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Editorial Views

The Norepinephrine Stores and Their Functional Compartments

RECENT advances in methodology make it possible to obtain reliable information on norepinephrine levels in the tissues as well as in the circulating blood. This issue of ANESTHESIOLOGY contains a report on catecholamine determinations carried out during heart surgery. Since it is likely that such problems will be of great interest to anesthesiologists, this opportunity may be taken to summarize and to reflect on our present knowledge of the norepinephrine stores of the peripheral autonomic nervous system.

When it was established that norepinephrine is the transmitter agent released from adrenergic fibers, it was soon found that the norepinephrine content of various organs is roughly proportional to the density of the sympathetic innervation. Apparently, a store of ready-made transmitter substance is present in the endings of adrenergic nerves. Further evidence for the view that the norepinephrine stores are related to sympathetic nerve endings came from the observation that chronic sympathetic denervation leads not only to the degeneration of nerve fibers but also to the loss of the endogenous norepinephrine.

In recent years electronmicroscopy and biochemistry have greatly increased our knowledge. Electronmicrographs revealed that both the chromaffin cells of the adrenal medulla and the endings of adrenergic nerves contain small intracellular structures which have been called "granules" or "vesicles." With biochemical methods it has been possible to isolate these granules which were found to be very rich in norepinephrine (or norepinephrine and epinephrine in the case of the adrenal medulla). Hence, these granules appear to be the major site of storage for catecholamines. In this connection it is of interest to note that these structures are also very rich in adenosine phosphates (predominantly ATP) which are believed by many to be responsible for the intragranular binding of norepinephrine. In fact, one molecule of ATP has been found to form a complex with four molecules of epinephrine; this ratio corresponds to that of the intragranular concentrations of ATP and catecholamines. Furthermore, prolonged nerve stimulation causes not only a fall in norepinephrine content of the adrenal medulla; there is also a corresponding loss of ATP.

If the catecholamines are bound in granular stores, how do they reach these sites? Two mechanisms have to be considered: synthesis and uptake. Adrenergic nerves possess enzymes necessary for the biosynthesis of norepinephrine: DOPA-decarboxylase is present in the cell plasma and converts DOPA to dopamine, while the next step, the conversion of dopamine to norepinephrine by dopamine-β-hydroxylase, appears to be carried out only inside the granules. It is of interest that various adrenergic nerves are quite rich in dopamine; in fact, some of them are so rich that it has been suggested that dopamine could fulfill the functions of a transmitter in addition to being the precursor of norepinephrine. The adrenal medulla is able to perform a third step by methylating norepinephrine to epinephrine. Consequently, this is the only organ which secretes appreciable amounts of epi-

nephrine, while the release from nerve terminals consists of nearly pure norepinephrine. Nevertheless, most organs contain small but significant amounts of epinephrine. The origin of this amine is not quite clear. It is quite possible that the epinephrine found in organs other than the adrenal medulla is not synthesized in the peripheral stores, but has been taken up from the circulation. The adrenal medulla is well known to have a resting secretion, *i.e.*, to liberate more or less continuously a mixture of epinephrine and norepinephrine. Since the stores of the nerve endings are able to take up considerable amounts of circulating amines, the epinephrine found in various organs may well be of adrenal medullary origin.

The ability of the norepinephrine stores to take up amines appears to be of great physiological and pharmacological importance. It is likely that this uptake limits the lifespan of injected or released norepinephrine. This constitutes an interesting difference to the other transmitter substance of the autonomic nervous system, acetylcholine. Whereas it is well established that the active life of acetylcholine is terminated by its hydrolysis by cholinesterases, metabolic enzymes do not seem to have the same biological importance for the termination of the action of norepinephrine. This is quite evident from the fact that cholinesterase inhibitors greatly potentiate the effects of injected or released acetylcholine, whereas inhibitors of either monoaminoxidase or of catechol-O-methyl transferase (the two enzymes responsible for the metabolism of norepinephrine) are not able to cause any appreciable potentiation of the effects of injected or released norepinephrine. Hence, there must be another mechanism responsible for the termination of the physiological and pharmacological actions of this amine; uptake into the stores is a likely candidate, since agents known to prevent uptake (cocaine, for instance) produce pronounced potentiation of the effects of norepinephrine.

From these considerations the following picture emerges: Adrenergic nerve terminals contain submicroscopic granules or vesicles in which the norepinephrine is bound in an inactive form (possibly to ATP) after having been synthesized within the nerve terminals. In response to nerve impulses both norepinephrine and ATP leave the stores. When the transmitter agent reaches the extracellular space, some of it acts on the receptor of the effector organ, some escapes into the circulation and some is taken up into the stores from which it had been released. It is evident that the level of circulating norepinephrine is determined both by the release and by the uptake of this amine; consequently, interference with either of these mechanisms increases or decreases the norepinephrine level in the blood. The metabolic enzymes, on the other hand, play a role in the eventual fate of norepinephrine but have no immediate influence on the concentration of norepinephrine near the receptors of the effector organ, *i.e.*, on the response of the organ.

Many experimental observations suggest that the norepinephrine stores are not homogeneous. The following observations may illustrate this point: After an injection of radioactive norepinephrine a considerable proportion of the injected material is taken up by the heart. Subsequently both the radioactivity and the norepinephrine content of the venous effluent were determined. It was found that the specific activity of the spontaneously released norepinephrine was much higher than the specific activity of the norepinephrine remaining in the heart; furthermore, on stimulation of the accelerans nerve the specific activity of the released norepinephrine was somewhere between these two extremes.[2] If the norepinephrine stores were homogeneous, the specific activity should not have differed; consequently, from a functional point of view, the norepinephrine stores appear to consist of various "compartments." Observations of this kind have caused a lively discussion of the postulated compartments. It is much too early for a generally accepted theory, but a few possible arrangements of these compartments may be enumerated. Norepinephrine may not only be bound in the granules, there may also be a fraction of free norepinephrine in the cell plasma. It is also possible that some of the bound norepinephrine is very loosely bound (and thus easily released), while part of the store is tightly bound (and thus not immediately available for release). As a third possibility, storage granules of different biochemistry could be

postulated. Finally, a different spatial arrangement of biochemically identical storage granules can be visualized: only those granules which are close to the surface of the nerve terminal may be involved in the uptake and the release of the transmitter, whereas the more centrally located granules do not immediately take place in these processes. Two factors could account for this: firstly, the distances which the norepinephrine has to travel by diffusion have to be considered, and secondly, the mitochondria of nerve terminals are rich in monoaminoxidase and may act as a "biological barrier" against deep penetration by norepinephrine taken up from the extracellular space.

This discussion brings us to a last point: If there are compartments of different functional importance, is the total norepinephrine content of an organ a good indicator of the functional condition of this organ? A valid answer cannot be given, but an extreme condition may be described to illustrate the difficulties of interpretation. Reserpine is well known to deplete the norepinephrine stores of the heart, and under such conditions tyramine then no longer causes a stimulation of the cardiac pacemaker, since this amine exerts its effects by liberating endogenous norepinephrine from the stores. However, the response of the cardiac pacemaker to tyramine can be restored by an infusion of norepinephrine which is believed to refill the previously depleted stores.[1] In similar experiments on isolated atria the norepinephrine content of the atria was determined before and after this "refilling" of previously depleted stores. Whereas the norepinephrine content increased from one to only two per cent of normal, this very small uptake of norepinephrine was enough to restore the response of the pacemaker to tyramine from zero to seventy per cent of normal.[3] In other words, this very small uptake of exogenous norepinephrine was of great functional importance, and there was no parallelism between changes in norepinephrine content and changes in function.

From such observations it is evident that a minute fraction of the total norepinephrine store may be sufficient for the release of adequate amounts of the transmitter agent, if the norepinephrine is available for release. This complex relation between compartments of different functional importance should be borne in mind, when determinations of the total norepinephrine stores are considered.

DR. ULLRICH G. TRENDELENBURG
Department of Pharmacology
Harvard Medical School

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DOUBLE ABSORBERS Reaction temperature is not a reliable criterion of carbon dioxide absorption. Higher reaction temperatures are observed in dry lime than in wet lime probably because a larger amount of heat is needed in wet lime for vaporization of water. Early warming of the second canister in a series occurs in spite of complete carbon dioxide absorption in the first canister due to water condensation on the granules in the second canister. During carbon dioxide absorption, soda lime loses up to 80 per cent of its moisture content. (*Lueder, M.: Experimental Investigations on Soda Lime Using Double Absorbers, Der Anaesthesist 12: 297 (Oct.) 1963.*)