ADVANCES IN

Pharmacology

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PREFACE

It is generally agreed that it is becoming increasingly difficult for the investigator today to keep abreast of the literature even in his own special field of interest; the investigator in pharmacology is no exception. In helping to alleviate this situation, the review article has assumed great importance since it enables the investigator to keep up to date in his special field and, if he is sufficiently conversant in other areas, to keep abreast of advances in these fields also. Unfortunately, even review articles are becoming difficult for the non-expert to digest since most are written by experts for the benefit of other experts.

Because of this, and because research in the broad aspects of pharmacology is expanding so rapidly, the editors of *Advances in Pharmacology* have attempted to orient this series in such a way that the chapters will not be reviews of the literature, but may be looked upon as a collection of monographs which will supply the expert with the most recent developments and will also allow the initiate to ground himself in new research areas. The various authors are encouraged to be selective in their definition of "advances" and to take the opportunity to freely formulate and consider hypotheses and concepts.

Since pharmacology is so closely allied to other disciplines of biological sciences, it is our hope that this series will be of interest not only to pharmacologists but to their colleagues in physiology, biochemistry, and other disciplines as well.

In this, the first volume, several important aspects of pharmacology are examined. The new concept of the functioning of the adrenergic nerve fiber is discussed in detail by J. H. Burn and M. J. Rand; different aspects of biogenic amines are presented in the chapters by J. P. Green and by B. J. Haverback and S. K. Wirtschafter. Some of the most interesting of the newer drugs and their possible mechanisms of action are discussed in the chapter on antihypertensive drugs by A. F. Green, in the chapter on psychotropic drugs by M. Shepherd and L. Wing, and in the chapter on the use of drugs in control of hyperlipidemia by D. Steinberg. Important pharmacological considerations in antithrombotic therapy are presented by M. Weiner, and the remarkable ways which drugs can increase or decrease the rate of drug metabolism are examined in the chapter by A. Conney and J. J. Burns.

SILVIO GARATTINI
PARKHURST A. SHORE

January, 1962

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A New Interpretation of the Adrenergic Nerve Fiber

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I. Introduction

The postganglionic sympathetic fibers have recently been shown to have peculiar properties not previously suspected. Tissues which they innervate can take up noradrenaline which is infused intravenously. When noradrenaline is taken up, stimulation of the postganglionic fibers becomes more effective than before, and the action of various amines such as tyramine also becomes more effective than before. Thus, postganglionic stimulation appears to liberate noradrenaline from a peripheral store.

Acetylcholine (in the presence of atropine) and nicotine also release noradrenaline from this peripheral store and imitate the effect of sympathetic stimulation.

Many sympathetic postganglionic nerves have long been known to contain cholinergic fibers, and investigation has now shown that all do. It is thus possible that a fiber liberating acetylcholine can act as an adrenergic fiber because the acetylcholine liberates noradrenaline from the store.

Observations with choline xylyl ether, bretylium, and especially hemicholinium indicate that all sympathetic postganglionic fibers act in the manner just described.

II. The Uptake of Noradrenaline from the Blood by Tissues with a Sympathetic Innervation

A. Tyramine and Adrenaline

When the different sympathomimetic amines were studied by Barger and Dale (1910), they were described as being qualitatively similar in action with certain exceptions, though quantitatively different. Tyramine for example, was described as having one twenty-fifth of the pressor action of adrenaline. It was seen later that tyramine differed from adrenaline not only quantitatively, but also qualitatively because of the effect of cocaine. Fröhlich and Loewi (1910) had shown that cocaine increased the pressor action of adrenaline; Tainter and Chang (1927) found that cocaine abolished the pressor action of tyramine. Another difference was demonstrated a few years later; Meltzer (1904)

had shown that the denervated iris of the cat was much more sensitive to the dilator action of adrenaline than was the normal iris. Burn and Tainter (1931) on the other hand, found that the denervated iris of the cat was insensitive to the dilator action of tyramine in doses many times greater than those sufficient to dilate the normal iris.

The difference between the action of adrenaline and that of tyramine was not solved until recently. The presence of extractable noradrenaline in the heart was demonstrated by Goodall (1951). In 1956, Bertler, Carlsson, and Rosengren made the observation that if a rabbit was injected with reserpine, then 16 hours later the heart had lost its extractable noradrenaline. In 1948, Schmiterlöw showed that arteries and veins also contained extractable noradrenaline. Burn and Rand (1958b) therefore injected rabbits and dogs with reserpine to see if the aortae of these animals would lose their extractable noradrenaline; they found that they did. In 1957, Carlsson and his colleagues showed that if a cat was treated with reserpine, then some hours later tyramine failed to cause a rise in blood pressure. This suggested to Burn and Rand that tyramine was able to act only when extractable noradrenaline was present, and that tyramine acted by releasing some of this noradrenaline.

Many years earlier Burn (1932) had studied the constrictor action of tyramine in the perfused hindlegs of the dog, and had found that the constrictor action was usually very feeble, though that of adrenaline was normal. He was, however, able to restore some of the constrictor action of tyramine by a steady addition of adrenaline to the circulating blood. Therefore, Burn and Rand (1958c), having confirmed the observation that in a reserpine-treated cat the pressor action of tyramine was almost absent, gave a slow intravenous infusion of noradrenaline into the cat, the total infused in 25 minutes being 0.25 mg. When the pressor effect of this infusion had passed off, they observed that the injection of tyramine once more caused a good rise of blood pressure. This observation supported their view that tyramine acted by releasing the extractable noradrenaline, and in addition showed that when the extractable noradrenaline was depleted by treating the animal with reserpine, the store could be made good by an intravenous infusion of noradrenaline.

B. The Effect of Infusing Noradrenaline Precursors

Not only in the cat but also in the rat, treatment with reserpine abolished the pressor action of tyramine, and subsequently an infusion of noradrenaline restored it. An infusion of adrenaline on the other hand had little or no effect in restoring it in either species. An infusion of 5 μg noradrenaline into a rat increased the pressor response to tyramine from 5 to 10 times; an infusion of this amount of adrenaline did

not increase it at all. Precursors of noradrenaline were shown to restore the pressor action of tyramine in the rat. Thus, dopamine infused in the amount of 50 μg restored it; L-dopa in the amount of 1 mg restored it; *meta*-tyrosine in the amount of 2 mg restored it, and finally phenylalanine in the amount of 25 mg restored it. The action of tyramine was also studied in the perfused hindleg of the dog. If the dog had been treated with reserpine, then tyramine when injected into the arterial cannula failed to constrict the vessels of the leg. Noradrenaline was then added drop by drop to the reservoir from which blood was carried to the leg; this caused the vessels to constrict. When the noradrenaline was cut off, the vessels slowly relaxed to the previous degree. The injection of tyramine then caused constriction. The same series of events was observed in dogs not previously treated with reserpine. When the hindleg was perfused, tyramine caused constriction at the beginning. After the addition of noradrenaline to the blood, the constrictor effect of tyramine was greatly increased. The action of tyramine was also studied in the heart-lung preparation of the dog, in which tyramine caused an increase in rate. But if the dog was previously treated with reserpine, tyramine had very little effect on the rate. An infusion of noradrenaline was then given during a period of 30 minutes, and after the effect of the infusion on the rate had subsided, the effect of tyramine on the rate was then greater and more prolonged (Bejrablaya *et al.*, 1958).

C. Measurement of Uptake of Noradrenaline

All these observations supported the view that the action of tyramine was indirect and was due to the release of noradrenaline from the store in the neighborhood of the sympathetic nerve endings. They further suggested that when noradrenaline was slowly infused into a vein it was not all destroyed as had generally been assumed, but some of it was taken up by tissues with a sympathetic innervation. Previous work had been done by Raab and Gigee (1955) in which they injected enormous amounts (10 mg per kg) of noradrenaline and of adrenaline into dogs and found evidence of uptake by the heart. von Euler (1956) however, using more reasonable quantities was not able to confirm their work.

Pennefather and Rand (1960) infused noradrenaline into a series of spinal eviscerated cats, giving 1 mg per cat during a period of 40 minutes. At the beginning of each experiment they took out one kidney, and one horn of the uterus. Then having given the infusion of noradrenaline and having waited 20 minutes, they removed the other kidney and

the other horn of the uterus. They estimated the extractable noradrenaline, making the estimation on the blood pressure of the pithed rat. The results of these experiments showed that the extractable noradrenaline in the kidney and in the horn of the uterus increased as a result of the infusion of noradrenaline, the increase in the kidney being in one experiment fourfold, and the mean increase in all experiments being 2.3-fold. Similar results were obtained in the horn of the uterus. Determination of the amount of noradrenaline in the blood showed that the rise in the blood as a consequence of the infusion was insignificant and that the increased amount in the kidney and the uterus was in no way explained by the amount in the blood within them. Pennefather and Rand carried out other experiments in which they infused dopamine instead of noradrenaline. In some of these experiments the infusion was followed by a rise in the extractable noradrenaline in the kidney and in the uterus, but only in a minority. They found that it was necessary to use cats which were not eviscerated, presumably so that the liver remained in the circulation. Further evidence of uptake of noradrenaline has been published by Whitby *et al.* (1960) who injected *dl*-β-H^3 noradrenaline into cats intravenously, and saw that the H^3noradrenaline was taken up by adrenal gland, heart, and spleen when these tissues were examined 1 hour after the injection of the noradrenaline. Small amounts were also taken up by liver and skeletal muscle.

These observations make it clear that when noradrenaline is infused intravenously, and presumably also when it is secreted into the blood by the adrenal medulla, some of it is not destroyed but is taken up by tissues with a sympathetic innervation. The fate of noradrenaline entering the blood is therefore determined only in part by its destruction.

D. Uptake by Denervated Tissues

When tissues with a sympathetic innervation were denervated, Burn and Rand (1960b) found that they did not take up noradrenaline. Thus, a cat was prepared by removing the superior cervical ganglion. Two weeks later it was injected with reserpine and was anesthetized on the following day to determine the effect of tyramine in dilating the pupils of the two eyes. At first tyramine failed to dilate either pupil. An infusion of noradrenaline was then given, and after the effect of the infusion had passed off, tyramine dilated the innervated pupil, but not the denervated pupil. Similarly, in two cats from which the stellate ganglion had been removed on one side, tyramine failed to constrict the vessels of the foreleg of the denervated side either before or after a long intravenous infusion of noradrenaline had been given.

III. The Action of the Sympathomimetic Amines

A. Release from Granules

The action of tyramine has been described and it has been shown that it appears to depend on the release of noradrenaline. Although all the evidence was consistent with this view, direct proof of noradrenaline release was not obtained. Schümann (1960) however, isolated the chromaffin granules from the adrenal medulla of the ox, and also the granular elements from the splenic nerves, by differential centrifugation, and studied the release of catcholamines from these granules in air at 37° C. He observed that the spontaneous release was increased by the addition of tyramine by about 100–150%. The smallest active concentration of tyramine was about 15 μg/ml. Schümann's evidence is the best so far obtained in favor of the view that tyramine acts by the release of noradrenaline. He also observed that ephedrine and phenylethylamine had a similar action on the granules, accelerating the release of catecholamines; dopamine however had no action of this sort.

B. Three Classes of Amines

These results agreed completely with the observations of the action of the sympathomimetic amines on the blood pressure of the spinal cat and on the rate of the heart-lung preparation of the dog. Phenylethylamine, ephedrine, and amphetamine had no action when the preparations were made on animals treated with reserpine, but their action was restored in the spinal cat after an intravenous infusion of noradrenaline. Similar observations were made in the perfused dog's hindleg where it was recorded that an infusion of 5-hydroxytryptamine had no effect in restoring the constrictor action of phenylethylamine while a subsequent infusion of noradrenaline, was effective. The action of the catecholamines, noradrenaline, adrenaline, and dopamine was quite different. In the spinal cat, or perfused dog's hindleg prepared from the animal after treatment with reserpine, the action of the catecholamines was greater than in the normal animal. Following an infusion of noradrenaline the action of the catecholamines was less. The conclusion was drawn that the pressor and constrictor actions of the catecholamines were direct actions on the heart and blood vessels, in contrast to the actions of derivatives of phenylethylamine which were due to the release of noradrenaline present in the heart and blood vessels. It was interesting to observe that the substance neosynephrine which is not a catecholamine, having in the ring one —OH group in the meta position, behaved like a catecholamine on the blood pressure of the spinal cat.

Samples of L-phenylethanolamine and D-phenylethanolamine were available for study, prepared by P. Pratesi and M. Grassi. The former substance, which had the same configuration as L-adrenaline, had a pressor action in the spinal cat which was only partly lost in the preparation made from a cat treated with reserpine. Thus, L-phenylethanolamine possessed some of the direct action of the catecholamines. The sample of D-phenylethanolamine on the other hand behaved like tyramine. Its pressor action in the reserpine-treated spinal cat was completely lost.

IV. The Effect of Noradrenaline on Sympathetic Stimulation

A. Blood Vessels

When a dog was treated with reserpine and on the following day its hindleg was perfused with blood, stimulation of the postganglionic fibers in the lumbar sympathetic chain caused vasodilatation of the leg vessels. When atropine was injected, stimulation was then without effect. After the addition of noradrenaline to the perfusing blood during a period of 30 minutes, stimulation of the sympathetic fibers caused constriction. Thus, just as the addition of noradrenaline to the blood restored the constrictor action of tyramine, in the same way it restored the constrictor action of sympathetic stimulation. This suggested that sympathetic stimulation and tyramine caused constriction by liberating noradrenaline from the same store.

The evidence that a simple infusion of noradrenaline into the blood would restore the effect of sympathetic stimulation was surprising. The failure of sympathetic stimulation after treatment with reserpine was not difficult to undersand. It seemed evident that reserpine could displace the noradrenaline in the terminations of the adrenergic fibers. But it was a completely new idea that these adrenergic fibers could not only synthesize noradrenaline as they are commonly believed to do, but could also take up noradrenaline from the circulating blood and hold it in such a way that it could be released by later stimulation. Burn and Rand (1960a) were able to show that this uptake could occur normally, that is to say without previous depletion of the store of noradrenaline by injecting reserpine.

Evidence was obtained both in the perfused dog's hindleg, and in the anesthetized dog. When the hindleg from a normal dog was perfused, a strength of stimulus was chosen which when applied to the postganglionic fibers caused a small constriction of the hindleg vessels. A total of 0.5 mg noradrenaline was then added at a uniform rate to the perfusing blood during 30 minutes; the preparation was left until