Br. J. Pharmac. Chemother. (1967), **30**, 4–10.

SUBCELLULAR DISTRIBUTION OF NORADRENALINE AFTER COLD EXPOSURE

BY

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(*Received July* 8, 1966)

Several studies in recent years have suggested that noradrenaline in tissues is present in more than one compartment (Trendelenburg, 1961 ; Potter, Axelrod & Kopin, 1962 ; Harrison, Chidsey & Braunwald, 1963). For instance, tyramine administered 30 min after injection of H^3-noradrenaline releases a large proportion of the labelled amine present in the heart while 24 or 48 hr after injection of H^3-noradrenaline a much smaller percentage of the H^3-noradrenaline is liberated (Potter *et al.*, 1962 ; Iversen & Whitby, 1963). When tachyphylaxis to tyramine is attained by prolonged infusion of this drug the response to nerve stimulation still persists (Harrison *et al.*, 1963).

Anatomical compartmentalization of noradrenaline has also been described: disruption of tissues by homogenization and differential centrifugation yield several subcellular fractions, each containing some of the noradrenaline. Noradrenaline-containing particles sedimented with the microsomal fraction have been tentatively identified with synaptic vesicles, seen in electron micrographs (Wolfe, Potter, Richardson & Axelrod, 1962 ; Burnstock & Holman, 1963). It has been suggested that release of noradrenaline from nerve endings proceeds in discrete " packages," similar to release of acetylcholine, and that these " packages " may be identical with the synaptic vesicles (Burnstock & Holman, 1962, 1963). However, this has not been directly demonstrated. Attempts to correlate the fraction of noradrenaline released by a drug (tyramine) with one of the subcellular fractions have given conflicting results (Campos, Stitzel & Shideman, 1963 ; Iversen & Whitby, 1963 ; Bhagat, 1964).

It seemed of interest, therefore, to investigate whether any changes in subcellular distribution of noradrenaline result from the release of noradrenaline induced by increased sympathetic discharge during acute exposure to cold—that is, whether the release is from a specific subcellular fraction.

METHODS

Male Sprague-Dawley rats weighing 250–300 g were used. A group of eight animals was used for each experiment; four rats were kept at room temperature (26° C) and four rats were exposed to cold (−15° C) for 90 min. The rats were killed by fracture of the neck. The hearts and spleens were placed into ice-cold medium, cut with scissors and then homogenized in an all-glass conical homogenizer. The hearts were homogenized for 2 min and the spleens for 40–50 sec.

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The medium consisted of 0.27 M sucrose containing 0.1% EDTA and 10^{-3} M β-phenylisopropylhydrazine. The homogenates were centrifuged for 10 min at 900 *g* and the sediment was separated (=coarse fraction). The supernatant was centrifuged at 100,000 *g* for 30 min and the pellet (=particulate fraction) was separated from the supernatant (=soluble fraction). Cold sucrose medium was added to the coarse fraction and to the particulate fraction to a final volume equal to that of the soluble fraction. All fractions were deproteinized with 0.2 volume of 10% metaphosphoric acid. The pH was then adjusted to 8.4 and the solutions were immediately poured on alumina columns. The catecholamines were eluted with 0.2 M acetic acid and assayed at pH 6.5 with the trihydroxyindole method according to Kahane & Vestergaard (1965) modified by oxidizing for 15 min in ice and irradiating for 7 min. Recovery of added CA ranged from 70–80%.

The proportion of adrenaline in heart and spleen is very small (4–7% of the total catecholamines (Leduc, 1961)); the results are therefore expressed as noradrenaline. Since the weight of the spleen varies considerably from animal to animal, and furthermore, the spleen contracts during exposure to cold, it seemed preferable to compare the total amounts of noradrenaline in the fractions from spleen rather than concentrations per gram spleen. Drugs: phenoxybenzamine (Dibenzyline= SK&F No. 688-A) was injected intraperitoneally 20 min before exposure to cold.

TABLE 1

NORADRENALINE CONTENT OF SUBCELLULAR FRACTIONS OF RAT HEART AND SPLEEN

	Coarse	Particulate	Soluble	Total
Heart	0·26±0·05	0·22±0·03	0·25±0·04	0·73±0·13
Spleen	0·99±0·23	0·58±0·10	0·46±0·12	2·03±0·32

The figures represent μg noradrenaline/g tissue for heart and μg noradrenaline/spleen for spleen. The values were derived from 7 experiments, uncorrected for recovery rate, and given as means ±S.E. The fractions were prepared as specified under Methods.

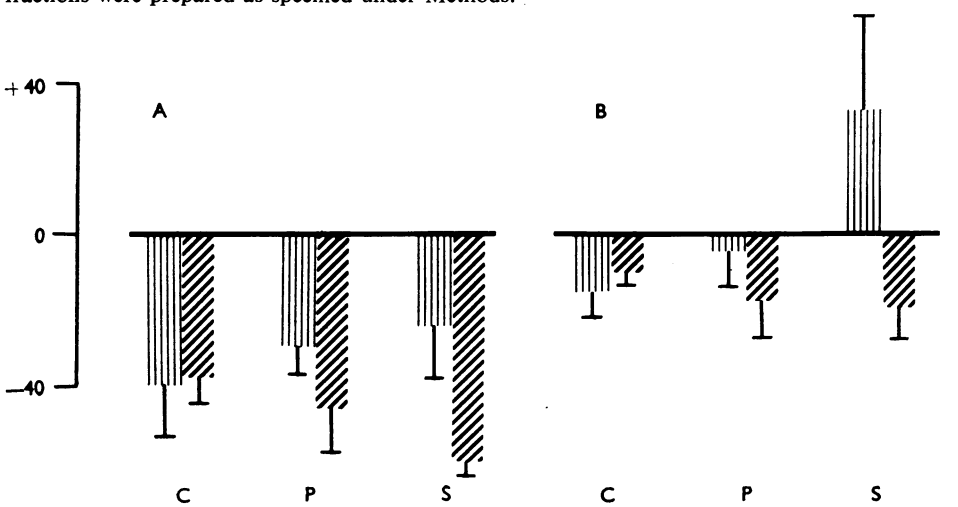

Fig. 1. Changes of noradrenaline in subcellular fractions of heart and spleen during cold exposure (vertical stripe columns) and during cold exposure under phenoxybenzamine, 10 mg/kg intraperitoneally (diagonal stripe columns). Ordinate—change of noradrenaline in the subcellular fraction from the experimental rats as % of the corresponding fraction from the controls. The differences between the values of the experiments with and without phenoxybenzamine are not statistically significant for the coarse and particulate fractions. For the soluble fraction the differences are significant ($P<0.05$) in both heart and spleen. O=control level. Vertical bars=standard error. A=heart; B=spleen; C=coarse fraction; P=particulate fraction; S=soluble fraction. Cold exposure=mean of 7 experiments; cold exposure under phenoxybenzamine=mean of 5 experiments.

RESULTS

The concentrations of noradrenaline in subcellular fractions of rat heart and spleen are given in Table 1. Acute exposure to cold caused depletion of 31% of the noradrenaline from heart while only 5% was released from the spleen. Noradrenaline was released from all three subcellular fractions of the heart, as seen in Fig. 1. The decrease in noradrenaline was largest in the coarse fraction. The small decrease of noradrenaline in the spleen was the net effect of a larger decrease in the coarse fraction and an increase in the soluble fraction, the particulate fraction showing insignificant change.

Since part of the noradrenaline released by nerve stimulation can be taken up again into tissue stores, particularly in spleen (Brown, 1965), we have carried out a second series of experiments under phenoxybenzamine which blocks tissue uptake of noradrenaline (Hertting, Axelrod & Whitby, 1961 ; Axelrod, Hertting & Potter, 1962).

Exposure to cold under these conditions resulted in depletion of 48% of the noradrenaline of heart and 14% of the noradrenaline of spleen. The pattern of release from the different subcellular fractions changed significantly as seen in Fig. 1. Thus, while the decrease in the coarse fraction was similar to that in the first series of experiments, the decrease in the soluble fraction was considerably larger, in both heart and spleen. The depletion of the particulate fraction was a little larger in both organs.

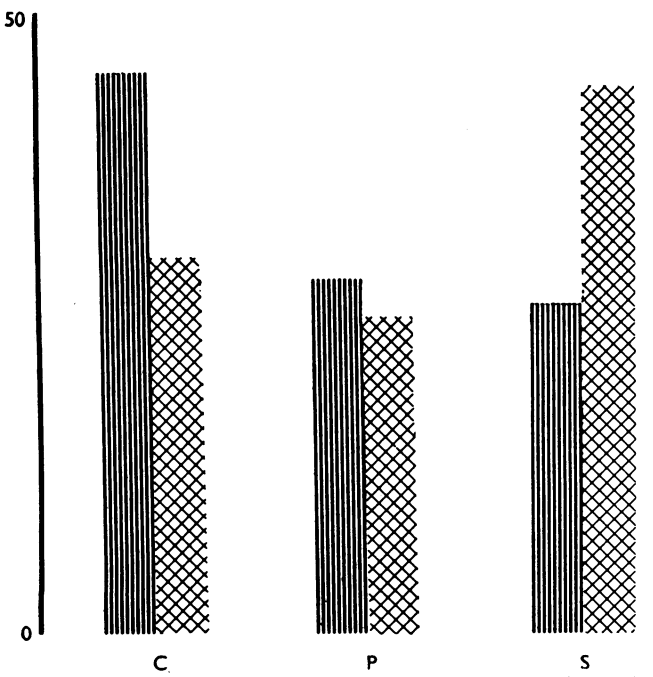

Fig. 2. Subcellular origin of noradrenaline released from rat heart during cold exposure (vertical columns, mean of 7 experiments) and during cold exposure under phenoxybenzamine, 10 mg/kg intraperitoneally (cross-hatched columns, mean of 5 experiments). Ordinate = % of total noradrenaline release. C = coarse fraction, P = particulate fraction, S = soluble fraction. Note that Fig. 1 presents changes in the subcellular distribution of NE *retained* in the tissues after cold exposure while Fig. 2 presents the subcellular origin of noradrenaline *released* from the tissues during cold exposure.

A more striking comparison is obtained on analysing the subcellular origin of the released noradrenaline rather than the distribution of noradrenaline retained in the organs. The results of this comparison for the heart are shown in Fig. 2. Here the contribution of each fraction to the total noradrenaline released is plotted for the two series of experiments, cold exposure and cold exposure under phenoxybenzamine. In cold exposure the contribution of the coarse fraction is larger and that of the soluble fraction is smaller than the contributions of the same fractions to noradrenaline release under phenoxybenzamine. The spleen showed a similar pattern: while >90% of noradrenaline released during cold exposure originated from the coarse fraction, the latter contributed only 26% of the noradrenaline released under phenoxybenzamine. On the other hand, the soluble noradrenaline fraction of spleen which was not depleted at all during cold exposure released noradrenaline under phenoxybenzamine to the extent of 35% of the total liberated. The particulate fraction of spleen also contributed a larger proportion under phenoxybenzamine (39%) compared to the experiments without this drug (7%).

Phenoxybenzamine itself can cause some depletion of tissue catecholamines (Leduc, 1961; Axelrod *et al.*, 1962). This may be attributed to the "spontaneous" release of noradrenaline due to impulses arriving at the nerve-endings. By blocking re-uptake of the released transmitter, phenoxybenzamine decreases tissue levels of noradrenaline. We have studied the contributions of the three subcellular fractions to the noradrenaline released at room temperature (26° C) under phenoxybenzamine to see whether the subcellular origin of noradrenaline discharged during "normal" sympathetic activity is different from that discharged during the increased activity induced by cold. Figure 3 shows the subcellular origin of noradrenaline released at room temperature under

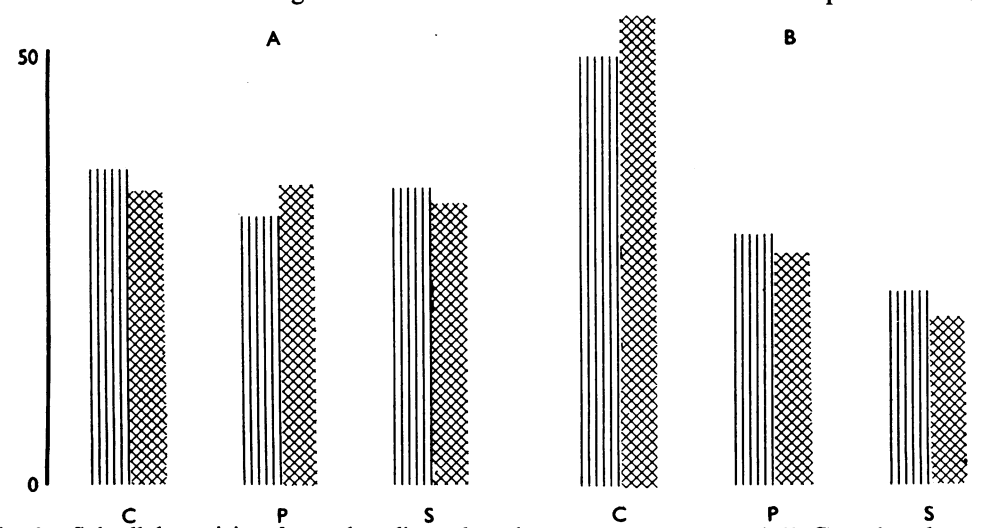

Fig. 3. Subcellular origin of noradrenaline released at room temperature (26° C) under phenoxybenzamine. Vertical stripe columns: initial distribution of noradrenaline in control organs; ordinate = % of total noradrenaline in the tissue. Cross-hatched columns: noradrenaline released in the experimental group; ordinate = % of total noradrenaline released. A = heart; B = spleen; C = coarse fraction; P = particulate fraction; S = soluble fraction. Note that the % of released noradrenaline originating from each fraction is not significantly different from the % of total tissue noradrenaline initially present in the same fraction.

phenoxybenzamine compared to the subcellular distribution of tissue noradrenaline in the same organs of the control animals. No significant difference in distribution was observed between the released noradrenaline and tissue noradrenaline in either heart or spleen.

DISCUSSION

Increased release of noradrenaline from tissue stores during cold exposure has previously been reported (Leduc, 1961). This is presumably due to increased sympathetic activity as suggested by blockade of the release when ganglion-blocking agents are administered (Leduc, 1961). The relatively small release of noradrenaline from the spleen compared to the heart in our experiments may be due to more intense sympathetic activity in the heart during cold exposure. However, the marked contraction of the spleen observed under these conditions indicates increased nerve impulses to the spleen too. According to recent hypotheses, released noradrenaline is partly inactivated not through metabolism but by re-uptake into tissue stores (Axelrod, 1965). A possible explanation for the difference in noradrenaline depletion between the heart and spleen may, therefore, be more efficient re-uptake of the released noradrenaline in the spleen. The contraction of the spleen may enhance the re-uptake process by decreasing the blood flow through this organ and thus diminishing the washout of liberated noradrenaline. The importance of the noradrenaline uptake process in spleen has been clearly demonstrated in the experiments of Brown (1965) and of Thoenen, Hürlimann & Haefely (1963). The spleens of rats exposed to cold under phenoxybenzamine were not contracted due to the adrenergic blocking effect of this drug.

Our experiments show that the noradrenaline released from the heart during cold exposure derives from all three subcellular fractions: coarse, particulate and soluble. This may indicate that nerve impulses do not liberate noradrenaline from a specific compartment within the cell, for example, the particulate fraction. However, it is also possible that the initial effect of nerve impulses is mainly on one compartment but that subsequent rapid redistribution of noradrenaline between all compartments masks the initial effect.

A further complication is the re-uptake process. Thus, after exposure to cold the noradrenaline in the soluble fraction from spleen increased, while it decreased in the other fractions (Fig. 1), a finding reminiscent of that reported by Bhagat for rat heart immediately after tyramine administration (Bhagat, 1964). The increase in the soluble noradrenaline fraction of spleen may have been due to an initial re-uptake of released noradrenaline into this fraction which later equilibrated with the other compartment(s).

This assumption was supported by the finding that after administration of a blocker of noradrenaline uptake (phenoxybenzamine) the contribution of the soluble fraction to the noradrenaline released during cold exposure increased considerably in both the heart (Fig. 2) and spleen. It seems, therefore, that re-uptake may result in an underestimate of the contribution of the soluble fraction to noradrenaline release during cold exposure.

It is noteworthy that noradrenaline released at room temperature following phenoxybenzamine administration derives from all three subcellular fractions proportionately to their noradrenaline content (Fig. 3). It seems difficult to explain the differences between the contribution of the subcellular fractions to the noradrenaline released at room temperature and that released during cold exposure. According to a recent report cold

exposure induces increased noradrenaline synthesis (Goldstein & Nakajima, 1966). Since the last step in noradrenaline synthesis (dopamine→noradrenaline) is considered to be carried out within the storage particles it is possible that increased synthesis during cold exposure under phenoxybenzamine could have replenished to some extent the depleted particulate fraction with the result that the apparent contribution of the soluble fraction to the net release of noradrenaline was increased, while at room temperature resynthesis might have been of lesser magnitude and, therefore, did not " distort " the contributions of the different fractions.

While our experiments were in progress Chang & Chang (1965) reported the selective release of noradrenaline from the particulate fraction of the isolated vas deferens of the rat following nerve stimulation. The lack of such selective release in our experiments during cold exposure may be due to differences in the organs studied (heart and spleen) or the methods used to induce release of noradrenaline (electrical stimulation of a nerve in the experiments of Chang & Chang, 1965). The use of an isolated organ *in vitro* also deprives the tissue of its normal circulation. One possible function of the circulation may be washout of noradrenaline released during stimulation. In *in vitro* experiments, washout would be dependent on diffusion only ; as this delays the removal of noradrenaline the re-uptake may become more effective and diminish the net loss from the soluble fraction (see our experiments with the spleen, Fig. 1). It is noteworthy that in the detailed distribution of noradrenaline in a sucrose gradient given by Chang & Chang there is a small increase in the soluble noradrenaline fraction from vas deferens following nerve stimulation.

Finally, we would like to draw attention to a subcellular fraction which has been neglected in many studies on noradrenaline distribution—that is, the coarse fraction. It is assumed that this fraction consists of incompletely broken cells, nuclei, and large fragments of cells. Presumably the coarse fraction contains both " particulate " and " soluble " noradrenaline. One would assume, therefore, that following partial depletion of the amine the effect on the " coarse fraction " would be intermediate between the effect on the particulate and that on the soluble fraction. Our experiments do not support this expectation: in both heart and spleen, especially in the latter, the contribution of the coarse fraction to noradrenaline release during exposure to cold was larger than its initial proportion of total noradrenaline. It is interesting that a similar result can be obtained on calculating the contribution of the coarse fraction from the data given by Chang & Chang (1965) in the studies of the rat vas deferens. Thus the contribution of the coarse fraction (= the difference between total noradrenaline and soluble + particulate noradrenaline) is 60% of the total noradrenaline released by nerve stimulation while the coarse fraction accounts for only 40% of the initial noradrenaline in the vas deferens. This shows a considerably larger contribution of the coarse fraction to noradrenaline release by nerve stimulation than would be expected from its proportion in the total noradrenaline in the tissue. However, the physiological role of this fraction, if any, is not clear. The coarse fraction may contain, for example, some undamaged cell membranes with noradrenaline-containing particles attached to it. Such membrane-bound particles may be the prime target for noradrenaline release induced by nerve stimulation. Further experiments are necessary to test this hypothesis and the possible significance of the noradrenaline in the coarse fraction.

SUMMARY

1. The subcellular distribution of noradrenaline in heart and spleen was studied in rats after cold exposure, with and without the administration of phenoxybenzamine.

2. Exposure to − 15° C for 90 min induced release of noradrenaline from the coarse, particulate, and soluble fractions of heart. The coarse fraction was affected most, the soluble fraction least. In the spleen, noradrenaline was released from the coarse fraction but the soluble noradrenaline fraction increased.

3. In cold exposure under phenoxybenzamine, the contribution of the soluble fraction to noradrenaline release increased considerably in both heart and spleen. The contribution of the coarse fraction did not change significantly. A small increase in noradrenaline release was found in the particulate fraction.

4. At room temperature phenoxybenzamine induced release of noradrenaline from the subcellular fractions of heart and spleen in proportion to their initial content of noradrenaline.

5. These observations are discussed in relation to the re-uptake of released noradrenaline and to its intracellular location before release.

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