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INTERACTION OF DIBENAMINE WITH THE PHOSPHOLIPIDS OF THE AORTA

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Abstract—Tritium labelled dibenamine interacts, *inter alia*, with the cephalin fraction of the rabbit aorta. This interaction is prevented by adrenaline. It is suggested that the protective effect of adrenaline towards the irreversible inhibition of alpha-receptors by dibenamine is mediated by phospholipid interactions.

DIBENAMINE (dibenzyl-beta-chloroethylamine hydrochloride) irreversibly inhibits the various receptors of the rabbit aorta. It has been shown by Furchgott[1] that the agonist of a receptor is able to exert some protective action against low concentrations of dibenamine. With adrenaline the protective effect on the alpha-receptor approaches 100%.

We have now studied the distribution of tritium-labelled dibenamine in various lipid fractions of the rabbit aorta and the effect of adrenaline upon their distribution. The salient point of such studies is the necessity to stick strictly to certain concentrations, such as described as a prerequisite for studying the protective effect of adrenaline.[1]

MATERIALS AND METHODS

Dibenamine hydrochloride (0·2 g) was labelled according to the Wilzbach method by equilibration with 7 c of tritium gas for 3 weeks. The material obtained was dissolved three times in 8 ml of concentrated hydrochloric acid and evaporated to dryness. Then it was recrystallized from cold acetone containing 1% HCl. The melting point was 192°, equal to that of the untreated sample. The activity in different samples varied from 30 to 80 μC/μM. The yield was 25%.

For incubation of the rabbit aorta 10 ml Krebs-bicarbonate solution oxygenated with 95% O_2 + 5% CO_2 was used at 37°. Through all experiments the concentration of dibenamine hydrochloride was 10 mcg/ml and that of adrenaline bitartarate 100 mcg/ml. The incubated aorta was washed at room temperature in 25-ml penicillin bottles with a magnetic stirrer in 5 ml of Krebs-bicarbonate solution (five times for 10 min each), a sixth wash of 1 hr was added finally (Flow Sheet No. 1).

For counting the radioactivity we used Bray's solution,[2] and the Tri-Carb Liquid Scintillation Counter. The efficiency of the counting was 5%.

Thin-layer chromatography was performed from the hot chloroform-methanol extract (2:1) of the aorta. The aorta was extracted with 10 ml of the boiling mixture for 2 min, the extract was then filtered and evaporated to dryness. The residue was

again dissolved in 0·25 ml of the same solvent and 0·05 ml of it were used for thin-layer chromatography. The plates were coated with Kieselgel-G in 0·01 M sodium acetate, the solvent system was a mixture of 75 ml chloroform, 22 ml methanol and 3

Flow Sheet No. 1

Step	Rabbit aorta cut into longitudinal halves	
1	Incubated 5′ with 100 mcg/ml adrenaline at 37° in Ringer solution	Incubated 5′ in Ringer solution
2	Radioactive dibenamine (10 mcg/ml) added	Radioactive dibenamine (10 mcg/ml) added
3	Incubated 30′ at 37°	Incubated 30′ at 37°
4	6 times washed in presence of 100 mcg/ml adrenaline	6 times washed in Ringer solution
5	Extracted with 10 ml chloroform-methanol (2:1)	Extracted with 10 ml chloroform-methanol (2:1)
6	Counting of the extract	Counting of the extract

ml water.[3] After running the extract, the plates were dried, stained by iodine vapour and the different brownish spots scraped directly into the counting tubes.

RESULTS

Table 1 shows the mean values of the radioactivity in the lipid fraction as determined in 13 experiments by counting 0·5 ml of the chloroform-methanol extract according to Flow Sheet No. 1. It is evident that in 10 out of 14 experiments adrenaline prevents the uptake of dibenamine into the lipid fraction.

TABLE 1. AVERAGE VALUES OF RADIOACTIVITY IN THE LIPID EXTRACT OF RABBIT AORTA AFTER ADDITION OF 100 MCG/ML ADRENALINE FOLLOWED BY 10 MCG/ML TRITIUM-LABELLED DIBENAMINE

	Counts/min per mg													
Incubated in presence of adrenaline	184	100	156	0	100	60	48	88	120	146	36	78	38	89
Incubated in absence of adrenaline	124	88	154	40	216	88	132	100	180	224	68	50	128	122

* The mean difference of 33 ± 13 calculated as difference of the 14 pairs is significant.

In the next stage of this work we chromatographed the lipid extract (Flow Sheet No. 1). In step 1 adrenaline had the possibility of catching its receptors, thus preventing the entrance of radioactive dibenamine (steps 2 and 4). In the following steps (4–6) that part of dibenamine firmly held by the lipid fraction of the aorta was determined.

The results are given in Table 2. It shows in the second column that radioactive decomposition products of dibenamine appear at R_f 0·1–0·4 and R_f 0·9–1·0 which

TABLE 2. RADIOACTIVITY IN THE VARIOUS LIPID FRACTIONS OF THE RABBIT AORTA INCUBATED ACCORDING TO FLOW SHEET NO. 1

R_f	Total count/min per segment of chromatogram						
	Control without aorta	Experiment 1 each half weighing 25 mgm		Experiment 2 each half weighing 30 mgm		Experiment 3 each half weighing 55 mgm	
		adrenaline absent	adrenaline present	adrenaline absent	adrenaline present	adrenaline absent	adrenaline present
0 –0·1	0	110	110	150	85	0	0
0·1–0·4	160	130	70	170	90	400	1200
0·4–0·7	0	80	20	150	60	650	50
0·7–0·9	0	135	135	210	260	30	50
0·9–1·0	1000	120	120	60	95	800	300
Total		575	455	740	590	1850	1600

means that we cannot evaluate the radioactivity at these spots since we have no knowledge of the extent of the decomposition of dibenamine under various conditions. In our system lecithin has a R_f of about 0·2 which means that we cannot derive any information from Table 2 as to whether lecithin has bound dibenamine or its metabolites. Phosphatidylserine and phosphatidylethanolamine enter the R_f range 0·4–0·7—the former occupying the lower part of that range—meaning that adrenaline prevents the binding of dibenamine to this (cephalin) fraction.

Flow Sheet No. 2

Rabbit aorta cut into longitudinal halves

Step		
1	Incubated with 100 mcg/ml adrenaline (5′ at 37°)	Incubated in Ringer solution (5′ at 37°)
2	Dibenamine added (10 mcg/ml not radioactive)	Dibenamine added (10 mcg/ml not radioactive)
3	Incubated 30′ at 37°	Incubated 30′ at 37°
4	Washed 10′ in Ringer solution	Washed 10′ in Ringer solution
5	Transferred to another incubation tube containing 10 mcg/ml radioactive dibenamine without adrenaline and incubated 30′ at 37°	Transferred to another incubation tube containing 10 mcg/ml radioactive dibenamine without adrenaline and incubated 30′ at 37°
6	6 times washed in Ringer solution.	6 times washed in Ringer solution
7	Extracted with chloroform-methanol (2:1)	Extracted with chloroform-methanol (2:1)
8	Chromatography	Chromatography

In the next stage we adopted Flow Sheet No. 2. In this set-up adrenaline should have been displaced by radioactive dibenamine. In step 1 adrenaline had the possibility of entering its receptors. In steps 2 and 3 the non-radioactive dibenamine

occupied all receptor sites not reacting with adrenaline. In step 4 the adrenaline and the dibenamine surplus were removed. In step 5 the radioactive dibenamine occupied the sites formerly held by adrenaline. Steps 6–8 served for the determination of the radioactive dibenamine. Table 3 shows that now indeed the tissue incubated in the

TABLE 3. RADIOACTIVITY IN VARIOUS LIPID FRACTIONS OF THE RABBIT AORTA INCUBATED ACCORDING TO FLOW SHEET NO. 2

R_f	Total count/min per segment of chromatogram					
	Experiment 1 each half weighing 30 mgm		Experiment 2 each half weighing 32 mgm		Experiment 3 each half weighing 40 mgm	
	adrenaline absent	adrenaline present	adrenaline absent	adrenaline present	adrenaline absent	adrenline present
0 –0·1	50	46	0	0	179	154
0·1–0·4	72	0	85	130	51	30
0·4–0·7	25	191	320	225	150	247
0·7–0·9	18	4	150	120	103	186
0·9–1·0	259	268	100	55	168	205
Total	424	509	565	530	651	822

presence of adrenaline contains an excess of radioactivity in the cephalin fraction (R_f 0·4–0·7). The other fractions contain unknown mixed decomposition products of dibenamine—as pointed out before. This experiment proves, also, that the bulk of the radioactivity is not firmly bound but is dissolved or held in an easily dissociable complex form since the total radioactivity per mg tissue lipid extract is in the same order of activity.

DISCUSSION

According to Belleau, the common receptor for dibenamine and for adrenaline should contain phosphate anions. He visualized adenosine triphosphate as an integral part of the adrenergic receptor. However, he could not present experimental evidence for this hypothesis.[4]

According to our experiments the phospholipids of the aorta simulate the behaviour of a hypothetical receptor by binding adrenaline. We feel that the series of convincing arguments presented by Belleau fit an active role of proteophospholipids just as well. Meanwhile we have obtained additional evidence for the importance of phospholipids in receptor functions. We have removed them by washing with dilute acetone—thus incapacitating receptor function—and we have restored them by phospholipids, thus reconstituting receptor function both for adrenaline and for acetylcholine.[5] Our hypothesis is, therefore, that a "receptor" is represented by a certain pattern of modification of electron transport in an osmo-chemical transducer system of the cell membrane. Such systems are known to require phospholipids for proper functioning.[6]

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REFERENCES

1. F. FURCHGOTT, *J. Pharmacol.* **111**, 265 (1954).
2. G. A. BRAY, *Ann. Biochem.* **1**, 279 (1960).
3. P. SKIPSKI, R. PETERSSON and M. BARCLAY, *J. Lipid Res.* **3**, 467 (1962).
4. B. BELLEAU, *First Inter. Pharmacol. Meeting*, Pergamon Press, **7**, 75 (1961).
5. S. DIKSTEIN and F. G. SULMAN, *Biochem. Pharmacol.* **14**, 739 (1965).
6. D. GREEN, *Biochem. biophys. Acta*, **70**, 554 (1963).