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Uptake, metabolism and release of [^{3}H]-adrenaline by human platelets

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Summary

1. Measurements were made of the uptake, metabolism and release of [^{3}H]-adrenaline by human platelets in citrated plasma or in an artificial medium.

2. Radioactive adrenaline was not taken up at 0–2° C. At 37° C there was a slow uptake which continued for at least 5 hours.

3. About half of the radioactivity in the platelets was intact adrenaline. The other half was an acidic metabolite from which adrenaline was released by acid hydrolysis.

4. The immediate uptake of adrenaline was proportional to its concentration in the plasma up to at least 1×10^{-5}M. Uptake measured after 1 h also increased linearly with concentration up to about 1×10^{-4}M but less with higher concentrations. The highest concentration ratio was about 12.

5. The concentration of metabolite in the platelets increased with the concentration of added adrenaline only up to about 2×10^{-4}M.

6. The immediate uptake of adrenaline was partially inhibited by phentolamine and dihydroergotamine. Measurement of uptake both immediately and after 1 h showed that the inhibition produced was not increased beyond about 50% by these drugs or by (±)-propranolol, chlorpromazine or amitriptyline up to 1×10^{-4}M.

7. Formation of the metabolite was inhibited by pyrogallol, 8-hydroxyquinoline, or tropolone. This inhibition was associated with a corresponding increase in the adrenaline accumulated intact. Formation of the metabolite was not inhibited by monoamine oxidase inhibitors.

8. Reserpine caused a small decrease in the uptake of adrenaline radioactivity in 1 h and a great increase in the proportion recovered as metabolite.

9. Thrombin caused the release from platelets of intact adrenaline but not of the metabolite.

10. Platelets of albino patients with spontaneous haemorrhages accumulated adrenaline radioactivity at the normal rate but this radioactivity was wholly accounted for by metabolite and not released by thrombin.

11. After taking up adrenaline, platelets resuspended in artificial medium at 37° C slowly released both adrenaline and its metabolite. At the same time, the intracellular adrenaline was slowly metabolized.

12. The results suggest that human platelets take up adrenaline by two processes, one of which is inhibited by both α- and β-adrenoceptor blocking agents as well as by phenothiazines; and that in the platelets adrenaline is partly stored in organelles from which, like 5-hydroxytryptamine, it can be specifically released.

Introduction

Adrenaline causes human platelets to aggregate (Clayton & Cross, 1963) and potentiates the aggregating effect of other substances such as ADP (Mills & Roberts, 1967). Indirect evidence suggests that the aggregating effect of thrombin on human platelets is potentiated by adrenaline contained in them (Thomas, 1967). Platelets of several mammalian species, including man, contain adrenaline in small amounts (Born, Hornykiewicz & Stafford, 1958; Markwardt, 1967). It has been claimed that platelets can take up adrenaline *in vitro* by an active transport (Sano, Kakimoto, Taniguchi & Takesada, 1959). We have investigated the uptake, storage, inactivation and release of adrenaline by human platelets.

Methods

Human platelet-rich plasma containing citrate as anticoagulant was prepared as already described (Born & Cross, 1963; Mills & Roberts, 1967); the concentration of platelets was determined by counting under the phase-contrast microscope. Samples (2·5 ml) of platelet-rich plasma or of resuspended platelets were incubated at 37° C in a water bath without shaking for different times with added [^{3}H]-adrenaline and/or with other substances, as described under **Results**.

After incubation, the plasma was centrifuged at 15,000 *g* for 5 min at 0° C. The supernatant was decanted and the inside of the centrifuge tube wiped with filter paper to remove any radioactivity remaining above the pellet. The extracellular space was determined in separate samples of platelet-rich plasma by adding [^{14}C]-inulin (0·01 μCi/ml). After centrifugation, the inulin radioactivity was determined in the platelet pellet. Inulin, like serum albumin, is apparently not adsorbed on to platelets nor does it enter them under the conditions of these experiments (Born & Bricknell, 1959).

For incubating platelets in artificial media, platelet-rich plasma was cooled to 2° C and cold EDTA (0·1 M) was added to a final concentration of 5 mM. The plasma was centrifuged in siliconized glass tubes for 10 min at 2,400 *g* in an angle head. The platelet-poor supernatant was decanted and the pellet of platelets re-suspended with a Pasteur pipette in the following medium (mM): NaCl, 134; Tris-HCl pH 7·4, 7·7; KCl, 7·7; D-glucose, 5·6.

For determining adrenaline and its breakdown products in the sediment of platelets, 0·5 ml ice-cold 2 N perchloric acid was added and the platelets were homogenized in a cold Teflon homogenizer. The volume of the homogenate was made up to 3 ml. After centrifugation, 2 ml of the clear supernatant was adjusted to pH 4–6 with 2 M potassium carbonate and the volume made up to 5 ml. The precipitate of potassium perchlorate was allowed to settle for 10 min at 0° C. Four ml of the supernatant was pipetted on to a Biorad AG 50W-X4 ion-exchange column (sodium form), 4 cm long and 4 mm internal diameter (Bertler, Carlsen & Rosen-

gren, 1958); the column was protected from light by aluminium foil to minimize the breakdown of adrenaline. As this column retains adrenaline but allows its acid and neutral metabolites to pass through, the effluent and a 10 ml wash of water were collected together for adrenaline metabolites and adrenaline was eluted with 25 ml N HCl. Radioactivities were determined on 0·5 ml samples of the aqueous solutions in 15 ml diotol (Herberg, 1960) in a Packard Model 3375 scintillation counter.

The recovery of 200–800 pmol of [^{3}H]-adrenaline, added to platelets and extracted as described, was 89 ± 2% in thirty determinations. The purity of [^{3}H]-adrenaline supplied was established by chromatography on columns and on cation-exchange paper (Roberts, 1962); it was never less than 95%. Experiments were almost always done in duplicate, which showed good agreement. Whenever necessary to establish a point, experiments were repeated with samples of platelets from different people.

Materials

[^{3}H]-adrenaline was obtained from the Radiochemical Centre, Amersham. The uptake experiments were done using three batches with specific activities of 2·56, 3·7 and 6·3 Ci/mmol (±)-adrenaline-7-T hydrochloride; and the release experiments with (±)-adrenaline (ring-T(G))-(±)-bitartrate with a specific activity of 0·2 Ci/mmol. ^{14}C-Inulin (1·54 Ci/mg) was obtained from Schwartz; pyrogallol, 8-hydroxyquinoline and (−)-adrenaline from British Drug Houses; mebanazine (Actomol oxalate) and propranolol (Inderal) from I.C.I.; chlorpromazine from May & Baker Ltd.; (±)-adrenaline from Sigma; reserpine (Serpasil) and phentolamine (Rogitine) from CIBA; amitriptyline (Laroxyl) and marsilid phosphate from Roche; dibenzyline from Smith, Kline & French; cellulose phosphate cation-exchange paper (P.81) from Reeve Angel Ltd.; thrombin from Parke Davis Ltd.; and silica-coated glass fibre paper (I.T.L.C.) from Gelman Ltd. (+)-adrenaline bitartrate was kindly supplied by Professor H. Blaschko; tropolone and tropolone 4-acetamide by Dr. D. Sharman and prostaglandin E_1 by Dr. J. E. Pike of V. Upjohn & Co. Ltd.

Results

Adrenaline uptake

Human platelets in citrated plasma containing [^{3}H]-adrenaline (10^{-6}M) did not take up radioactivity at 0° C. At 37° C the platelets took up radioactivity at a rate which decreased slightly and continuously over 5 h (Fig. 1). Radioactivity extracted from platelets was separated into two fractions, one consisting of intact adrenaline and another of an acidic metabolite. The radioactivity due to intact adrenaline increased with time about half as rapidly as the total radioactivity. The highest concentration ratio (Conc. inside/Conc. outside) found for adrenaline was 12.

Partial identification of the metabolite

Platelets which had been allowed to accumulate radioactivity were centrifuged and extracted as described under **Methods**. Samples of the extracts were chromato-

graphed on cellulose phosphate cation-exchange paper with 0·2 M acetate buffer pH 6·0 : isopropanol (3 : 1 v/v) (Roberts, 1962). The paper was cut into sections and the radioactivity determined directly in diotol. The radioactivity had 2 peaks, one (R_f 0·32) corresponding to adrenaline and the other (R_f 0·84) indicating the presence of an acidic metabolite (Fig. 2). In three experiments the radioactivity of the metabolite accounted for between 40 and 50% of the total.

Samples of the extract were passed through columns before and after hydrolysis in 1 N HCl at 100° C for 10 min. In four experiments the radioactivities retained as adrenaline were $50 \pm 4\%$ before hydrolysis and $89 \pm 2\%$ after hydrolysis. The radioactive metabolite, isolated by column chromatography, was also re-chromatographed on cellulose phosphate paper before and after hydrolysis. After hydrolysis the metabolite had the same R_f value as adrenaline. Similar results were obtained on I.T.L.C. paper developed with *n*-butanol : acetic acid : water (7 : 15 : 15). These results showed that the metabolite is an adrenaline conjugate.

Effect of concentration of added adrenaline on the accumulation of adrenaline and its metabolite

Initial uptake. First, the uptake was measured as rapidly as possible by adding [^{3}H]-adrenaline to platelet rich plasma, inverting the tubes rapidly six times against parafilm, cooling immediately in ice-water and centrifuging at 0° C and 10,000 *g* for 3 min ; the whole operation took about 5 min. Under these conditions, the uptake of radioactivity was directly proportional to the concentration of added adrenaline between 10^{-8} and 10^{-5}M.

Continued uptake. In later experiments uptake was determined after incubation for 1 h. As adrenaline can cause aggregation of platelets in plasma, prostaglandin E_1 ($2{\cdot}8 \times 10^{-7}$M) which had no effect on adrenaline uptake (Table 1) was added to

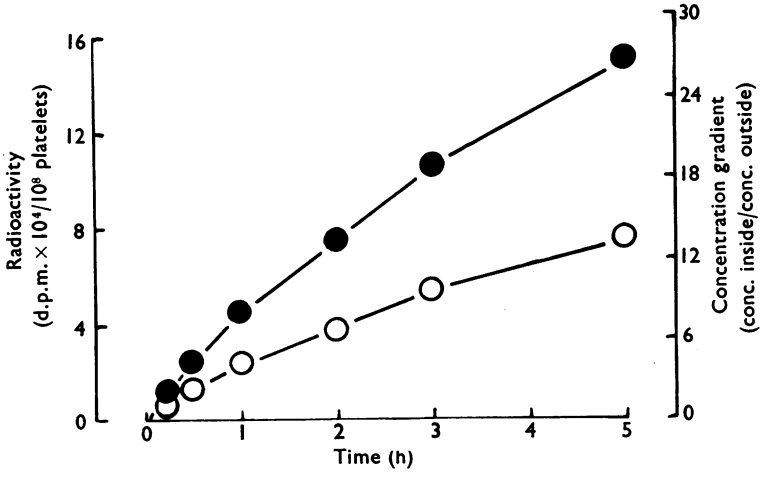

FIG. 1. Uptake of total radioactivity (●——●) and of intact [^{3}H]-adrenaline (○——○) as a function of time. Platelet-rich plasma ($3{\cdot}98 \times 10^8$ platelets/ml) was incubated with 10^{-6}M [^{3}H]-adrenaline (2·56 Ci/mmol) for increasing times. Results are corrected for an extracellular space of $0{\cdot}5 \times 10^4$ d.p.m./10^8 platelets.

the platelet-rich plasma to inhibit aggregation (Emmons, Hampton, Harrison, Honour & Mitchell, 1967). The uptake of intact [^{3}H]-adrenaline was proportional to the concentration of extracellular adrenaline up to about 1×10^{-4}M; with higher concentrations the proportionality decreased (Fig. 3). The concentration of metabolite in the platelets was also proportional at low concentrations of added adrena-

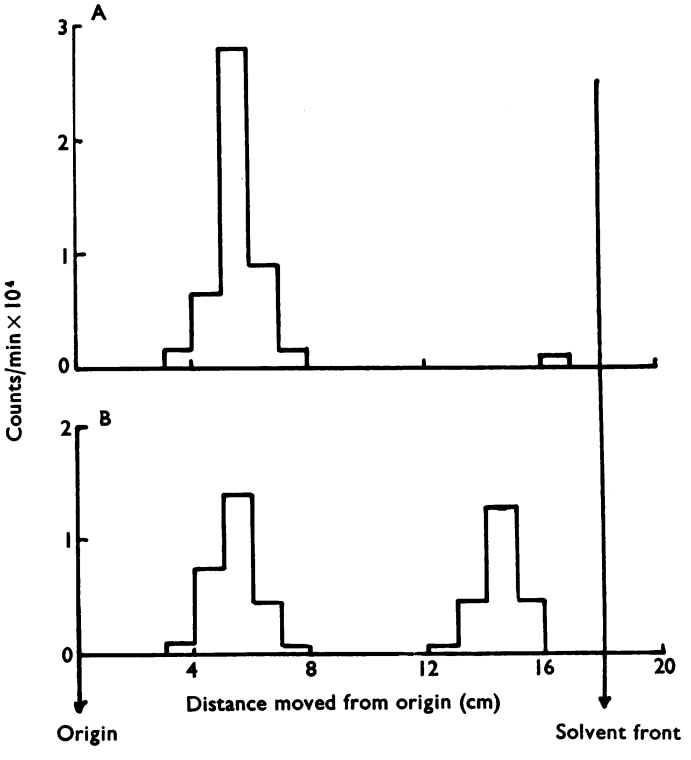

FIG. 2. Separation of [^{3}H]-adrenaline from a radioactive metabolite in extracts from platelets incubated for 2 h with [^{3}H]-adrenaline (5×10^{-7}M). The figure shows the distribution of radioactivity after chromatography on cellular phosphate cation-exchange paper. A, Control run with [^{3}H]-adrenaline; B, platelet extract.

TABLE 1. *Effect of different substances on the uptake of adrenaline radioactivity and on its intracellular metabolism*

Substance	Concentration M	Uptake of radioactivity: 10^{-12} mol/10^8 platelets	Uptake of radioactivity: % of saline control	% of uptake present as adrenaline	% of uptake present as metabolite
Saline	—	6·08	100	51	49
(±)-Propranolol	5×10^{-5}	3·34	55	19	81
Dibenzyline	5×10^{-5}	3·02	50	22	78
Amitriptyline	5×10^{-5}	2·92	48	32	68
Chlorpromazine	5×10^{-5}	2·87	47	30	70
Prostaglandin E_1	$2{\cdot}8 \times 10^{-7}$	5·95	98	51	49

Plasma containing $3{\cdot}42 \times 10^8$ platelets/ml was incubated at 37° C for 1 h with 5×10^{-7}M [^{3}H]-adrenaline (2·56 Ci/mmol) in the presence of the substances shown. The results are corrected for an extracellular space of $0{\cdot}71 \times 10^{-12}$ mol/10^8 platelets.

line but this changed at higher concentrations and above about 2×10^{-4}M adrenaline showed no further increase. When this experiment was done using carrier adrenaline, i.e. (±), (+) or (−), the results were similar. When the concentration of added adrenaline was increased the concentration of the metabolite increased more slowly with time than the concentration of intact adrenaline (Fig. 4). This suggested that, at the higher adrenaline concentrations, some of the metabolite was released from the platelets.

Inhibition of adrenaline uptake

Initial uptake. Pharmacological antagonists of adrenaline were mixed with platelet-rich plasma just before addition of [^{3}H]-adrenaline and its initial uptake was measured as described. The results of two experiments with phentolamine were very similar and so were the results of two experiments with dihydroergotamine; one result with each of these is shown in Fig. 5. Both substances inhibited the initial adrenaline uptake as their concentrations increased up to about 5×10^{-6}M. At higher concentrations, the inhibition by phentolamine increased little whereas that by dihydroergotamine actually decreased. Even at 1×10^{-4}M phentolamine inhibited the uptake by only about 50%.

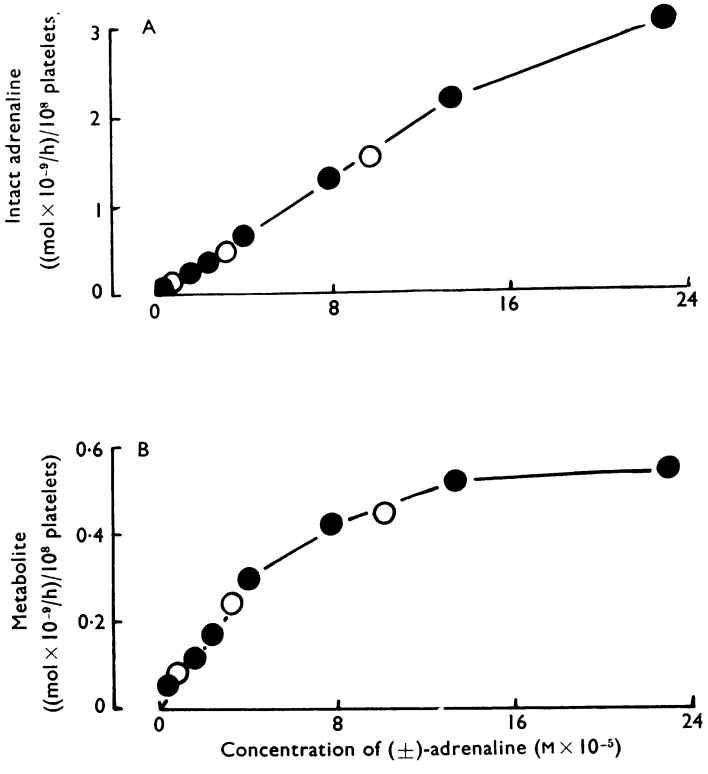

FIG. 3. Accumulation by platelets of [^{3}H]-adrenaline and of its radioactive metabolite at 37° C, as a function of the [^{3}H]-adrenaline concentration in the plasma. Plasma from two donors was used containing $3{\cdot}20 \times 10^8$ (○——○) and $3{\cdot}86 \times 10^8$ (●——●) platelets/ml respectively. A, Intact adrenaline; B, metabolite.

The initial uptake of adrenaline was inhibited also by (±)-propranolol but less effectively.

Continued uptake: Platelet-rich plasma was incubated with [^{3}H]-adrenaline for 1 h in the absence and presence of several antagonists of the actions or uptake of adrenaline. Table 1 shows that (±)-propranolol, dibenzyline, amitriptyline and chlorpromazine all inhibited the continued uptake of adrenaline by platelets with similar potencies. The diminished uptake was associated with an increased proportion of adrenaline metabolized inside the platelets.

Inhibition of the formation of metabolite

Simultaneous incubation of platelets with [^{3}H]-adrenaline and pyrogallol inhibited formation of the metabolite and caused a corresponding increase in the adrenaline accumulated intact (Table 2). Other catechol-*o*-methyl transferase inhibitors—8-hydroxyquinoline, tropolone and tropolone 4-acetamide (Ross & Haljasmaa, 1964)—also inhibited production of the metabolite but less effectively. This inhibition

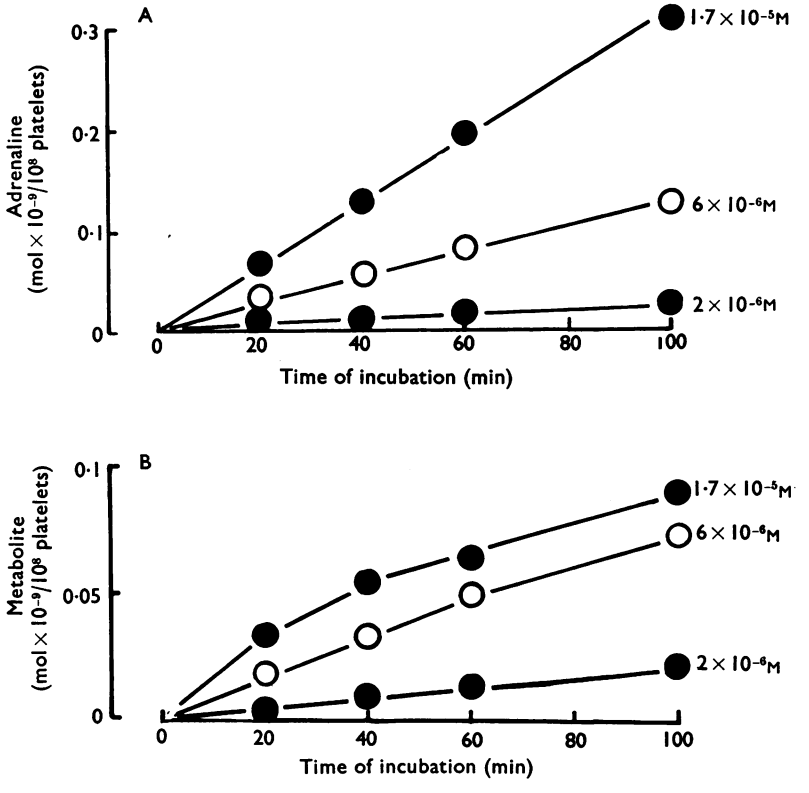

FIG. 4. Accumulation of [^{3}H]-adrenaline and of its radioactive metabolite with time at increasing concentrations of added [^{3}H]-adrenaline. Plasma from two donors was used containing $4{\cdot}00 \times 10^8$ (○——○) and $4{\cdot}51 \times 10^8$ (●——●) platelets/ml respectively. A, Intact adrenaline; B, metabolite.

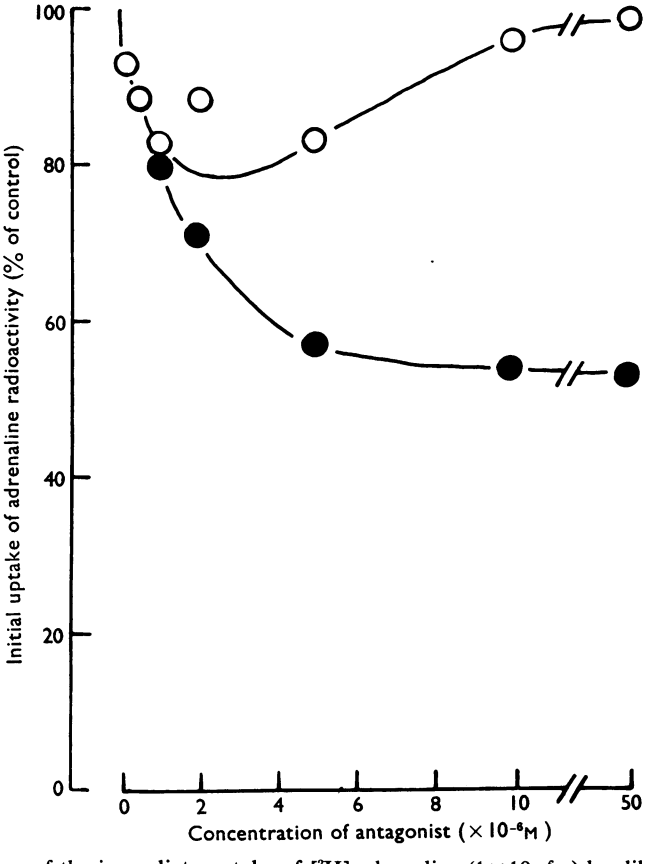

FIG. 5. Inhibition of the immediate uptake of [^{3}H]-adrenaline (1×10^{-6}M) by dihydroergotamine (○——○) or phentolamine (●——●), in different plasmas containing 3·41 and $3{\cdot}13 \times 10^8$ platelets/ml respectively. The results were corrected for the extracellular spaces in the platelet pellets.

TABLE 2. *Effect of inhibitors of amine metabolism on the uptake of adrenaline radioactivity and on its intracellular metabolism*

Substance	Concentration M	Uptake of radioactivity: 10^{-12} mol/10^8 platelets	Uptake of radioactivity: % of saline control	% of uptake present as adrenaline	% of uptake present as metabolite
Experiment *A*					
Saline	—	4·29	100	63	37
Pyrogallol	1×10^{-4}	4·37	102	88	12
8-Hydroxyquinoline	4×10^{-5}	3·60	84	77	23
Tropolone	1×10^{-4}	4·42	103	71	29
Tropolone 4-acetamide	1×10^{-4}	3·75	87	66	34
Marsilid	1×10^{-4}	4·15	95	62	38
Mebanazine	1×10^{-4}	4·58	107	62	38
Experiment *B*					
Saline	—	7·18	100	61	39
Phentolamine	5×10^{-5}	3·08	43	45	55
Phentolamine	1×10^{-4}	2·64	37	40	60
8-Hydroxyquinoline	4×10^{-5}	6·71	93	79	21
8-Hydroxyquinoline	8×10^{-5}	5·87	82	82	18
8-Hydroxyquinoline + phentolamine	4×10^{-5} + 5×10^{-5}	2·26	37	68	32

Plasma containing $4{\cdot}98 \times 10^8$ platelets/ml (experiment *A*) and $2{\cdot}90 \times 10^8$ platelets/ml (experiment *B*) was incubated for 1 h at 37° C with $6{\cdot}4 \times 10^{-7}$M [^{3}H]-adrenaline (6·3 Ci/mmol) in the presence of the substances mentioned. The results are corrected for an extracellular space of $0{\cdot}25 \times 10^{-12}$ mol/10^8 platelets.

of metabolite formation could also be shown when the uptake of adrenaline had been inhibited by phentolamine. No inhibition of metabolite formation occurred when platelets were incubated with the monoamine oxidase inhibitors mebanazine or marsilid. These results suggest that formation of the metabolite involves conjugation at a phenolic hydroxyl group.

Release of platelet adrenaline by thrombin

Platelets were incubated in plasma with [^{3}H]-adrenaline, resuspended in artificial media and incubated for 10 min without and with thrombin. After centrifugation, [^{3}H]-adrenaline and its metabolite were determined in the platelets and in the supernatant.

In the control, of the total radioactivity 53% was found in the platelets as adrenaline and 35% as metabolite, whereas platelets treated with thrombin contained only 6% as adrenaline but still 28% as metabolite. There were corresponding increases of adrenaline and the metabolite in the supernatants (Fig. 6).

When pyrogallol was added to the same platelet-rich plasma to inhibit the formation of the metabolite, thrombin again caused the release of about 90% of intact

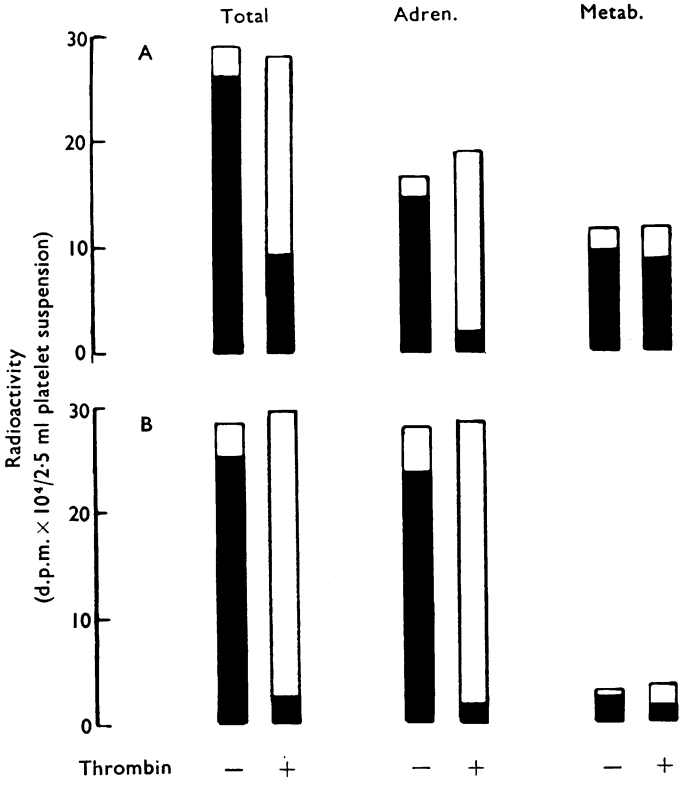

FIG. 6. Effect of thrombin on the content of [^{3}H]-adrenaline and of its metabolite in platelets. Plasma containing $3{\cdot}22\times10^8$ platelets/ml was incubated with 10^{-6}M [^{3}H]-adrenaline (0·2 Ci/mmol) at 37° C for 2 h (A) without and (B) with pyrogallol (1×10^{-4}M). The platelets were resuspended in artificial medium and reincubated for 10 min without or with thrombin at 1 u./ml. Radioactivities in the sedimented platelets are shown as closed columns and in the supernatants as open columns.

adrenaline but the release of more radioactivity than that from platelets not treated with pyrogallol (Fig. 6). The specificity of this effect was shown by the release from the same platelets of 82% of 5-hydroxytryptamine but only about 2% of adenylate kinase (Haslam & Mills, 1966).

Certain albino patients suffer from a tendency to bleed; their platelets are abnormal in that they cannot store 5-hydroxytryptamine (Mills & Hardisty, 1970). When such platelets were incubated with [^{3}H]-adrenaline they accumulated radioactivity almost as rapidly as normal platelets. However, this radioactivity was accounted for by metabolite and was not released by thrombin. These results provide evidence that normal platelets accumulate intact adrenaline in organelles from which it can be released by thrombin in a specific release reaction similar to that for 5-hydroxytryptamine (Grette, 1962).

Effect of reserpine

Platelets were incubated at 37° C in plasma with [^{3}H]-adrenaline without and with reserpine for 1 h. The concentrations of intact adrenaline and of its metabolite were determined in the platelets. Figure 7 shows one of two experiments with very similar results. In the presence of reserpine, the total radioactivity of the platelet was diminished by about 10% and, of this total, about 90% was metabolite. This showed that reserpine blocked the uptake of [^{3}H]-adrenaline into the storage organelles but did not interfere with its metabolism in the platelets.

Spontaneous release of adrenaline

Platelets incubated with [^{3}H]-adrenaline were centrifuged and suspended in artificial medium and reincubated at 37° C. After increasing times, samples were centrifuged and the platelets extracted as already described. The platelets lost radioactivity progressively, which was wholly accounted for by a decrease in their content of intact adrenaline; the intracellular level of metabolite remained approximately

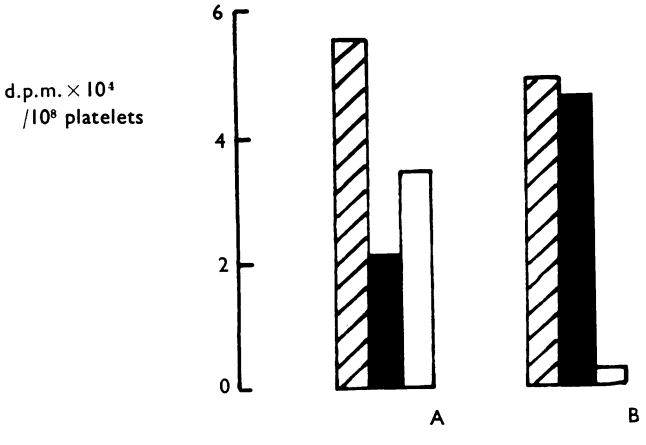

FIG. 7. Uptake of radioactivity (hatched columns) and contents of [^{3}H]-adrenaline (open columns) and its radioactive metabolite (closed columns) in platelets after incubation of plasma containing $4{\cdot}98\times10^8$ platelets/ml with 6×10^{-7}M [^{3}H]-adrenaline (6·3 Ci/mmol) at 37° C for 1 h in the absence (A) and presence (B) of reserpine (2 μg/ml). The values for the uptakes of radioactivity and [^{3}H]-adrenaline are corrected for an extracellular space of $0{\cdot}5\times10^4$ d.p.m./10^8 platelets.

constant (Fig. 8). Analysis of the released radioactivity indicated that both metabolite and adrenaline were lost (Table 3). Therefore, intracellular adrenaline was being slowly metabolized.

Resuspension of platelets preincubated with [^{3}H]-adrenaline in a medium containing non-radioactive adrenaline did not affect the release of radioactivity (Table 3). As resuspended platelets were still able to take up and metabolize adrenaline, it may be slowly released from platelets in this way *in vivo*.

Discussion

The results show that human platelets at body temperature can take up adrenaline at a slow rate for long periods of time. About half of the adrenaline was metabo-

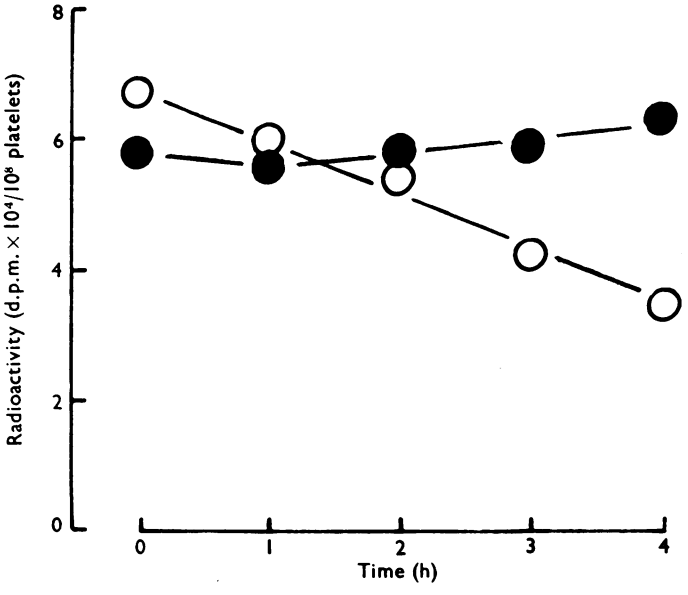

FIG. 8. Changes in the contents of [^{3}H]-adrenaline (○——○) and its metabolite (●——●) in platelets during reincubation in an artificial medium. Platelets suspended in artificial medium ($3{\cdot}86\times10^8$ platelets/ml) were incubated at 37° C for 2 h with 5×10^{-7}M [^{3}H]-adrenaline. The platelets were centrifuged, resuspended and reincubated (at 37° C) for increasing times.

TABLE 3. *Release of adrenaline and of its metabolite from platelets during reincubation*

Time reincubated (h)	Adrenaline addition	Radioactivity in platelets			Radioactivity in supernatant		
		Total	As adrenaline	As metabolite	Total	As adrenaline	As metabolite
0	—	79·1	54·3	24·8	14·4	5·9	8·5
2	—	59·2	38·5	20·7	31·5	16·9	14·6
4	—	52·6	27·6	25·0	42·3	21·0	21·3
2	+	59·5	44·3	15·2	31·9	18·7	13·2
4	+	52·6	36·0	16·7	37·5	23·1	14·4

Plasma containing $4{\cdot}08\times10^8$ platelets/ml was incubated at 37° C for 1 h with 8×10^{-7}M [^{3}H]-adrenaline (6·3 Ci/mmol). The platelets were resuspended and reincubated without and with (±)-adrenaline (2×10^{-5}M). Results are expressed as d.p.m. $\times10^4$/2·5 ml platelet suspension.

lized to a derivative from which adrenaline could be released again by acid hydrolysis. With concentrations of adrenaline higher than 4×10^{-5}M the proportion present as metabolite decreased and the total radioactivity taken up also decreased. These results suggest that the metabolite can diffuse out of the platelets; this was confirmed with resuspended platelets which also lost intact adrenaline at a slow rate.

In the light of our observations, it is instructive to consider the similarities and differences in the uptake, storage, inactivation, and release by platelets of adrenaline on the one hand and of 5-hydroxytryptamine on the other. *Uptake* of either amine occurs at 37° C but not at 0° C. However, at the same concentration, for example 5×10^{-6}M, the rate of uptake of 5-hydroxytryptamine is about 50 times faster than that of adrenaline. Furthermore, the uptake of 5-hydroxytryptamine follows saturation kinetics with a *Km* of about 5×10^{-6}M, whereas that of adrenaline does not, at least up to 1×10^{-4}M. Lastly, platelets can clear plasma completely of 5-hydroxytryptamine (Born & Gillson, 1959), so that the concentration ratio becomes infinite. This does not happen with adrenaline, which diffuses slowly out of the platelets; and the highest concentration ratio observed in our experiments was 12.

The uptake of both amines is inhibited by phenothiazines and related compounds such as imipramine (Long & Lessin, 1962) but they inhibit the uptake of 5-hydroxytryptamine more effectively than that of adrenaline. Indeed all the antagonists tested, in concentrations up to 1×10^{-4}M, inhibited the uptake of adrenaline by no more than 50–60%, whether it was measured immediately or after one hour. This suggests that platelets take up adrenaline by two processes only one of which is susceptible to inhibition by these drugs and is in that way similar to the process responsible for the non-diffusional uptake (Born & Bricknell, 1959) of 5-hydroxytryptamine.

Storage. As with 5-hydroxytryptamine, reserpine diminished the proportion of adrenaline in platelets and increased that of the metabolite (Pletscher, 1968). Thrombin released adrenaline but not its metabolite, which is analogous to the release of platelet 5-hydroxytryptamine by thrombin. These results are evidence that, like 5-hydroxytryptamine, adrenaline is stored intact in intracellular organelles. The similarity of the effect of thrombin suggests that both amines are stored in similar if not identical organelles.

Inactivation. Platelets inactivate 5-hydroxytryptamine by metabolizing a small amount of it to both 5-hydroxytryptophol and 5-hydroxyindoleacetic acid (Pletscher, 1968). Adrenaline, on the other hand, is inactivated to a greater extent, apparently by conjugation. The metabolite in platelets has not yet been fully identified; a conjugate of adrenaline hydrolysable by acid has been demonstrated in human blood plasma (Häggendal, 1963).

Release. Both amines are released in a specific release reaction which can be produced in many species by thrombin and in some species by one or more other agents including ADP, 5-hydroxytryptamine, and adrenaline itself (see Mills, 1970). Adrenaline is also released spontaneously from platelets resuspended in artificial media; whether platelets in their original plasma release adrenaline is not known. There is no evidence that undamaged platelets release 5-hydroxytryptamine spontaneously; if such release occurs it must be extremely slow.

The observations made so far can be accounted for by the following hypothesis (Fig. 9). Human platelets take up adrenaline by two different processes. In the first process the adrenaline crosses the platelet membrane with a high chance of being taken up intact into storage organelles. This process can be blocked at the platelet membrane by α-adrenoceptor blocking agents such as dihydroergotamine and phentolamine, by β-adrenoceptor blocking agents such as propranolol, and by chlorpromazine and related substances. This pathway is also blocked at another point by reserpine, presumably at the level of the storage organelle, thereby making the adrenaline available to the metabolizing system elsewhere in the platelet. The second uptake process is not or little affected by the drugs which inhibit the first. This process makes the adrenaline available to metabolic inactivation which can be inhibited by pyrogallol, 8-hydroxyquinoline and tropolone. When these drugs block its metabolism, more adrenaline taken up by the second process is available for accumulation in the storage organelles.

The highest concentration ratio for the distribution of intact adrenaline between platelets and the plasma in which they were suspended was about 12. When the pH of the medium is 7·4, the intracellular pH of human platelets is about 7·1 (Zieve & Solomon, 1966); therefore, the concentration ratio for adrenaline is higher than can be accounted for by a Donnan distribution. On the other hand, the ratio is much lower than the very high ratios for 5-hydroxytryptamine which are due to its accumulation in the organelles. The ratio for adrenaline is presumably also accounted for by its uptake into storage organelles. However, either far fewer organelles are formed (Berneis, Da Prada & Pletscher, 1969) when adrenaline is

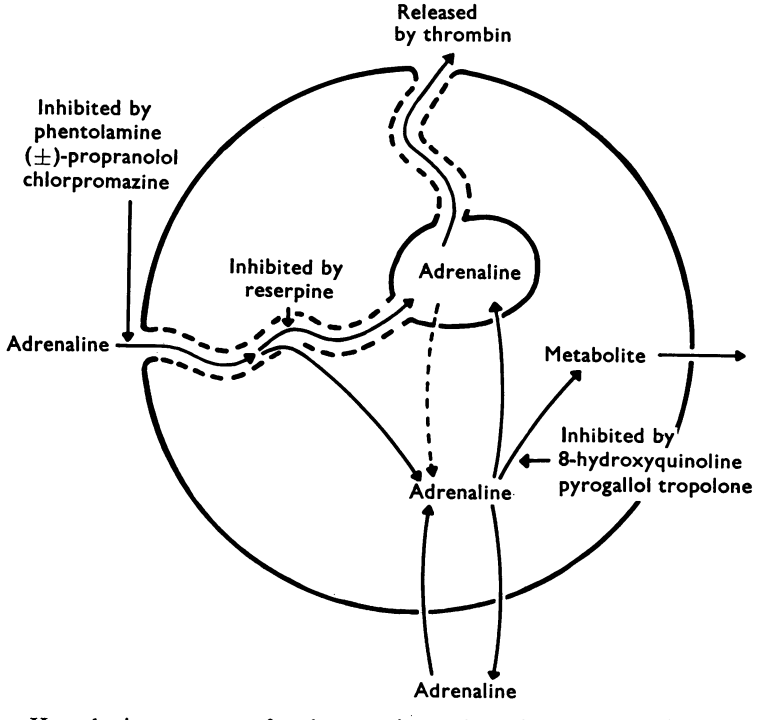

FIG. 9. Hypothesis to account for the experimental results. For description see text.

taken up than when 5-hydroxytryptamine is taken up; or pre-existing organelles have a much lower capacity for binding adrenaline than for binding 5-hydroxytryptamine. These problems have still to be solved.

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