Effects of amphotericin B and phospholipase C on the uptake of catecholamines

SIR,—Lipids appear to play an important role in transport processes across cell membranes. Phospholipase C reduces the activity of some membrane-bound ATPases (Cuthbert, 1967) and inhibits the accumulation of iodide by thyroid gland (Larsen & Wolff, 1967). Amphotericin B appears to act by interacting with membrane sterols producing downhill ion movements (Cuthbert, 1967) and an inhibition of the accumulation of iodide by thyroid gland (Shishiba & Solomon, 1967).

Recent investigations have indicated that catecholamine retention by adrenergic neurons is linked to the transport of Na^+ and K^+ (Gillis & Paton, 1967; Bogdanski, Tissari & Brodie, 1968; Paton, 1968). In view of this cation dependence and the possible relation of retention to the membrane-bound ATPase (Berti & Shore, 1967) the effect of phospholipase C and of amphotericin B on the retention of [^{3}H]noradrenaline and [^{3}H]metaraminol by rabbit ventricular slices has been examined.

Male New Zealand white rabbits were killed by a blow on the head after which their hearts were excised rapidly and slices of left ventricle prepared as described by Gillis & Paton (1967). Slices were incubated in Krebs Ringer medium (Paton, 1968) at 37° and gassed with carbon dioxide 5% in oxygen. After varying durations of preincubation described below, either [^{3}H]noradrenaline or [^{3}H]-metaraminol was added so as to achieve a final concentration of 3·5 and 6·6 ng of the base per ml respectively and the incubation continued for a further 45 min. Retention of [^{3}H]noradrenaline and [^{3}H]metaraminol was then measured as described by Paton (1968) and expressed as a ratio (R) calculated by dividing the [^{3}H] counts/min/g of slice by [^{3}H] counts/min/ml of medium.

When amphotericin B was used, control slices were incubated in Krebs Ringer medium containing proportionate amounts of sodium deoxycholate, dibasic sodium phosphate and monobasic sodium phosphate to that contained in the commercial preparation of amphotericin B used. Amphotericin B was added to the test group for 15 min of preincubation and during incubation with the amines.

Slices were preincubated with phospholipase C for 60 min, rinsed twice in Krebs Ringer medium and then incubated for 20 min in Krebs Ringer medium to complete the wash; the tritiated amines were then added. Control slices from the same animals were preincubated in Krebs Ringer medium for the same time duration.

(±)-Metaraminol-7-[^{3}H]hydrochloride and (±)-noradrenaline-7-[^{3}H]hydrochloride* with specific activities of 6·7 and 9·7 c/mmole respectively were obtained from the New England Nuclear Corporation. Amphotericin B was obtained from E. R. Squibb & Sons Ltd. and phospholipase C from the Sigma Chemical Company. Since neither the molecular weight nor the purity of the enzyme were known, doses were expressed *in μg of the total material per ml.*

The results obtained are presented in Table 1. Both amphotericin B (10–50 μg/ml) and phospholipase C (200 μg/ml) produced a significant reduction in retention of [^{3}H]noradrenaline and [^{3}H]metaraminol.

Noradrenaline appears to be retained as the result of the operation of a two-stage process. The first involves passage of the amine across the membrane (uptake phase) and the second, binding in or to subcellular granules (storage phase). Metaraminol is retained in a manner essentially similar to noradrenaline; however, unlike noradrenaline, it is not a substrate for monoamine oxidase and can be retained by tissues pretreated with reserpine (Giachetti & Shore, 1966).

* 2-Amino-1-(3,4-dihydroxyphenyl)-[1-^{3}H]ethanol.

TABLE 1. EFFECT OF AMPHOTERICIN B AND PHOSPHOLIPASE C ON RETENTION OF TRITIATED AMINES BY RABBIT VENTRICULAR SLICES

Drug	Conc. (μg/ml)	No. of slices in each group	R Value (mean ± s.e.) Control	Experimental	*t*-test
A. Retention of [^{3}H]noradrenaline					
Amphotericin B	2	5	3·49 ± 0·32	3·03 ± 0·10	NS
Amphotericin B	10	6	3·78 ± 0·50	2·45 ± 0·17	$P < 0.05$
Amphotericin B	50	6	3·78 ± 0·50	2·04 ± 0·26	$P < 0.02$
Phospholipase C	10	10	5·66 ± 0·58	6·27 ± 0·26	NS
Phospholipase C	100	10	6·66 ± 0·58	4·42 ± 0·30	NS
Phospholipase C	200	14	5·42 ± 0·45	2·28 ± 0·10	$P < 0.001$
B. Retention of [^{3}H]metaraminol					
Amphotericin B	50	26	5·68 ± 0·43	3·41 ± 0·10	$P < 0.001$
Phospholipase C	200	6	5·07 ± 0·36	2·17 ± 0·10	$P < 0.001$

Consequently these authors have proposed that reserpine prevents the storage phase only; metaraminol cannot be stored in reserpine pretreated tissues but, since it is not deaminated, it can accumulate within the axoplasm. Thus any substance which inhibits the retention of metaraminol *in vitro* can be presumed to act on the uptake phase (Giachetti & Shore, 1966). My investigations would therefore suggest that both agents used are inhibiting the uptake of catecholamines by adrenergic neurons.

How this inhibition is produced is as yet uncertain. Amphotericin B decreases K^+ and increases Na^+ concentrations in thyroid gland without inhibiting the membrane ATPase (Shishiba & Solomon, 1967). If this also occurs in adrenergic neurons it would support the hypothesis of Bogdanski & others, who have suggested that amines are transported by a carrier whose affinity for amines is increased by Na^+. The affinity for amines would normally thus be high on the extracellular surface of the membrane and low on the intracellular surface resulting in release of the amine into the axoplasm and subsequent binding. In this model, the membrane ATPase would have an essential but secondary role (Bogdanski & others, 1968). In terms of their model, should amphotericin B in fact produce an increased intracellular Na^+ concentration, then the affinity of the carrier would rise intracellularly resulting in an inhibition of uptake.

As phospholipase C inhibits certain membrane ATPases (Cuthbert, 1967) but does not appear to act in this way on thyroid gland (Larsen & Wolff, 1967); it is possible that it reduces the uptake of amines by either inhibiting the axonal membrane ATPase or by an action on a phospholipid component of the carrier for amines. However, further work is required before either of these possibilities can be excluded. Since the concentration of phospholipase C required to inhibit uptake was high, it is possible that impurities could have contributed to the effect seen.

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The Histamine-binding property of serum

SIR,—Parrot & Laborde (1953) found that human and rat serum *in vitro* bind histamine and they suggested that γ-globulin is probably responsible for this action. We now find that the non-protein fraction of serum also contains a substance which has the power to bind histamine.

Rats of the R-Amsterdam strain of either sex (170–190 g) were stunned and bled from the carotid arteries into collecting test tubes and the blood was allowed to clot for 2 hr at room temperature. After separation of the serum by centrifugation, 7 ml was transferred to a Sephadex G-25 column (diameter 3 cm, length 60 cm), which was eluted with a solution of sodium chloride (0·1 M, pH 6·7, temperature 4°) passing at a rate of 30 ml/hr. The eluate was collected in 5 ml samples and tested both for protein, using paper electrophoresis, paper chromatography, and light absorption at 280 mμ, and for polypeptides by thin-layer chromatography. The serum proteins were detected in the first 9 samples (45 ml) but the next 8 samples were free from nitrogenous material. Polypeptides of low molecular weight (1,000–5,000) were eluted in the next 8 samples, the peak concentration, as measured by the intensity of the ninhydrin reaction, being in sample No. 20 (that is, after 100 ml of solution had been collected).

Each sample was subsequently tested for its ability to bind histamine. This was determined by mixing aliquots of 1 ml with a solution of histamine (1·2 μg base) and incubating the mixture at 37° for 20 sec. The free histamine was then estimated on the atropinized guinea-pig ileum using a four-point assay procedure, and the amount of histamine combined with the eluate sample was calculated and expressed as a percentage of that added. The first 17 samples, of which the earlier ones contained the free serum proteins, did not bind histamine but much of that present in the samples containing the polypeptides (Nos 18–25) was not free. The sample with the peak concentration of polypeptides (usually No. 20) contained the highest amount of combined histamine (up to 40% of that added). All the eluates from the columns had no effect by themselves on the isolated ileum and hence contained no kinin. The possibility that an antihistamine-like substance derived from serum was present in these samples cannot entirely be ruled out for hydrolysis of the samples before incubation with histamine removed most, but not all, of the power to bind histamine.

When fresh normal human serum was treated in a similar way, only the polypeptide fractions had the ability to bind histamine. The pathophysiological significance of this finding may be important in the field of allergy.

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