the blood vessel wall is of particular importance for a better understanding of a series of problems concerning cardiovascular

regulation, especially the maintenance of vascular tone, both in health and in disease. An eventual physiological role of blood vessels in regulating plasma levels of catecholamines of sympathoadrenal origin strongly suggests itself, and may well be altered in some pathological states, e.g. hypertensive disease, as discussed later on. On the other hand, a number of drugs, some of which are of appreciable therapeutic relevance, interfere with inactivation of catecholamines and thus may exert effects of therapeutic and/or toxicological consequence. For all these reasons, in this chapter an attempt will be made to review these interrelated topics.

MECHANISMS OF INACTIVATION

As stated above, the blood vessels so far studied appear to possess all enzymatic and non-enzymatic mechanisms which play relevant roles in the inactivation of catecholamines. Detailed description of these mechanisms is beyond the scope of this short review, but may be found in the reviews quoted in the Introduction. In short, the following mechanisms must be considered:

1. Diffusion. By definition a passive, gradient-dependent mechanism, diffusion occurs in every vascular tissue. Its importance depends on the structural characteristics of the vessel under study and on the experimental setup. In incubation experiments, the highly active sites of loss present in the tissue generate concentration gradients, i.e. the amine concentration throughout the extracellular space is not homogeneous (Trendelenburg, 1984). In the rabbit aorta (a much used experimental model), for example, the tissue characteristics of a large average neuromuscular distance and a strictly adventio-medial innervation create for this relatively thick vessel conditions which favour much higher diffusion constants in the adventitia than in the media (Török and Bevan, 1971, Henseling, 1983). Thus, it is understandable that if the rabbit aorta is incubated for short periods with low concentrations of amine, neuronal mechanisms of inactivation will be overestimated and extraneuronal mechanisms underestimated, because of the rapid and easy equilibration of the neuronal sites with the medium offered to the adjacent adventitia, whereas access to the media is diffusion limited (Trendelenburg, 1984).

2. Uptake processes. Active transmembraneous transport mechanisms have been described which are able to translocate the highly polar catecholamines from the extracellular space to the interior of neuronal and nonneuronal cellular elements. These mechanisms have been described as neuronal (uptake 1) and extraneuronal (uptake 2) processes (Paton, 1960, Iversen, 1967, 1971; reviews by Paton 1976 and Trendelenburg, 1980). These mechanisms must play a prominent role in inactivation of catecholamines, since they precede metabolism, which occurs exclusively in the cells themselves (see below, under 4). These mechanisms are saturable at quite different amine concentrations, the K_m of the extraneuronal uptake being about 100 times higher than that of the neuronal uptake; relatively specific inhibitors of these uptakes have been extensively studied (e.g. cocaine and tricyclic antidepressant drugs for neuronal uptake, corticosteroid Δ, O-methylated derivatives of catecholamines and oestradiol for extraneuronal uptake). Uptake represents in itself an inactivation mechanism, because it contributed to clear the amines from the biophase (where receptors are present); however, if the enzymatic machinery behind the uptake process is not functioning, (i.e. is inhibited by suitable drugs) uptake may function as a temporary loss, because the intact amine is again able to cross the membrane and reach the biophase. This has been demonstrated to occur in the extraneuronal (but not in the neuronal) system, when its enzymes are blocked (Trendelenburg 1974, Guimarães and Paiva 1977, Henseling 1980).

3. Binding to tissue constituents. First described to occur by Avakian and Gillespie (1968), binding of intact molecules to collagen, elastin, endothelial cells, fibroblasts and nuclei may occur in a variety of tissues, including the vascular ones (review by Gillespie, 1976). Very little is known about the importance of this mechanism, but the fact that the total volume of these tissue constituents is much higher than that of nerve terminals and smooth muscle cells taken together (Coimbra, Ribeiro-Silva and Osswald, 1974, Branco et al. 1981) should encourage more detailed investigations into the role played by these mechanisms. Since the initial studies were made with the fluorescence technique, which requires the use of high concentrations of amines, it was thought that the process only played a role for these high, unphysiologic concentrations. However, resorting to autoradiography allowed Branco et al (1981) to conclude that this "binding" occurs even for relatively low concentrations of isoprenaline and may represent the structural counterpart of the corticosteroid-resistant, non O-methylating site of loss described in the rabbit aorta (Henseling 1980). This corticosteroid-resistant site of loss

and its metabolizing capacity appear to present wide interorgan variations. It is practically nonexistent in the rat heart (Bönisch and Trendelenburg, 1974) and increases in importance in the following order: canine mesenteric arteries (Osswald, Garrett and Guimarães, 1975), dog saphenous vein (Azevedo and Osswald, 1976, Paiva and Guimarães, 1978, Branco and Osswald, 1980), rabbit aorta (Henseling, 1980, Branco et al 1981, Sweet and Levin, 1983, Levin and Wilson, 1983, Magill and Levin, 1984) and rabbit ear artery, where it predominates (Head et al, 1980). A recent critical appraisal of this corticosteroid-resistant site of loss is to be found in the review by Trendelenburg (1984).

4. Enzymatic breakdown. Oxidative deamination through monoamine oxidase (MAO) and O-methylation through catechol O-methyl transferase (COMT) result in the formation of metabolites which are much less active than the parent compounds (in the order of 100 to 1000 times less: Holtz, Osswald and Stock, 1960, Renson, Weissbach and Udenfriend, 1964). These enzymatic mechanisms can only operate after entry of the amines into the cell (by active uptake - see section 2 - or by passive entry, due to pinocytosis - Azevedo, Teixeira and Sarmento, 1984 - or diffusion, depending on the relatively low lipophilicity of the catecholamines - Mack and Bönisch, 1979). Since the active uptake processes play the major role in most tissues studied so far, the sequence of uptake and intracellular metabolism represents, in fact, a "metabolizing system" or a "pump and enzyme system" (Kurahashi, Rawlow and Trendelenburg, 1980, Trendelenburg, 1984). It is evident that the intact system can not be described in terms of kinetic constants, since the constants of both the uptake process and the enzyme influence the function of the entire system. It is also evident that blockade of either the uptake or the enzyme may have very different consequences on the inactivation of amines. For example, for the extraneuronal O-methylating system it has been demonstrated that it functions as an "irreversible site of loss" when the enzyme is intact or not saturated, but that it functions as a "reversible site of loss" when the enzyme is saturated or inhibited (Bönisch, 1978). Thus, the system is transformed from a "pump and enzyme" one into a "pump and leak" system; a mathematical model has been developed (Kurahashi et al., 1980) which well agree with experimental data (Bryan, 1984, Kurahashi, et al., 1984).

The concept of metabolizing systems is not only helpful in our progressive understanding of the function of these sites of loss in the inactivation of catecholamines but also allows us to understand why it is so difficult to compare results obtained in intact tissues with those gained in experiments in which diffusion and uptake processes are excluded

(homogenates, purified enzyme preparations). Another important point which must not be forgotten in the attempts to define relative roles of mechanisms of inactivation is that of the relationship between the two enzymes, MAO and COMT. Firstly, the metabolites resulting from the action of MAO on catecholamines (dihydroxyphenylglycol - DOPEG and dihydroxymandelic acid - DOMA, which result from reduction and oxidation, respectively, of the unstable aldehyde formed by oxidative deamination of adrenaline and noradrenaline; dihydroxyphenylethanol - DOPET and dihydroxyphenylacetic acid - DOPAC, which stem in a parallel way from dopamine) are substrates of COMT and give rise, through O-methylation, to the corresponding derivatives (methoxyhydroxyphenylglycol - MOPEG, methoxyhydroxymandelic or "vanillylmandelic" acid - VMA, methoxyhydroxyphenylethanol - MOPET and homovanillic acid - HVA). On the other hand, the O-methylated compounds resulting from the action of COMT (normetanephrine - NMN, metanephrine - MN, methoxytyramine - MT) are substrates of MAO and thus give rise to MOPEG, VMA, MOPET and HVA. The O-methylated and deaminated metabolites (OMDA) thus represent the final metabolites of the catecholamines and should be the only ones found if the tissue contains both MAO and COMT, the enzymes are not saturated and transport mechanisms would allow for an even distribution of the amines and metabolites to the intracellular enzymatic sites. As discussed further on, this does not happen under most experimental conditions; the efflux of metabolites from the cells depend very much on their physicochemical properties (review by Trendelenburg et al., 1979).

A further complication lies in the fact that two different types of MAO have been demonstrated to coexist in blood vessels (Caramona, 1983, 1983). As reviewed by Mantle and Tipton (1982), the evidence accumulated since Johnston (1968) first suggested the presence in tissues of more than one form of MAO has led to the present definition of two forms of MAO, namely A and B, which may be studied with the help of specific inhibitors and of preferential substrates (Kinemuchi, Fowler and Tipton, 1984) and differ in structure and amino acid sequence (Denney and Abell, 1984). In the lateral saphenous vein of the dog, both types of MAO are represented, the ratio of MAO-A/MAO-B (as indicated by the ratio of V_{max} for 5-hydroxytryptamine/V_{max} for B-phenylethylamine) being of 0.51. Tyramine, which is a substrate devoid of specificity, is deaminated, in saphenous vein homogenates, by both forms of MAO, although the activity of MAO-B predominates (Caramona, 1982). Since MAO is present both in nerve terminals and extraneuronal cells (review by Fowler, Magnusson and Ross 1984) it is of interest to pinpoint the localization of the two types of

MAO. Recent studies from this laboratory (Caramona, 1983; Caramona and Soares-da-Silva, 1984) on several normal and denervated venous and arterial vessels allowed to conclude that there is a very good correlation between adrenergic innervation and MAO-A content (but not MAO-B content) and that MAO-A is located both inside and outside of the nerve terminals, whereas MAO-B is entirely extraneuronal. Thus, the suggestion that neuronal MAO belongs to the A type (Goridis and Neff, 1971, Vanhoutte, et al., 1977) could be confirmed. However, it should be stressed that this does not mean that a "transmitter-specific" form of MAO (Goridis and Neff, 1971) exists, since intra- and extraneuronal MAO-A did not differ in their sensitivity to inhibitors (Osswald and Caramona, 1984).

Another point of interest in the inter-relationship of the two enzymes (COMT and MAO) is that pertaining to its localization in cells. For neuronal elements, all existing evidence points to the presence of MAO (type A - see above) but not of COMT (review by Trendelenburg, 1980). For extraneuronal cells, however, the situation is quite different, since, as aptly discussed by Trendelenburg (1984), the results obtained in a number of experiments are consistent with the co-existence of COMT and MAO (type A and B - see above) in the same cells. However, we do not know if <u>all</u> extraneuronal cells contain both types of enzymes or if the distribution of enzyme activity in different cell types is homogeneous, although it is evident that the smooth muscle cells of the rabbit aorta are endowed with both COMT and MAO (Levin and Wilson, 1977).

5. <u>Nonenzymatic formation of condensation products</u>. Catecholamines may easily form aldehyde condensation products, the tetrahydroisoquinolines (TIQs). Thus, the condensation of parent amines with unstable and highly reactive aldehydes originated from them by oxidative deamination (by MAO) represents at least a theoretical possibility. Holtz, Stock and Westermann (1963) identified such a product (tetrahydropapaveroline - THP) as resulting from the reaction of dopamine with the corresponding aldehyde, due to the action of MAO. More recently, Teixeira and Macedo (1981) found that the relaxant effect of dopamine on vascular smooth muscle contracted by prostaglandin $F_2\alpha$ in the presence of an α-adrenoceptor blocking agent appeared to be due to activation of dopamine and β-adrenoceptors by a substance formed through the action of MAO. The use of inhibitors of MAO and of the formation of THP, as well as incubation experiments (Teixeira, Almeida and Figueiredo, 1983) led the authors to suggest that nonenzymatic formation of THP is an important pathway in vascular tissue (for dopamine, but not for adrenaline).

MORPHOLOGICAL ASPECTS

The question of topographical localization of inactivation mechanisms has already been raised in the preceding section. However, it is felt that a very short review of the sites of inactivation and of their interrelations should be attempted at this place. It is by no means clearly established where and in which proportion the inactivation proceeds, since most studies conducted on this subject have dealt with entire segments of blood vessels or with perfused vascular areas and only global inactivation can be measured in most of these experimental setups. Nevertheless, some indications allow the summarization of this question in the following manner :

Most arterial and venous vessels are characterized by the existence of an adventiomedial nervous ground plexus, although the adrenergic innervation pattern may differ and take the form of medial innervation, i.e. of a distribution of nerve terminals throughout the media. This also means that mean neuromuscular distance may vary markedly (from 60 to 4000 nm - see review by Osswald and Guimarães, 1983) from vessels showing close apposition (as some arterioles and veins) to large arteries where no true synaptic cleft exists. These facts are of obvious importance for the fate of the transmitter, after its release from the nerve terminals. The type and density of innervation is also of importance for the disposition of exogenous or blood-borne catecholamines. In the lateral saphenous vein of the dog, nervous tissue represents 7.9% of the wall (Coimbra et al., 1974), whereas in the rabbit thoracic aorta nervous elements account only for 0.5% of the volume (Branco et al., 1981); it is evident that in the former vessel neuronal systems of inactivation (uptake, metabolism by MAO-A, storage in dense-core vesicles) must play a more important role than in the rabbit aorta, and this is actually what happens, as discussed below.

Smooth muscle cells are endowed with both the O-methylating and the deaminating system; in the rabbit aorta the adventitia (containing all nervous elements) can be separated from the media (the endothelium can easily be eliminated by rubbing) as described by Maxwell, Eckhardt and Wastila (1968). This preparation of the isolated (noninnervated) media has been used by several authors (Levin 1974, Levin and Wilson, 1977, 1983, Henseling and Trendelenburg 1978, Henseling 1980, Branco et al., 1981, Sweet and Levin 1983, Magill and Levin 1984) and shown to be a good model for the extraneuronal metabolizing systems, i.e. of the "pump and

enzyme" systems described in the previous section.

As far as the endothelium is concerned, there is some evidence pointing in the sense of the presence of uptake and metabolic processes in at least some endothelial cells, e.g. those of the cerebral microcirculation (Owman, Edvinsson and Hardebo, 1980) or of the lung vessels (Gillis, 1980). However, a number of observations suggest that these properties are not restricted to the pulmonary and cerebral endothelium (Bevan and Török, 1970, Török and Bevan, 1971, Osswald, Guimarães and Coimbra, 1971). Azevedo and Osswald (1976) described an active uptake mechanism (blocked by cortexone) in the endothelium of the saphenous vein; on the other hand, Lowe and Creveling (1979) and Lowe (1979), in immunocytochemical and ultrastructural autoradiography studies, described the presence of COMT and MAO in endothelial cells of the rat aorta and of capillaries of the heart. Other cellular elements present in blood vessels (fibroblasts, Schwann cells, fat cells), although capable of accumulating and metabolizing isoprenaline (Branco et al., 1981) probably play only a minor role in the inactivation of catecholamines (Osswald and Guimarães, 1983).

In this respect, it is important to state that we are just beginning to realize that the spatial relationship (i.e. the relative vicinity) of some sites of loss with the source of the amine or the receptors of the effector cell may be of paramount significance for the relevance of these sites of loss in the inactivation of that amine. For example, although fibroblasts to not appear to play a quantitatively important role in metabolism of noradrenaline, their close vicinity to nerve endings in several vessels (Azevedo and Soares-da-Silva, 1981, Soares-da-Silva and Azevedo, 1985) makes them suitable candidates for a role in regulation of transmitter concentration in the juxtaneuronal biophase. On the other hand, it has been unequivocally proven that α- and β-adrenoceptors show a differential localization in relation to nerve terminals and COMT activity, α-adrenoceptors being more closely related to neuronal elements and β-adrenoceptors to sites of COMT activity (Guimarães, 1975, Guimarães et al., 1975, Belfrage, Fredholm and Rosell, 1977, Guimarães and Paiva, 1977, Bevan et al., 1978, Winquist and Bevan, 1979). These observations led to the concept of two different biophases for α- and β-adrenoceptors (Guimarães, 1982, Guimarães and Paiva, 1981 a and b). Thus it is clear that the blockade of one site of loss or enzymatic system may alter the