EFFECT OF SOME DRUGS ON THE RELEASE OF NORADRENALINE FROM ISOLATED NERVE GRANULES *

U. S. von EULER and F. LISHAJKO

From the Physiology Department, Karolinska Institute, Stockholm, Sweden

THE discovery by Blaschko and Welch (1953) and, independently, by Hillarp *et al.* (1953) that the catecholamines in adrenal medullary cells occur, to the largest part, in subcellular granules, raised the question whether such storage granules may also occur in adrenergic nerves. Several facts pointed to this possibility, among others the fact that the transmitter is unevenly distributed in the axons, being accumulated in the nerve terminals in considerable amount.

1. PREPARATION OF NERVE STORAGE GRANULES

Bovine splenic nerves were homogenated in 0·3 M sucrose and cells and larger particle removed by centrifugation at 1000 × *g*. Subsequent high speed centrifugation yielded a fine granular sediment containing noradrenaline in a concentration 10–20 times higher than in the original material (Euler and Hillarp 1956).

It was later found that squeezing of the gauze-wrapped nerves between nylon cylinders yielded a press juice which contained the particle-bound noradrenaline (Euler 1958). Instead of sucrose, which was unsatisfactory for fluorimetric assay purposes, ice-cold isotonic (0·13 M) neutral potassium phosphate was used for washing the nerves during squeezing. The diluted press juice was freed from small amounts of cell-débris by 5 min centrifugation at about 1000 × *g*. Upon high *g* centrifugation, the supernatant from the low speed centrifugation yielded a small, almost colorless and translucent sediment, which contained noradrenaline in a granule bound form.

The noradrenaline present in the sediment was not oxidized by manganese peroxide and was not adsorbed on alumina, indicating that it was not occurring in free form.

* Supported by grants from the U.S.A.F. Office of Aerospace Research through the European Office under contract AF 61(052)-309 and from The Swedish Medical Research Council.

2. ESTIMATION OF FREE AND BOUND NORADRENALINE IN GRANULE SUSPENSIONS

The noradrenaline present in the granules can be estimated after centrifugation of the suspension at + 4° C for 30 min at 50,000 × *g* and extraction of the sediment-pellet with 0·1 N HCl at pH 2. By addition of a small amount of 2·5% HPO_3 flocculation occurs and the extracted granules can be sedimented by recentrifugation at 10,000 × *g* for 5 min. The noradrenaline extracted into the clear supernatant is estimated by fluorimetric technique according to Euler and Lishajko (1961 b). The free noradrenaline in the supernatant from the original suspension is determined after adsorption on alumina and subsequent elution with acetic acid.

The noradrenaline, released spontaneously or as a result of various factors from resuspended granules, can be directly estimated in the supernatant after resedimentation.

3. YIELD OF BOUND NORADRENALINE FROM SPLENIC NERVES

Using the press juice technique, the total amount of noradrenaline in the original suspension has been 8·7 μg/g nerves, which approximates the amounts found on extraction of splenic nerves (Euler 1949). The mean recovery in the sediment was 28 ± 4·5% of the total amount (Euler and Lishajko 1961 a).

4. SPONTANEOUS RELEASE OF NORADRENALINE FROM GRANULES AT DIFFERENT TEMPERATURES

The granule suspension is stable at 0–4°C for several hours in neutral isotonic potassium phosphate solution. At higher temperatures

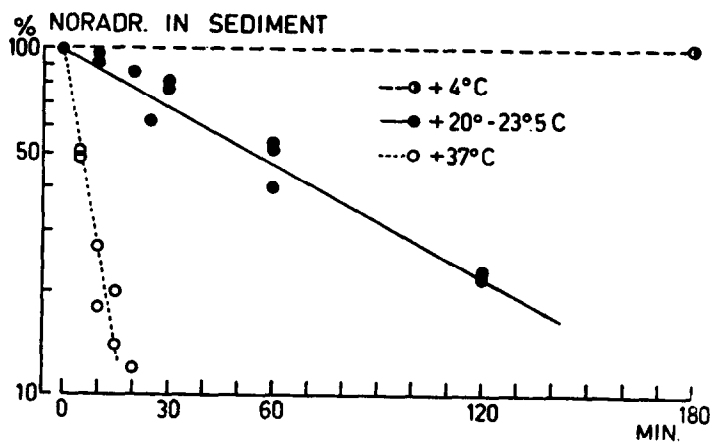

FIG. 1. Effect of temperature on noradrenaline release from isolated bovine splenic nerve granules, incubated in 0·13 M potassium phosphate or 0·15 M NaCl at pH 6·8–7·7. Ordinate: noradrenaline in sediment in per cent of original amount. (From Euler and Lishajko, *Acta physiol. scand.* (1961a, **51** 193).

a spontaneous release is observed. At + 20°C about 70–80 per cent of the bound noradrenaline is liberated in 2 hr into the incubation fluid and can be recovered quantitatively. The release occurs according to an exponential curve (Fig. 1). The rate of release increases rapidly with temperature, thus at 37°C about 70 per cent of the noradrenaline is liberated in 10 min. In most experiments the release has been studied at + 20°C which gives a convenient liberation rate.

5. EFFECTS OF ACIDS, DETERGENTS, OSMOLARITY

Moderate changes in osmolarity in the suspension do not influence the spontaneous rate of release of catecholamine from the nerve as strongly as from the adrenal medullary granules, and the same is true for freezing and thawing.

Addition of acid causes an immediate release of all the stored transmitter even at low temperature when the reaction is at pH 3 or less. At pH 5 the releasing effect is very weak.

Detergents cause a rapid liberation of the amine from the granules, presumably by disorganizing the membrane. Sodium lauryl sulphonate in 10^{-3} M solution causes more than 90 per cent release in 30 min at + 20°C.

6. EFFECT OF RESERPINE

Incubation of the original suspension or of resuspended bovine splenic nerve granules with reserpine in concentrations of $0{\cdot}075–0{\cdot}75 \times 10^{-3}$ M causes an increased rate of release (Euler and Lishajko 1960 a). In lower concentrations, however, reserpine exerts the opposite effect, causing a very marked inhibition of the spontaneous release. This inhibitory effect was maximal in the concentration range of $10^{-5}–10^{-6}$ M, but was observed even in concentrations as low as about 10^{-8} M when the granules were subjected to a preincubation period of 30 min at + 4°C, which in itself does not cause any release (Euler and Lishajko 1961 c).

The effect of reserpine has also been studied in relation to tyramine. This amine causes a release of catecholamines from isolated granules both from adrenal medullary cells and from nerves as found by Schümann (1960) and confirmed by ourselves (Euler and Lishajko 1960 b). It was now observed that the releasing effect of tyramine was completely blocked by reserpine in higher concentrations (about 10^{-5} M). In the lower concentration area ($10^{-7}–10^{-8}$ M) reserpine had a marked inhibitory effect of its own, but did not prevent the releasing effect of tyramine. A competitive effect of tyramine and reserpine was therefore apparent (Fig. 2).

The inhibitory action of reserpine could also be demonstrated on adrenal medullary granules from cat and rabbit, although it was much

less marked than on the nerve granules. The average percentage release, in 2 hr at +20°C, for adrenaline in the cat adrenal was 82 per cent, and with reserpine $1{\cdot}6 \times 10^{-5}$ M 74 per cent. For noradrenaline no inhibitory effect of reserpine could be observed on the release rate.

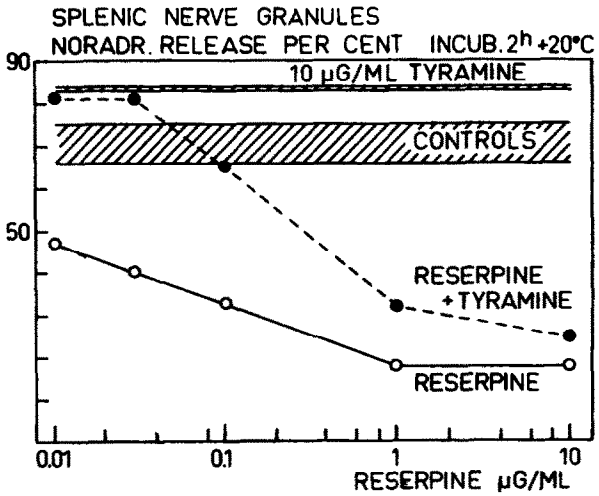

FIG. 2. Ordinate: Per cent release of original amount of noradrenaline in isolated bovine splenic nerve granules on incubation for 2 hr at +20 °C in isotonic neutral potassium phosphate. Enhancing effect of tyramine and inhibitory effect of reserpine in comparison with controls. Low concentrations of reserpine do not influence tyramine action, while higher concentrations block the tyramine effect. (From Euler and Lishajko, *Acta physiol. scand.* (1961 c, **52** 137).

In experiments on isolated adrenal medullary granules from the rabbit the effect of reserpine was more marked. Here, too, the spontaneous rate of release was considerably slower. Reserpine in $1{\cdot}6 \times 10^{-5}$ M concentration lowered the release from 41 per cent (2 hr, + 20°C) to 17 per cent during the same time.

The releasing effect of tyramine could also be partially blocked by reserpine. After an i. v. injection of dopa, 100 mg/kg, the spontaneous rate of release of adrenaline from rabbit adrenal granules was greatly enhanced. At the same time the inhibitory effect of reserpine was diminished.

7. OTHER DRUGS

The effects of acetylcholine, histamine, nicotine, 5-hydroxytryptamine and GABA in different concentrations were also tested on the noradrenaline release rate in isolated splenic nerve granules. No change in release rate was observed, indicating that, *in vivo*, the catecholamine liberating effect of acetylcholine, nicotine and histamine is not on the granules but presumably on the axon membrane.

A number of other drugs have also been tested with regard to their effects on the rate of release, in particular some compounds known to exert an influence on the release of the transmitter *in vivo*, such as bretylium, guanethidine xylocholine, and phenoxybenzamine.

Guanethidine (Ismelin), Bretylium (Darenthin)

Neither bretylium nor guanethidine in the approximate concentration range of 10^{-5}–10^{-3} M had any definite effect on the noradrenaline release rate, either on isolated nerve granules or on adrenal medullary granules from rabbit.

Xylocholine (TM-10) and Related Drugs

Xylocholine exerted a marked inhibitory effect on the spontaneous release of noradrenaline from the nerve granules in a concentration of 0·05 M. A related compound, β-methyl TM-10, had a stronger effect of this kind, causing a marked inhibition in 0·01 M concentration. TM-6 (*p*-tolyl-choline-ether bromide) had a similar action in 0·01 M concentration. In higher concentrations (0·05–0·1 M) both of these compounds caused an increased rate of release. Tyramine ($7{\cdot}3 \times 10^{-5}$ M) reduced the inhibitory effect of β-methyl TM-10.

Phenoxybenzamine (Dibenyline)

This compound inhibited the noradrenaline release from granules to a very marked degree, in concentrations of 10^{-3}–10^{-4} M, but had a definite effect even in concentrations less than 10^{-5} M. No certain effect was observed with $1{\cdot}5 \times 10^{-4}$ M on adrenal medullary granules from rabbit. When isolated granules from the bovine adrenal medulla were used, phenoxybenzamine caused a very strong release of both adrenaline and noradrenaline, less than 10 per cent of the control amount being left in the granules after 4 hr incubation at 20 °C.

Cocaine, Xylocaine

In higher concentrations, about 0·1 M, the local anesthetics cocaine and xylocaine caused a rapid release of the transmitter in the granules. At lower concentrations this effect was reversed, a marked inhibitory effect being noticed in 10^{-2} M solution. In 10^{-4} M solution no consistent effect was observed, however. Xylocaine (Lidocaine) and cocaine had quite similar effects.

Tetracaine and procaine had a marked inhibitory effect on the release, in $0{\cdot}3 \times 10^{-2}$ M solution, tetracaine also in $0{\cdot}3 \times 10^{-3}$ M, but none of them had any definite effect in $0{\cdot}3 \times 10^{-4}$ M.

Uptake of Catecholamines in Nerve Granules

Incubation of bovine splenic nerve granules, with noradrenaline (10 μg/ml) almost wholly prevents the depletion seen on incubation for

2 hr at +20 °C. This may be interpreted as an equilibrium between uptake and loss, or explained by ussuming a blocking effect of exogenous noradrenaline on the release.

After partial depletion of bovine splenic nerve granules by incubation at +20 °C for 90 min, causing a loss of some 70 per cent of the original amount, addition of noradrenaline (10 μg/ml) and incubation for 30 min at +20 °C caused a net uptake of approximately 50 per cent of the amount of noradrenaline left in granules. The net uptake was measured as the difference between the total uptake and the amount adhering to the granules, estimated after immediate centrifugation at low temperature where uptake is small. Addition of adrenaline caused an uptake of this amine in the granules to a similar absolute amount.

The figure given for the uptake of noradrenaline is probably a minimum figure, since some depletion might occur concomitantly with the uptake during the 30 min incubation with noradrenaline. Furthermore the net figure is probably somewhat too low since even at low temperature a small uptake may take place, the figure subtracted to give the net uptake is probably a little too high.

No evidence for an uptake was observed, when the noradrenaline concentration in the incubation fluid was 1 μg per ml or less.

The uptake of noradrenaline into depleted granules exposed to noradrenaline (10 μg/ml) in the incubation fluid was not diminished in the presence of reserpine ($0{\cdot}6 \times 10^{-5}$ M).

DISCUSSION

The results indicate that the rate of spontaneous release of noradrenaline, from a suspension of nerve catecholamine storage granules at 20 °C, can be readily influenced by a number of drugs. It is noteworthy that autonomic drugs like acetylcholine, nicotine and histamine have no effect in this respect and thus presumably do not exert their action on the granules. Other drugs like reserpine, xylocholine and others have, at least in lower concentrations, an effect which may be regarded as specific and possibly related to the known pharmacodynamical effect of these substances on the transmitter release *in vivo*. On the other hand it appears probable that the effects, seen on incubation with, for example, reserpine and cocaine in higher concentrations, are to a large part unspecific and presumably of limited significance.

As to the effect of reserpine, the inhibitory action on the release can be clearly seen in concentrations down to 10^{-8} M. Whether this inhibitory effect can contribute to the understanding of the depleting effect of reserpine on the catecholamine content of organs is not clear. It is tempting to assume, however, that this alkaloid causes a disturbance of the normal transfer of amines across the granule border by interfering with some vital process. The fact that reserpine also prevents

the exchange release action caused by tyramine speaks in favour of this assumption.

On the adrenal medullary granules the effect of reserpine was much less apparent; this, again might be connected with the well known fact that reserpine has a much weaker effect on the depletion of catecholamines from the adrenal medulla than from the organs. This was particularly well seen with granules from the cat and less so from the rabbit, which is in harmony with the difference in action of reserpine on the adrenal medullary stores in these two animals (Kroneberg and Schümann 1957).

The action of phenoxybenzamine is also noteworthy, an inhibitory action of the noradrenaline release being observed in concentrations of less than 10^{-5} M or 1 μg per ml. No other substances were found to be active in such low concentrations except reserpine.

SUMMARY

Noradrenaline containing storage granules, prepared from bovine splenic nerves, retain their content in neutral isotonic potassium phosphate for several hours at 0–4 °C but give off noradrenaline at increasing rates at higher temperatures.

This spontaneous release can be enhanced by tyramine in $0{\cdot}2 \times 10^{-4}$–10^{-3} M concentration.

The release rate at +20 °C is increased by a number of drugs such as reserpine (10^{-3} M), xylocholine and β-methyl xylocholine (10^{-1} M), cocaine and xylocaine (Lidocaine) (10^{-1} M).

An inhibition of the release rate at +20 °C is observed with reserpine (10^{-8}–10^{-6} M), β-methylxylocholine (10^{-2} M), cocaine and xylocaine (10^{-3} M), and phenoxybenzamine (10^{-5}–10^{-3} M).

Phenoxybenzamine exerts a releasing action in 10^{-3} M concentration on isolated granules from rabbit's and cow's adrenal medulla.

After spontaneous depletion at +20 °C, isolated bovine splenic nerve granules are able to take up adrenaline as well as noradrenaline when these amines are present in the incubation fluid in concentrations of 5–20 μg/ml. This uptake is not prevented by reserpine in $0{\cdot}6 \times 10^{-5}$ M solution.

REFERENCES

BLASCHKO, H. and WELCH, A. D (1953) *Arch. Exp. Path. Pharmak.* **219** 17–22.
VON EULER, U. S., (1949) *Acta Physiol. Scand.* **19** 207–214.
VON EULER, U. S., (1958) *Acta Physiol. Scand.* **43** 155–166.
VON EULER, U. S. and HILLARP, N.-Å. (1956) *Nature, Lond.* **177** 44–45.
VON EULER, U. S. and LISHAJKO, F. (1960 a) *Science* **132** 351–352.
VON EULER, U. S. and LISHAJKO, F. (1960 b) *Experientia* **16** 376–377.
VON EULER, U. S. and LISHAJKO, F. (1961 a) *Acta Physiol. Scand.* **51** 193–203.
VON EULER, U. S. and LISHAJKO, F. (1961 b) *Acta Physiol. Scand.* **51** 348–355.

VON EULER, U. S. and LISHAJKO, F. (1961 c) *Acta Physiol. Scand.* **52** 137–145.
HILLARP, N.-Å., LAGERSTEDT, S. and NILSON. B. (1953) *Acta Physiol. Scand.* **29** 251–263.
KRONEBERG, G. and SCHÜMANN, H. J. (1957) *Arch. Exp. Path. Pharmak.* **231** 349–360.
SCHÜMANN, H. J. (1960) *Arch. Exp. Path. Pharmak.* **238** 41.