Brit. J. Pharmacol. (1965), **25**, 758–762.

A CHANGE IN THE SUBCELLULAR DISTRIBUTION OF NORADRENALINE IN THE RAT ISOLATED VAS DEFERENS EFFECTED BY NERVE STIMULATION

BY

C. C. CHANG AND J. C. CHANG

From the Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan, China

(*Received June* 14, 1965)

Kernell & Sedvall (1964) have recently demonstrated a decrease of the noradrenaline content in skeletal muscle on sympathetic stimulation, while Luco & Gõni (1949) were unable to show any change in the catechol amine content. On the other hand, noradrenaline in heart, vas deferens (Potter & Axelrod, 1963) and splenic nerve (Euler & Hillarp, 1956) is stored in subcellular vesicles. Since nerve impulses probably release the neurotransmitter from a particular storage compartment of endogenous noradrenaline (see Kopin, 1964) we decided to study the effect of nerve stimulation upon the pattern of subcellular distribution of noradrenaline stored in the rat vas deferens.

The results reveal that the decrease of noradrenaline content in rat vas deferens induced by coaxial stimulation is largely due to a release of the amine from the particulate fraction.

METHODS

Vas deferens preparation

Both vasa deferentia were isolated from Long Evans rats, weighing about 250 g, and suspended in an organ-bath containing 50 ml. of Krebs-bicarbonate solution. The bath solution was kept at 35° C and gassed with 95% oxygen and 5% carbon dioxide. One vas deferens was stimulated coaxially as described by Birmingham & Wilson (1963) with rectangular pulses (0.2 msec duration) of nearly maximal intensity at 50 shocks/sec. The contralateral vas deferens was kept in exactly the same condition but without stimulation, and served as a control. The contraction induced by coaxial stimulation was readily blocked by the addition of guanethidine or bretylium but not by hexamethonium, indicating that the stimulation was on the postganglionic fibres, as shown by Birmingham & Wilson (1963). The stimulation was maintained continuously for 1 hr.

Noradrenaline assay

Total noradrenaline content of the vas deferens was assayed according to a trihydroxyindole method (Chang, 1964), by homogenizing the tissue directly into ten volumes of butanol. Small amounts of sodium chloride were added to increase the extraction of the amine into butanol for the assay of noradrenaline in the fractions separated by sucrose gradient centrifugation, since it was found that sucrose in the solution tended to retain water and thus to interfere with the extraction.

Sucrose density gradient centrifugation

The vas deferens was homogenized in the cold in a glass homogenizer with about thirty volumes of 0.25 M-sucrose containing 0.001 M-magnesium chloride. Homogenization was limited to 30 sec. The

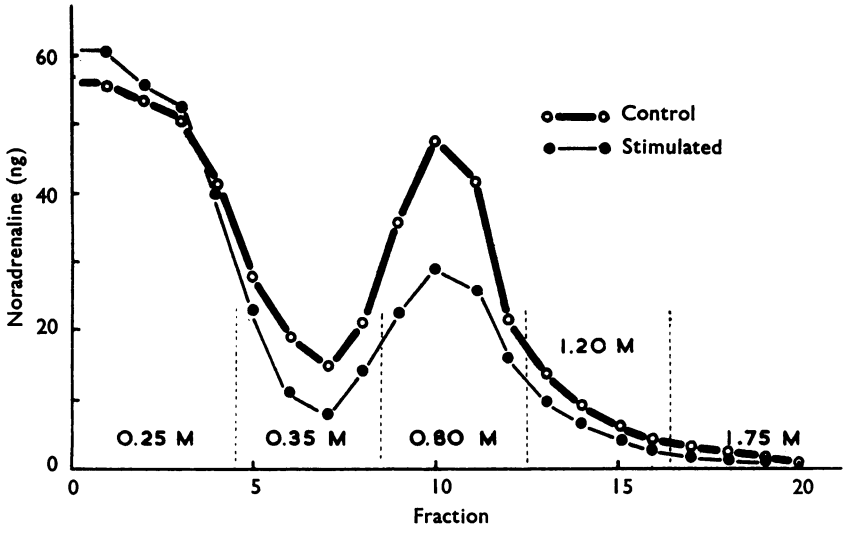

Fig. 1. Effect of sympathetic stimulation on the pattern of subcellular distribution of noradrenaline in the isolated vas deferens of rats, assessed by sucrose density gradient centrifugation. Each point represents the mean of seven or eight experiments. Sucrose concentrations (M) are shown on the graph.

homogenate was first centrifuged at 1,000 *g* for 5 min to remove the unbroken cells and nuclei. An aliquot (1 ml.) of the supernatant fluid was layered on a freshly made sucrose density gradient which consisted of 1 ml. each of 0.35, 0.8, 1.2 and 1.75 M-sucrose. It was then centrifuged at 39,000 revs/min (125,000 *g*) for 45 min in a Spinco Model L preparative ultracentrifuge with swinging bucket rotor SW 39.

The cellulose tube was then punctured at the bottom with a needle and the content was separated into about twenty fractions (twelve drops in each fraction). The sucrose gradient used in our experiments had a layer of 0.35 M-sucrose between the 0.25 M- and the 0.8 M-sucrose and gave a distribution pattern of noradrenaline similar to that obtained with the exponential gradient used by Potter & Axelrod (1963). In addition, as shown in Fig. 1, it provided a more clear-cut separation of supernatant noradrenaline from the particulate fraction of endogenous amine.

RESULTS

Prolonged coaxial stimulation and muscle response

The height of contraction slowly declined during the continuing stimulation of 1 hr and, at the end of stimulation, was reduced to about one-third. If the stimulation was then stopped, the response to coaxial stimulation of 10 sec duration returned gradually to almost the original height within the next hour. This indicates that the process of fatigue induced by stimulation is reversible. In contrast, addition of noradrenaline (0.4 μg/ml.) to the bath always elicited a constant height of contraction even after such exhaustive stimulation, an indication that the fatigue takes place at the site of nervous structures and not at the muscle.

The responsiveness of the preparation to tyramine (4 μg/ml.) was also tested to see whether the action of indirectly acting amine was affected by the exhaustive stimulation. Immediately after the electrical stimulation, tyramine still produced a contraction as high as the control response even though the effect of coaxial stimulation was much depressed at this time.

Decrease in the total noradrenaline content

In Table 1, the noradrenaline content of a vas deferens stimulated for 1 hr is compared with that of the unstimulated contralateral organ. The results show that the mean noradrenaline content of the stimulated vas deferens was 27% lower than that of control. There was no significant decrease of noradrenaline content when the preparation was stimulated for only 10 min. The decrease in noradrenaline content therefore depends on the duration of stimulation. In some experiments after the 1-hr period of stimulation, the vas deferens was kept unstimulated in the bath for another hour. Although the response to electrical stimulation recovered, no detectable increase in noradrenaline content was found.

TABLE 1

EFFECT OF SYMPATHETIC NERVE STIMULATION ON THE NORADRENALINE CONTENTS OF THE ISOLATED VAS DEFERENS OF RATS

* $P<0.01$

No. of expt.	Noradrenaline content (μg/g)		(C−S)/C
	Control (C)	Stimulated (S)	
1	15·5	11·3	0·27
2	21·0	15·1	0·28
3	21·9	15·6	0·29
4	23·4	17·0	0·27
5	14·4	11·3	0·22
Mean	19·2	14·4	0·27*
Standard error	±1·7	±1·2	±0·029

Change in the subcellular distribution of noradrenaline

Analysis of the subcellular distribution pattern of noradrenaline revealed that the noradrenaline content in the particulate fractions of the stimulated vasa deferentia was considerably lower than that of control preparations, while the noradrenaline content of the supernatant fluid was not changed. In Table 2 the noradrenaline contents in the fractions 1 to 5 were added together and considered to represent supernatant noradrenaline, and the sum of fractions 8 to 12 was considered to be the particulate noradrenaline. The results show that nerve stimulation does not change the noradrenaline content of the supernatant

TABLE 2

EFFECT OF SYMPATHETIC NERVE STIMULATION ON THE SUBCELLULAR DISTRIBUTION OF NORADRENALINE IN THE VAS DEFERENS OF RATS

Noradrenaline contents in fractions 1 to 5 (Fig. 1) were added together and considered as supernatant noradrenaline, and those in fractions 8 to 12 as particulate noradrenaline. The amounts of noradrenaline (means and standard errors) were those found in one centrifuge tube, that is the noradrenaline contained in 33 mg of wet tissue. Figures in parentheses indicate the numbers of experiments

Condition	Noradrenaline (ng) in		Ratio P/S
	Supernatant (S)	Particulate (P)	
Control (8)	222±22·2	161±14·4	0·72±0·028
Stimulated (7)	213±16·7	107± 8·5	0·47±0·021
Deviation from control (%)	−4·2	−33·7	−33·8
Significance (P)	>0·7	<0·002	<0·001

fluid, but it decreases by about 34% the noradrenaline content of the particulate fraction. The ratio of particulate noradrenaline to supernatant noradrenaline decreased significantly from 0.72 to 0.47.

DISCUSSION

The change in noradrenaline content produced by coaxial stimulation of the vas deferens might be due to damage to the tissue caused by electric stimulation. However, some of our experiments speak against this possibility: the duration of each rectangular pulse was only 0.2 msec; the response evoked by electrical stimulation was easily blocked by low concentrations of bretylium and guanethidine; the decline of response to coaxial stimulation was reversed in about 1 hr; and the response to application of noradrenaline was not changed by the coaxial stimulation. These results indicate that the muscle is not affected by the stimulation and that the decline of the response must be due to an action on the nerve endings. The unchanged responsiveness to tyramine, an indirectly acting sympathomimetic drug, provides additional support for the view that the noradrenaline storing site of the nerve ending is not impaired by electric current and that the decline of responsiveness to coaxial stimulation is due to a fatigue phenomenon induced by an excessive activity of the nerve.

It follows therefore that the decrease of noradrenaline in the vas deferens is a result of continuous release of the amine caused by repetitive stimulation and that fatigue is due to a decrease of stored noradrenaline. Similar results were obtained by Kernell & Sedvall (1964) in skeletal muscle. Furthermore, the present experiment reveals that the release of noradrenaline induced by nerve impulses is mainly from the particulate fraction. It is reasonable to assume that the particulate noradrenaline may contain several noradrenaline storage compartments and that nerve impulses cannot mobilize all of them, since when fatigue occurred there were still two-thirds of the original noradrenaline in the particulate fraction. However, this result does not necessarily mean that the compartment available to release by nerve impulses is one-third of the particulate noradrenaline. Indeed this compartment might be smaller in size, as generally considered (Gaffney, Chidsey & Braunwald, 1963), and be in equilibrium with other compartments. During the 1-hr period of stimulation, noradrenaline from other compartments may move continuously and become available for the release by nerve impulses. As a result, there would be a greater decrease of noradrenaline content than would be expected from the actual size of the compartment. This kind of movement, however, may not be rapid enough to meet the need of excessive nerve activity in the vas deferens.

It is interesting that tyramine could induce a response immediately after the exhaustive stimulation, although the responsiveness to electrical stimulation was greatly reduced and the noradrenaline content was decreased. This probably indicates that different compartments of noradrenaline are involved in the action of tyramine and in nerve stimulation. This view is further supported by the observations that nerve stimulation can evoke a full response in a preparation which is made tachyphylactic to tyramine (Harrison, Chidsey & Braunwald, 1963) or to amphetamine (Cowan, Cannon, Koppanyi & Maengwyn-Davies, 1961). In this regard, the suggestion made by Campos & Shideman (1962), and Campos, Stitzel & Shideman (1963), that tyramine releases noradrenaline primarily from the "soluble fraction," agrees with our results, although tyramine may also have an effect on the particulate fraction (Bhagat, 1964).

SUMMARY

1. Isolated vas deferens preparations of rats were coaxially stimulated for 1 hr to determine the effect of nerve impulses on the noradrenaline stores.

2. During the stimulation the height of contraction markedly declined, but, after cessation of stimulation, the responsiveness to coaxial stimulation recovered within the next hour.

3. The response to addition of either noradrenaline or tyramine was not reduced even immediately after the stimulation.

4. Coaxial stimulation caused a 27% decrease in total noradrenaline content in 1 hr.

5. Studies of the subcellular distribution of noradrenaline revealed that the amine in the " microsomal fraction " was decreased by 34% whereas that in the supernatant fluid was not affected.

6. It is concluded that the noradrenaline available for release by nerve impulses is in a compartment of particulate amine pools which is different from that on which tyramine acts.

We wish to express our thanks to the China Medical Board of New York for the donation of the Aminco-Bowman spectrophotofluorometer and other equipments used in this experiment. This work was supported in part by National Council on Science Development, Republic of China.

REFERENCES

BHAGAT, B. (1964). Tyramine-induced depletion of cardiac catecholamines and the effects of cocaine and bretylium. *Arch. int. Pharmacodyn.*, **147**, 26–35.

BIRMINGHAM, A. T. & WILSON, A. B. (1963). Preganglionic and postganglionic stimulation of the guinea-pig isolated vas deferens preparation. *Brit. J. Pharmacol.*, **21**, 569–580.

CAMPOS, H. A. & SHIDEMAN, F. E. (1962). Subcellular distribution of catecholamines in the dog heart. *Int. J. Neuropharmacol.*, **1**, 13–22.

CAMPOS, H. A., STITZEL, R. E. & SHIDEMAN, F. E. (1963). Actions of tyramine and cocaine on catecholamine levels in subcellular fractions of the isolated cat heart. *J. Pharmacol. exp. Ther.*, **141**, 290–300.

CHANG, C. C. (1964). A sensitive method for spectrophotofluorometric assay of catecholamines. *Int. J. Neuropharmacol.*, **3**, 643–649.

COWAN, F. F., CANNON, C., KOPPANYI, T. & MAENGWYN-DAVIES, G. D. (1961). Reversal of phenylalkylamine tachyphylaxis by norepinephrine. *Science*, **134**, 1069–1070.

EULER, U. S. VON & HILLARP, N.-Å. (1956). Evidence for the presence of noradrenaline in submicroscopic structures of adrenergic axons. *Nature* (*Lond.*), **177**, 44–45.

GAFFNEY, T. E., CHIDSEY, C. A. & BRAUNWALD, E. (1963). Study of the relationship between the neurotransmitter store and adrenergic nerve block induced by reserpine and guanethidine. *Circulat. Res.*, **12**, 264–268.

HARRISON, D. C., CHIDSEY, C. A. & BRAUNWALD, E. (1963). Circulatory response to cardioaccelerator nerve stimulation after induction of tyramine tachyphylaxis. *Proc. Soc. exp. Biol.* (*N.Y.*), **112**, 37–38.

KERNELL, D. & SEDVALL, G. (1964). Reduction of the noradrenaline content of skeletal muscle by sympathetic stimulation. *Acta physiol. scand.*, **61**, 201–202.

KOPIN, I. J. (1964). Storage and metabolism of catecholamines: the role of monoamine oxidase. *Pharmacol. Rev.*, **16**, 179–191.

LUCO, J. V. & GÕNI, F. (1948). Synaptic fatigue and chemical mediators of the post-ganglionic fibres. *J. Neurophysiol.*, **11**, 497–500.

POTTER, L. T. & AXELROD, J. (1963). Subcellular localization of catecholamines in tissues of the rat. *J. Pharmacol. exp. Ther.*, **142**, 291–298.