J. Pharm. Pharmac., 1967, **19**, 313–317 Received November 18, 1966

Noradrenaline-phospholipid interactions

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The uptake of (±)-[^{3}H]noradrenaline from an aqueous phase to an ether phase containing dissolved lecithin has been measured. No differences between the behaviour of (+)- or (−)-noradrenaline in this system could be detected. The biological implications of this finding are discussed.

ASSOCIATION of noradrenaline with phospholipids has received sporadic attention. It was found that phospholipids were able to remove catecholamines from the aqueous to the hydrophobic phase in biphasic systems containing chloroform (Kendall, 1942) or ether (Euler, 1946a, b; Norlander, 1950). Others (Mass & Colburn, 1965) found that noradrenaline interacted with phospholipids and metal ions to give ether-soluble complexes.

More recently membrane lipids have been implicated in the interactions of catecholamines with their receptors. For instance, Dikstein & Sulman (1965) found that when rabbit aortic strips were treated with dibenamine the drug was bound to a cephalin fraction. Adrenaline was able to protect the cephalin from interactions with dibenamine. Most recently, Naylor (1966) found that catecholamines facilitated the transfer of calcium ions to the chloroform phase in a biphasic system containing heart lipids. The opposite effect was observed with the β-blocking drugs, propranolol and pronethalol.

Thus, various authors have concluded that lipids are involved in catecholamine uptake, storage and receptor interaction. One simple way to test this hypothesis is to examine whether the two stereoisomers of noradrenaline show unequal properties in model systems. The interaction between catecholamines and their receptors is certainly stereospecific, as is the uptake of catecholamines into sympathetic nerve terminals at physiological concentrations (Iversen, 1963; Beaven & Maickel, 1964).

Experimental

Two types of experiments were made. First, the uptake of [^{3}H]-noradrenaline by lecithin in ether at a constant catecholamine concentration, but with a varying ratio of labelled to unlabelled material, was measured. Second, the uptake by lecithin of [^{3}H]noradrenaline at increasing concentrations of unlabelled noradrenaline was determined, the concentration of the labelled material remaining constant.

In all instances the biphasic system consisted of 4 ml lecithin-ether solution (10 mg/ml) with 10 ml of aqueous phase. The aqueous phase consisted of 5 ml 0·2M phosphate buffer (pH 6·5) and 5 ml 0·01N hydrochloric acid containing the dissolved noradrenaline. After preparation the systems were shaken gently for 15 min and then allowed to separate into two phases. One ml samples of the ether phase were added to 3 ml ethanol and 10 ml phosphor [toluene with 4 g/litre 2,5-diphenyl-

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oxazole and 100 mg/litre 1,4-bis-2(5-phenyloxazolyl)-benzene] and the radioactivity measured in a liquid scintillation counter (Nuclear Chicago). Controls for uptake of radioactivity by ether alone, inherent radioactivity of ether and lecithin and for variations in counting efficiency were made.

The lecithin used was highly purified egg lecithin supplied by Mann Research Laboratories Inc. The [^{3}H]noradrenaline was supplied by the Radiochemical Centre, Amersham, and was the racemic form.

Results

UPTAKE OF [^{3}H]NORADRENALINE BY LECITHIN AT CONSTANT TOTAL NORADRENALINE CONCENTRATION

In these experiments the total noradrenaline concentration was 200 ng/ml in the aqueous phase of which the labelled material constituted 0·395 to 12·6 ng/ml (2·5 to 80 mμc/ml). In separate experiments the unlabelled noradrenaline was either the (+)- or (−)-isomer. In Fig. 1 the uptake of [^{3}H]noradrenaline in the presence of (+)- and (−)-noradrenaline is shown, and is compared with that obtained in the absence of unlabelled noradrenaline.

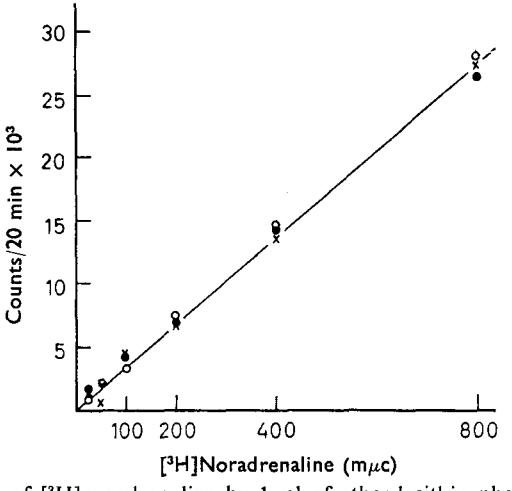

FIG. 1. Uptake of [^{3}H]noradrenaline by 1 ml of ether-lecithin phase with varying concentrations of [^{3}H]noradrenaline in the aqueous phase. ● [^{3}H]noradrenaline alone, ○ in the presence of (−)-noradrenaline and × in the presence of (+)-noradrenaline.

It is obvious that the uptake of [^{3}H]noradrenaline by lecithin is the same in the presence of (+)- or (−)-noradrenaline, or in their absence. From this certain conclusions can be drawn. First, from the linear uptake curve obtained in the absence of unlabelled noradrenaline it is clear that,

$$\text{Uptake} = K \times [[^3\text{H}]\text{noradrenaline}]$$

Provided the concentration of noradrenaline is much smaller than the concentration required to produce saturation then uptake in the presence of unlabelled noradrenaline will be,

$$\text{Uptake} = K \times [[^3\text{H}]\text{noradrenaline}] + K' \times [(-)\text{-noradrenaline}] + K'' \times [(+)\text{-noradrenaline}]$$

that is, the uptake of radioactivity will be unaltered. As the total amount of catecholamines remained constant in these experiments, Fig. 1 may be interpreted to mean that (*a*) (+)- and (−)-noradrenaline show an equal affinity for lecithin, or (*b*) either (+)- or (−)-noradrenaline show no affinity for lecithin, that is the curve obtained is identical with that for [^{3}H]noradrenaline alone.

Although the latter possibility is unlikely, the data of Fig. 1 must be analysed further. Conversion of counts/min to disintegrations/min allows the uptake of [^{3}H]noradrenaline to be calculated as a percentage of the total catecholamine present. The results are presented in Fig. 2.

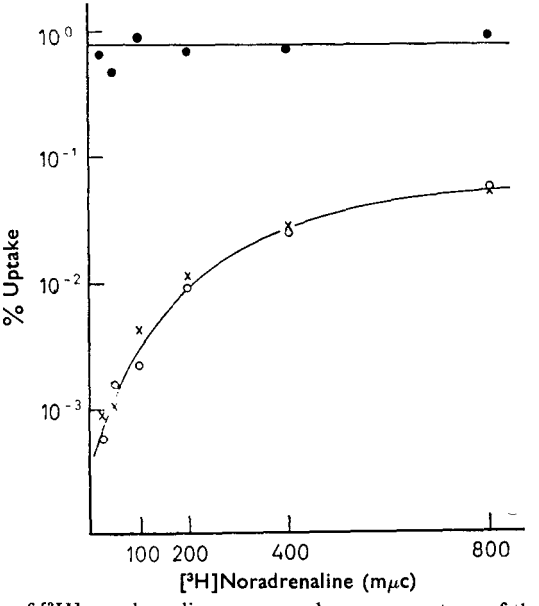

FIG. 2. Uptake of [^{3}H]noradrenaline, expressed as a percentage of the total amount of catecholamine present, with varying concentrations of [^{3}H]noradrenaline. ● [^{3}H]noradrenaline alone, ○ in the presence of (−)-noradrenaline, and × in the presence of (+)-noradrenaline.

In the absence of unlabelled noradrenaline the percentage uptake of tritiated material remained constant, 1 ml of the ether-lecithin phase taking up 0·8% of the total catecholamine present in the aqueous phase. In the presence of (+)- or (−)-noradrenaline the percentage uptake of labelled material was reduced by equal amounts. From this it must be concluded that both (+)- and (−)-noradrenaline show an equal affinity for lecithin.

UPTAKE OF [^{3}H]NORADRENALINE AT CONSTANT CONCENTRATION BY LECITHIN WITH INCREASING CONCENTRATIONS OF UNLABELLED (+)- OR (−)-NORADRENALINE

In these experiments the concentration of [^{3}H]noradrenaline in the aqueous phase remained constant at 10 mμc/ml. Noradrenaline in the aqueous phase is able to react reversibly with lecithin dissolved in

the ether phase thus,

$$L + N \rightleftharpoons LN$$

Control experiments showed that ether alone was only able to take up minute amounts of noradrenaline from the aqueous phase at pH 6·5, so that almost all the noradrenaline taken up by the ether phase is associated with lecithin. From mass action considerations it is known that the linear relation between uptake and concentration will no longer hold as saturation is approached. The decrease of the proportionality constant is related to the reduced uptake of [^{3}H]noradrenaline in the presence of increasing concentrations of (−)-noradrenaline. The reduction in uptake of [^{3}H]noradrenaline in the presence of increasing concentrations of (−)-noradrenaline is shown in Fig. 3. Assuming an initial proportionality constant of 0·8% (from Fig. 2) the amount of (−)-noradrenaline taken up per ml of ether-lecithin solution can be calculated for various concentrations of (−)-noradrenaline. The calculated values are shown in Table 1 and have been plotted as a second curve in Fig. 3.

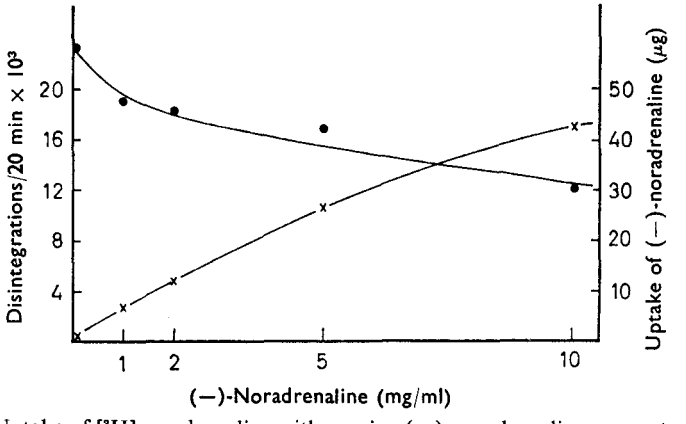

FIG. 3. Uptake of [^{3}H]noradrenaline with varying (−)-noradrenaline concentrations (●). Calculated uptake of (−)-noradrenaline per ml of ether-lecithin phase from 1 ml aqueous phase with varying (−)-noradrenaline concentrations (×).

From the uptake curve of Fig. 3 it is clear that the system is not fully saturated even when the concentration of (−)-noradrenaline in the aqueous phase was 10 mg/ml. From these results it is concluded that the concentration of (−)-noradrenaline producing 50% saturation of lecithin is at least 5 mg/ml.

TABLE 1.

Concentration of (−)-noradrenaline in aqueous phase mg/ml	[^{3}H]noradrenaline uptake % compared with value obtained in absence of (−)-noradrenaline	Calculated uptake of (−)-noradrenaline (μg) per ml ether-lecithin from 1 ml aqueous phase
0	100·0	0
1	83·5	6·7
2	76·2	12·2
5	66·7	26·7
10	53·3	42·6

Discussion

These experiments have shown that (+)- and (−)-noradrenaline have an equal ability to associate with lecithin. If this is indeed the case for other compound lipids then little biological significance can be attached to these interactions if the biological process itself shows stereospecificity.

Calculation of the equilibrium constant for the reaction

$$\text{lecithin} + \text{noradrenaline} \rightleftharpoons \text{lecithin-noradrenaline complex}$$

shows this to be around 33, which makes the standard free energy of formation of the complex (ΔF°) −2·0 kcal. Free energies of this order imply that only weak physical forces, such as van der Waals' forces, are involved; also it is unlikely that the formation of such a complex would be stereospecific.

The uptake of catecholamines into sympathetic nerve terminals when the former are in high concentration shows no stereospecificity (Iversen, 1965). It is possible that complex formation between phospholipids and noradrenaline play a part in this process.

It can be argued that the uptake of noradrenaline by our system is due to the solubilization of the aqueous phase by lecithin micelles, such a process would not be expected to show stereospecificity. We can make a reasonable estimate of the extent of solubilization in the following way. Elworthy & McIntosh (1964) showed that lecithin micelles in benzene could solubilize water to the extent of 0·33 g/g lecithin. If we assume this figure for our results then the percentage of uptake due to solubilization varies from 5 to 8%. Consider for instance the uptake of noradrenaline by 1 ml of lecithin-ether solution from 10 ml of noradrenaline solution (10 mg/ml). This has been estimated as 426 μg. One ml of lecithin-ether solution solubilizes 3·3 mg of aqueous phase, that is, 33 μg noradrenaline or 7·7% of the total uptake. Even if this percentage was much higher, the amount of noradrenaline not taken up in bulk water would be large enough to detect differences between the binding of the two isomers. We conclude therefore, that uptake by solubilization does not alter the interpretation of the results.

Finally, it is interesting to consider the stoichiometry of the lecithin-noradrenaline interaction. If we assume a molecular weight of 750 for lecithin, then at a noradrenaline concentration of 10 mg/ml the complex has a formula of 5·7 lecithin molecules to each noradrenaline molecule.

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