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ACCUMULATION, STORAGE AND RELEASE OF ADRENERGIC NEURON BLOCKING AGENTS AND RELATED DRUGS BY HUMAN PLATELETS*

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Abstract—Human platelets suspended in plasma or Krebs solution were incubated with radioactive guanethidine, debrisoquin, amiloride or guanidine and the platelet-bound radioactivity was measured. Guanethidine and debrisoquin were taken up by a saturable energy-dependent process inhibited by 5-hydroxytryptamine (5-HT), desipramine, amphetamine, cocaine and quinidine. Amiloride and guanidine entered by a non-saturable mechanism not affected by metabolic-inhibitors, 5-HT or drugs which interfere with the 5-HT transport process. Unlike guanethidine, amiloride released 5-HT from loaded platelets and accumulation was partly blocked by reserpine. We suggest that guanethidine and debrisoquin probably utilize the 5-HT transport mechanism while amiloride and guanidine enter the cells by diffusion. However, because amiloride released 5-HT from loaded cells some of the bound amiloride may be related to 5-HT binding sites.

Evidence for intracellular binding was obtained in efflux experiments. When platelets loaded with amiloride or guanethidine were resuspended and incubated in drug-free plasma, most drug was retained and efflux was not affected by metabolic inhibitors. When the effects of 5-HT, reserpine and amphetamine were tested for their ability to enhance efflux of guanethidine and amiloride we found that only amphetamine was active. It released guanethidine but not amiloride, thus suggesting that a specific type of binding site may be involved. Clearly accumulation of these drugs by platelets is not by means of a continuous uptake and loss mechanism in the outer membrane.

There is now considerable evidence supporting the view that the platelet may serve as a model for the uptake and binding systems in serotonergic and dopaminergic nerve endings in the brain and noradrenergic nerve endings in the periphery.[1–5] Use of the model need not be limited to studies of biogenic amines but may be extended to various drugs which affect these systems. The first reports on platelet uptake of substances other than endogenous amines included reserpine and guanethidine.[6–8] Both drugs have potent effects on nerve cells and are accumulated by them.[9–12] Since such neuropharmacologically-active compounds are taken up by platelets, further studies in this area were warranted. We have previously reported the accumulation of the adrenergic neuron blocking agents guanethidine,[7,8] debrisoquin[13] and the diuretic amiloride[14] by platelets. This work is an extension of these earlier findings, as we

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wished to find out some more information on the mechanism of accumulation and retention and observe effects on endogenous 5-HT stores. Because the above substances all contain the guanidine-moiety we included guanidine in our experiments.

MATERIALS AND METHODS

Preparation of platelets. Blood was obtained and prepared by the method of Boullin and O'Brien[8] from normal human volunteers of either sex using polycarbonate laboratory utensils. The packed platelet volume was measured as described by Hardisty and Stacy.[15]

Uptake experiments. One ml of platelet-rich-plasma was incubated with various drugs, added in a volume of 10–50 μl of distilled water. Incubations were carried out in a Dubnoff metabolic incubator in an atmosphere of 5% CO_2 in O_2 at 4, 22 or 37°. Prior to the addition of drugs, the platelet samples were first equilibrated by incubation for 10 min. In experiments where the effects of potential inhibitors of uptake were investigated, these substances were added after the 10 min equilibration period and incubated for 1–60 min before the addition of the radioactively-labeled compound whose uptake was under study.

Efflux experiments. After cells were loaded with drug by incubation for 60–90 min, they were centrifuged at 8,000 *g* for 5 min; the resulting cell-free supernatant was decanted and the tubes wiped out with absorbent tissue. Sufficient plasma or Krebs solution [16] was added to restore the incubation volume to the original value, and then the platelets were resuspended by agitation on a Vortex mixer. The platelets were then reincubated for various times, as described in Results.

Estimation of uptake. At the end of the incubation period the platelet suspension was centrifuged at 20,000 *g* to form a dense button. The incubation medium was decanted and the tube interior wiped dry. One ml of distilled water was added and each pellet was disrupted by sonication with a "Biosonik 2" sonifier, probe setting 50 (Bronwill Sci., Rochester, N.Y.). The drug content in the pellet extract was estimated by liquid scintillation spectrometry using a Triton–X 100: toluene based scintillator and a Beckman LS–250 liquid scintillation spectrometer. To allow for the quantity of drug trapped in the interstices between cells we incubated samples of platelet-rich plasma with [^{14}C]carboxylic acid–inulin (specific activity 3·08 μC_i/mg, New England Nuclear Corp, Boston, Mass.). The amount of radioactivity recovered in the platelet pellet was taken as the "trapped cell volume". This volume was in the range 0·5–1·0 μl/μl packed cells. Uptake of drugs is expressed as nmoles/ml packed platelets.

5-HT estimation. In some experiments cells were loaded with 5-HT by incubation in 10^{-5} M 5-HT for 90 min. The platelet 5-HT content was determined by spectrophotofluorimetry.[17]

Drugs. The following were used: [^{14}C]amiloride (N-amidino-3,5-diamino-6-chloro [$^{14}C_3$]pyrazine carboxamide hydrochloride), 0·89 m Ci/mmole, (Merck and Co., Inc., Phila., Pa.); cocaine hydrochloride; [^{14}C]-debrisoquin (3,4-dihydro-2[lH] isoquinoline [^{14}C]carboxamidine sulfate), (0·2. m Ci/mmole) (Roche Products, Ltd., Welwyn Garden City, Herts, England); desipramine hydrochloride, dexamphetamine sulfate, dinitrophenol; [^{14}C]guanethidine (2-(octahydro)-1-azocinyl), ethyl [^{14}C]-guanidine) sulfate (0·93 m Ci/mmole) and [^{3}H]guanethidine (2-(octahydro-1-[$^{3}H_2$]-azocinyl) [$^{3}H_1$]ethyl guanidine sulfate (32–64 m Ci/mmole), (Ciba Laboratories, Ltd., Horsham, Sussex, England); and [^{14}C]guanidine hydrochloride (50 m Ci/mmole, Tracerlab, Waltham, Mass.); 5-hydroxytryptamine creatinine sulfate (Regis Chem. Co., Chicago, Ill.), iodoacetic acid; N-ethyl-maleimide (N–EM); ouabain, quinidine sulfate; and reserpine sulfate.

RESULTS

When platelets were suspended in plasma and incubated with ^{14}C-labeled debrisoquin, guanethidine, amiloride or guanidine for up to 90 min, the cells accumulated radioactivity. The concentration of drug in the platelet (nmole/ml packed cells, C_i) always exceeded the final plasma concentration (nmole/ml, C_f (Fig. 1). Amiloride, debrisoquin and guanethidine have a basic structural similarity being heterocyclic substituted guanidines, and in each case C_i/C_f was high, in the range 6–20:1 after 90 min incubation. In contrast, platelets concentrated guandidine itself only two-fold. The actual quantities of drug accumulated are given in Table 1; uptake of guanethidine and debrisoquin was considerably greater than the other substances.

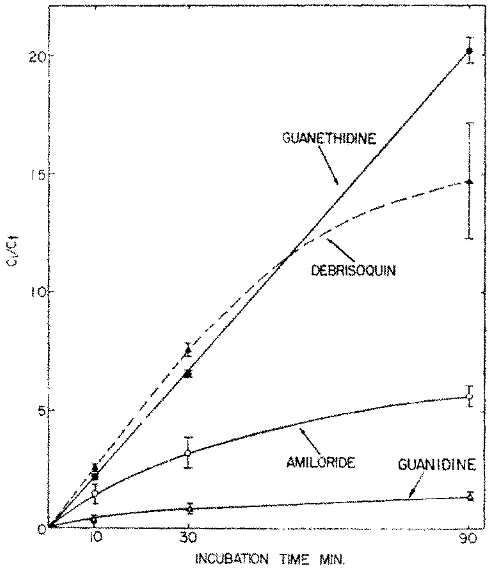

Fig. 1. The uptake of guanidine and derivatives by platelets. Platelets in plasma were incubated with 10^{-5} M ^{3}H-guanethidine (●), ^{14}C-debrisoquin (▲), ^{14}C-amiloride (○) or ^{14}C-guanidine (△) for up to 90 min. Results are plotted as the ratio of the concentration of drug in platelets (c_i) to the final plasma concentration at the end of incubation (C_f) in relation to time. Values are the mean ± S.E. of 6–9 determinations.

These data suggested that amiloride, debrisoquin and guanethidine were actively accumulated by energy-dependent mechanisms whereas guanidine entered platelets by diffusion. Support for this view was obtained in the case of debrisoquin and guanethidine from plotting C_i/C_f against C_f. With these two drugs, C_i/C_f declined with increasing C_f (Fig. 2), indicating also a saturable component in their uptake processes. On the other hand, C_i/C_f for amiloride and guanidine did not change with C_f, suggesting that, in fact, their uptake was by passive diffusion and did not involve saturable mechanisms.

TABLE 1. ACCUMULATION OF GUANIDINE-CONTAINING DRUGS BY PLATELETS*

	Uptake (nmoles/ml packed platelets)		
Plasma concentration	10^{-6} M	10^{-5} M	10^{-4} M
Guanethidine	21·7 ± 1·6	179·0 ± 7·5	1094·0 ± 101·3
Debrisoquin	N.D.	147·5 ± 10·7	978·0 ± 85·5
Amiloride	†	56·4 ± 3·8	509·2 ± 51·3
Guanidine	1·35 ± 0·17	13·4 ± 1·3	186·1 ± 19·3

* Platelets in plasma were incubated for 90 min with labeled drugs. Values are mean ± standard error of the mean (S.E.) of 4–8 determinations.

† Uptake at 5×10^{-6} M was 27·4 ± 3·2 nmoles/ml packed platelets. N.D. —not determined.

In the interpretations of results of the accumulation of drugs by cells, it is important to know the degree of binding to plasma proteins because this will affect the free drug concentration in plasma. In particular, if a drug is bound to plasma proteins to any significant extent, C_f may be much lower than is actually apparent. Therefore, we measured drug uptake by platelets in Krebs solution, and compared the results with those obtained with plasma. Uptake of debrisoquin and amiloride was not decreased in plasma compared with Krebs solution; it is already known that guanethidine binding to plasma proteins does not exceed 4 per cent.[11]

Mechanism of uptake. Further information on the nature of the uptake processes involved was obtained by studying the effects of metabolic inhibitors and low temperatures (Table 2). Incubation at 4° caused complete inhibition of the uptake of all drugs,

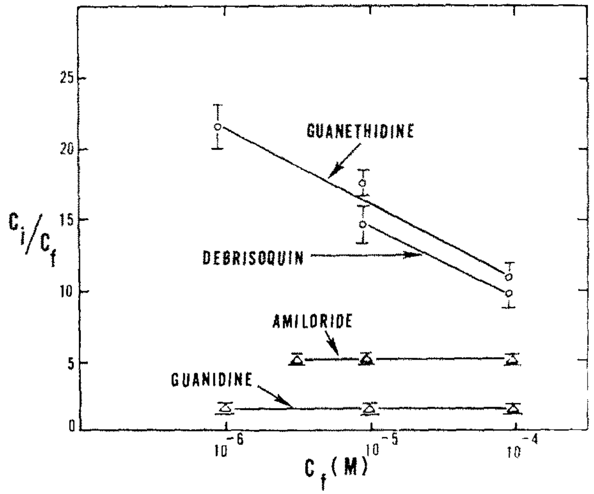

FIG. 2. Relationship between concentration of amiloride, debrisoquin, guanethidine and guanidine in platelets (C_i) and the final plasma concentration after 90 min incubation (C_f). C_i/C_f is plotted arithmetically (ordinate) against log C_f (abscissa). Results are the mean ± S.E. of 5–10 determinations.

TABLE 2. EFFECT OF FACTORS WHICH INHIBIT ENERGY-DEPENDENT PROCESSES ON THE UPTAKE OF GUANIDINE AND ITS DERIVATIVES*

Experiment	% Inhibition Guanethidine	 Debrisoquin	 Amiloride	 Guanidine
4°	98·0 ± 0·1	97·5 ± 1·3	81·0 ± 1·2	88·3 ± 4·1
22°	87·6 ± 1·1	72·1 ± 6·9	95·0 ± 3·7	36·7 ± 4·4
Ouabain 10^{-5} M	52·0 ± 2·9	31·3 ± 6·7	30·9 ± 5·5	49·3 ± 3·0
Iodoacetate and DNP 10^{-3} M	75·6 ± 6·3	71·0 ± 2·4	30·8 ± 6·6	30·7 ± 4·1
N-ethylmaleimide 10^{-4} M	91·9 ± 1·5	81·2 ± 0·8	8·0 ± 0·3	38·7 ± 3·3

* The potential inhibitors were incubated with platelet-rich-plasma for 60 min before addition of labeled drug (10^{-5} M). Incubation was then continued a further 90 min. The uptake (nmoles/ml packed platelets) in the absence of inhibitor was: guanethidine, 184·0 ± 8·9; debrisoquin, 139·9 ± 9·8; amiloride, 61·3 ± 5·8; and guanidine; 12·2 ± 1·0. Results are mean S.E. of 4–6 determinations.

but incubation at 22° produced more selective effects; 72–95 per cent inhibition of uptake of the heterocyclic guanidine derivatives, but much less blockade of guanidine accumulation.

The metabolic inhibitors caused variable responses, but generally the uptake of guanethidine and debrisoquin was inhibited to a much greater degree than amiloride or guanidine.

Unfortunately, we were unable to draw any firm conclusion from these results regarding the involvement of metabolic processes in the accumulation of amiloride and guanidine, although the effects observed in regard to debrisoquin and guanethidine agreed with the idea that accumulation was energy-requiring. Accordingly, we turned our attention to other indications of metabolic processes in platelets, by observing the effects of 5-HT; of inhibitors of 5-HT uptake by platelets[18–20] and of inhibitors of noradrenaline uptake into sympathetic nerves[21] upon the accumulation of the four drugs we were investigating. The rationale for this approach was that 5-HT accumulation is known to involve an energy-requiring mechanism.[2,3]

We incubated platelets with desipramine, cocaine, dexamphetamine, guanidine or 5-HT for 20 min before addition of guanethidine, debrisoquin or amiloride. Apart

TABLE 3. EFFECT OF DRUGS ON UPTAKE OF ANTIHYPERTENSIVE AGENTS

Experiment	% Inhibition of uptake Guanethidine	 Debrisoquin	 Amiloride
5-HT 10^{-5} M	80·0 ± 4·1	80·8 ± 5·9	41·9 ± 3·7
Desipramine 10^{-5} M	79·0 ± 3·3	60·9 ± 1·9	2·1 ± 0·9*(43)
Cocaine 10^{-5}	68·0 ± 4·1	59·8 ± 4·2	0·8 ± 1·3*(21)
Dexamphetamine 10^{-5} M	61·0 ± 3·2	61·0 ± 7·7	59·1 ± 1·8
Quinidine 10^{-5} M	59·4 ± 1·2	61·4 ± 6·3	24·1 ± 1·8

The potential inhibitory drugs were added to platelet-rich-plasma 20 min before incubation with labeled compound (10^{-5} M). Incubation was then continued for a further 90 min. Results are mean ± standard error of 4–6 determinations, and are expressed as nmoles/ml packed platelets.

* Values in parentheses refer to the percentage inhibition obtained with 10^{-4} M inhibitor.

from dexamphetamine, the pharmacological antagonist of guanethidine[22,23] each substance inhibited guanethidine and debrisoquin uptake to a much greater degree than amiloride (Table 3). These experiments suggest that while guanethidine and debrisoquin may utilize a transport mechanism at the outer platelet membrane which is very similar to that for 5-HT, amiloride transport may be of a different nature.

Drug efflux. Our next experiments were designed to give an indication of the affinity of amiloride and the adrenergic neuron blocking agents for binding sites on the platelet membrane or within the interior of the cell. Unfortunately, efflux studies with debrisoquin were not possible due to the low specific activity of the radioactive compound. We loaded platelets with amiloride or guanethidine and then resuspended them in fresh medium and observed the rate and time course efflux.

The initial efflux caused by the procedure of resuspension carried out at 2° (see Methods) gives an indication of the localization of drugs within the platelet membrane. This loss was greater in the case of guanethidine (15·2 ± 4·4 nmole/ml cells, five experiments) than amiloride (6·15 ± 1·07 nmole/ml cells, five experiments), but when efflux was expressed as a percentage of the drug content of the loaded cells the difference was minimized. Thus, the loaded platelets contained 245 ± 12·1 nmole/ml cells of guanethidine and lost 10·3 ± 1·8 per cent, whereas the amiloride content was 59·7 ± 8·5 nmole/ml cells and only 6·2 ± 1·8 per cent was lost during resuspension.

These small losses suggest that both amiloride and guanethidine were firmly bound to platelets, and that the small amounts lost were from such readily accessible sites as the platelet membrane.

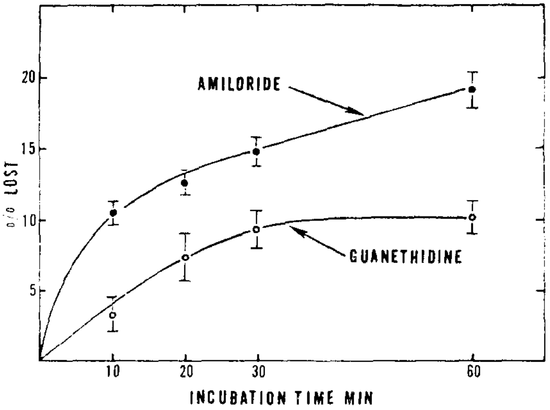

Fig. 3. Efflux of guanethidine and amiloride from platelets. The cells were labeled with ^{3}H-guanethidine or ^{14}C-amiloride by incubation in 10^{-5} M for 90 min and resuspended as stated in Methods. Before resuspension the platelets contained 245·0 ± 12·1 nmoles guanethidine or 59·7 ± 8·5 nmoles amiloride per ml packed platelets. The resuspension procedure alone caused the loss of 10·3 ± 1·8 per cent guanethidine and 6·2 ± 1·8% amiloride in addition to that shown here. Each result is the mean ± S.E. of 5 determinations.

The subsequent efflux occurring when loaded cells were reincubated for 10–60 min is shown in Fig. 3. In both experiments only a small proportion of the bound substance leaves the cells, indicating that amiloride and guanethidine are firmly bound to platelets, probably having an intracellular localization. Further evidence for this view

TABLE 4. FACTORS AFFECTING GUANETHIDINE AND AMILORIDE EFFLUX FROM HUMAN PLATELETS

Experiment	% Loss of drug Guanethidine	Amiloride
Control (no drug)	11·6 ± 0·5	14·8 ± 1·1
Ouabain 10^{-4} M	10·5 ± 1·3	17·0 ± 1·9
Iodoacetate and dinitrophenol 10^{-3} M	13·0 ± 2·1	15·6 ± 2·1
5-HT 10^{-4} M	11·3 ± 0·9	17·3 ± 2·6
Dexamphetamine 10^{-4} M	18·5 ± 0·5*	15·0 ± 1·6
Reserpine 10^{-4} M	10·9 ± 0·3	13·1 ± 1·1

Platelets were labeled with drug and resuspended as described in Methods, and incubated for 30 min either with or without a metabolic inhibitor or other drug.

* Values are mean ± S.E. of 6–8 determinations. $P < 0·05$.

is given by the results of Table 4, which show that metabolic inhibitors, 5-HT and reserpine all fail to release either guanethidine or amiloride. However, amphetamine did produce a significant increase in the loss of guanethidine in a concentration ten times less than that required to release platelet 5-HT[24] (Table 4, see ref. 8).

The releasing properties of reserpine and 5-HT were also examined to see if the guanidines were bound at the same subcellular sites as 5-HT. Neither reserpine nor 5-HT had an effect (Table 4). Reserpine is known to damage 5-HT binding sites in cells[25–27] and would therefore be expected to release drugs from these sites, whereas 5-HT would produce the same effect by displacement.

Another way of studying the question of the relationship of guanethidine and amiloride binding sites to 5-HT stores was to see what accumulation took place after alteration of the 5-HT storage sites by reserpine. Therefore, we measured the uptake of guanethidine and amiloride by platelets which had been preincubated with 10^{-5} M reserpine for 2 hours. Table 5 shows that at this concentration reserpine produced considerable blockade of amiloride accumulation while having no effect on guanethidine. This suggests that amiloride may be binding to the 5-HT storage sites. Table 6 gives further evidence for this. Thus 10^{-5} M amiloride released 39·6 per cent of the 5-HT from ^{14}C-5-HT loaded platelets but guanethidine had no effect in concentrations

TABLE 5. EFFECT OF RESERPINE ON THE UPTAKE OF AMILORIDE AND GUANETHIDINE

Reserpine concentration (M)	Uptake (nmoles/ml packed platelets) Amiloride	Guanethidine
0	60·1 ± 7·4	145·0 ± 11·2
10^{-5}	14·2 ± 0·9	152·0 ± 17·9

Platelets were incubated in plasma for 2 hr with 10^{-5} M reserpine; 10^{-5} M [^{3}H] guanethidine (or ^{14}C-amiloride), was then added and incubation continued for 90 min. Results are mean ± S.E. of 4 determinations.

TABLE 6. EFFECT OF GUANETHIDINE AND AMILORIDE ON PLATELET 5-HT CONTENT

		5-HT Content (umoles/ml packed platelets) Loaded	Unloaded
Initial		1·87 ± 0·30	0·28 ± 0·02
After incubation with amiloride	10^{-5} M	1·13 ± 0·20†	0·28 ± 0·02*
After incubation with guanethidine	10^{-5} M	1·87 ± 0·25*	0·28 ± 0·01*
	10^{-4} M	1·87 ± 0·20*	0·28 ± 0·01*

Platelets were loaded with 5-HT by incubation in 10^{-5} M for 90 min. After resuspension, incubation was carried out in plasma with guanethidine 10^{-5} M or 10^{-4} or 10^{-5} amiloride. Amiloride, 10^{-4} M could not be tested since it interfered with the 5-HT spectrophotofluorometric assay. Values are the mean ± S.E. of 4–5 determinations.

* Not significantly different from initial value.

† $P < 0·05$.

up to 10^{-4} M. 10^{-4} M Amiloride could not be tested since this concentration interfered with the spectrophotofluorometric assay for 5-HT.

In 5-HT loaded platelets, amiloride uptake was inhibited to a significantly greater degree (33 per cent) than guanethidine (16 per cent) (Table 7) implying that at least a fraction of the amiloride may be binding to 5-HT storage sites.

TABLE 7. EFFECT OF 5-HT LOADING ON GUANETHIDINE AND AMILORIDE UPTAKE

	% Uptake of drug 5-HT loaded	Unloaded
Guanethidine 10^{-5} M	83·3 ± 6·9	100
Amiloride 10^{-5} M	67 ± 5·8	100

Platelets were loaded with 5-HT (see Table 6), resuspended and incubated in plasma containing 10^{-5} M ^{14}C-guanethidine or ^{14}C-amiloride for 90 min. Values are the mean ± S.E. of 4–5 experiments.

DISCUSSION

Although none of the substituted guanidines are accumulated to the extent of 5-HT (i.e. $C_i/C_f = 1000:1$), there is much evidence that guanethidine and debrisoquin utilize the 5-HT transport system, or some system that is very similar. Thus uptake is blocked by cocaine, desipramine, dexamphetamine and quinidine which are all potent inhibitors of 5-HT uptake; and 5-HT itself is the most active inhibitor. Although the uptake of guanethidine plotted as 1/V against 1/S did not conform to the Michaelis–Menten equation for saturable enzymatic reactions, there is really no reason why it should. Figure 2 does indicate that there is a saturable component involved in the uptake process because C_i/C_f declines as C_f is increased. This is also true for debrisoquin.

On the other hand, amiloride did not appear to utilize a saturable uptake component, since C_i/C_f did not change with increasing C_f.

The effects of metabolic inhibitors also support the contention that amiloride uptake was by diffusion because the inhibitory effects of ouabain, DNP + IAC and NEM did not exceed 30 per cent (Table 2). However, low temperatures did produce very considerable inhibition. These effects are difficult to explain on any simple basis. It is quite conceivable that low temperatures affect intracellular binding in addition to membrane configuration, metabolic transport processes and the overall shape of the cell.

The actual binding sites for the substituted guanidines are not known, although the efflux data tell us several things about the mechanism of retention. The strong attachment of these drugs is comparable to that of 5-HT[28,30] and suggests that like 5-HT they are bound intracellularly. Since there is no increase in efflux in the presence of metabolic inhibitors, this is further grounds for belief that amiloride and guanethidine are bound within the cell and are not retained by an energy-dependent pumping process located in the platelet membrane. Evidence that guanethidine does not use 5-HT binding sites is substantial: guanethidine does not release endogenous or exogenous 5-HT; it is not released by 5-HT or reserpine. Moreover, guanethidine uptake is not reduced by previous saturation of the 5-HT binding sites. The objection to this view is that amphetamine did release some guanethidine. However this may merely indicate that amphetamine acts upon more than one type of storage site. Indeed, most of the work concerning interactions of guanethidine and amphetamine involve noradrenaline stores and not 5-HT. Also the concentration of amphetamine required to release 5-HT from platelets was approximately ten times greater than that required to release guanethidine,[24] possibly indicating great potency in releasing substances from other types of stores.

Although most of the evidence suggests that amiloride does not have the same subcellular localization in platelets as 5-HT (i.e. does not release endogenous 5-HT, is not released itself by 5-HT, amphetamine or reserpine), the strong effect of reserpine on amiloride accumulation (Table 5) cannot be ignored and may allude to the fact that a fraction of the amiloride may be associated with 5-HT storage organelles.

Some of our results with debrisoquin[20] have been confirmed and extended by Pocelinko and Solomon.[29] They confirmed that uptake was energy-dependent and utilized a saturable process. Additionally, they showed by kinetic experiments that debrisoquin, guanethidine and 5-HT compete for the same uptake mechanism. Of special interest was their finding that debrisoquin was lost rapidly from loaded cells. This is in contrast to our findings with guanethidine, which was tightly bound. However, since debrisoquin releases 5-HT[31] and guanethidine does not (this work), they most likely do not exhibit the same intracellular localization.

The large amount of guanethidine accumulated in contrast to debrisoquin and the other drugs (Table 1) suggests that it might be the most likely platelet-bound drug to play a pharmacological role *in vivo* as we have discussed earlier.[8]

A study of drug effects on platelet function has yielded results which support some of our findings on drug localization. Zbinden and Tomlin[32] incubated platelets with debrisoquin and guanethidine for one hour, which we find is sufficient time for a considerable intracellular accumulation. They found no effect on platelet adhesiveness, platelet aggregation, release of platelet factor 3 or clot retraction. However, adhesiveness was enhanced when measured immediately after addition of drugs. This is the only observation which can be explained on the basis of guanethidine being localized in the outer platelet membrane. Since platelet adhesion is essentially a membrane phenomenon, their other experiments using this criterion certainly suggest that very little guanethidine or debrisoquin was present in the platelet membrane after 60 min incubation.

As the characteristics of accumulation of amiloride are rather different from the other drugs it is reasonable to suppose that this is due to differences in chemical structure. Hence the amidine group in amiloride appears to give it an entirely different chemical reactivity from either guanethidine or debrisoquin. The straight-chain guanidine moiety itself may not be essential since guanidine was distributed only by diffusion. However, combination of this guanidine grouping with some types of heterocyclic ring system seems essential although a larger series of compounds would have to be investigated to test this hypothesis.

We conclude that our experiments show that although guanethidine and debrisoquin are taken up by platelets by an energy dependent system related to the 5-HT system, their storage sites are different. Amiloride enters platelets by diffusion and like guanethidine is firmly bound by the platelet, but not in the outer membrane.

Storage of both drugs is largely intracellular involving more than one site, one of which may be the 5-HT stores.

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