Biochimica et Biophysica Acta, 691 (1982) 106–114
Elsevier Biomedical Press

BBA 71352

THE EFFECT OF VERAPAMIL, LANTHANUM AND LOCAL ANESTHETICS ON SEROTONIN RELEASE FROM RABBIT PLATELETS

YAEL ZILBERMAN, YEHUDA GUTMAN and RUTH KOREN *

Department of Pharmacology, Hebrew University, Hadassah Medical School, Jerusalem 91010 (Israel)

(Received March 3rd, 1982)
(Revised manuscript received June 21st, 1982)

Key words: Verapamil; La^{3+}; Anesthetic; Serotonin release; (Rabbit platelet)

The effect of calcium blockers (verapamil, local anesthetics and lanthanum chloride) on serotonin release from rabbit platelets was studied. The following results were obtained: (1) Verapamil and tetracaine (but not lanthanum) caused a time- and dose-dependent release of serotonin. The curves describing the time-course and those describing the concentration dependence of the release were sigmoid, suggesting cooperativity. (2) Thrombin-induced release from the platelets was dependent upon extracellular sodium ions, while no dependence was observed for the drug-induced release. (3) The release by verapamil was partially inhibited by prostaglandin E_1 and theophylline which are known to raise intracellular cAMP levels, but was unaffected by the prostaglandin-synthesis inhibitor, indomethacin. (4) Verapamil, tetracaine and lanthanum inhibited thrombin-induced release of serotonin. The curve of dose dependence of the inhibition by verapamil and tetracaine was not sigmoid. The inhibition by verapamil and tetracaine was reversed by extracellular calcium ions, but no effect of this ion on the drug-induced release reaction was observed. It is concluded that the serotonin release induced by some calcium blockers and the inhibition of the thrombin-induced release by the same drugs are two separate phenomena. It is suggested that verapamil and tetracaine-induced release are mediated by exocytotic processes brought about by the interference of the drugs with calcium distribution between the cytosol and storage compartments within the platelet.

Introduction

The release of serotonin, nucleotides and calcium from platelets induced by various agents is an exocytotic process, for which an increase in intracellular free calcium ion concentration is an essential requirement [1]. On the other hand, neither thrombin-induced nor ionophore A23187-induced release require extracellular calcium, though the release is enhanced in the presence of calcium [2–4]. It has been proposed that thrombin, as well as ionophore A23187, can mobilize calcium from intracellular storage sites, probably the dense tubular system, thus elevating its concentration in the cytosol, leading to serotonin release [5,6]. The advanced stages of platelet activation by thrombin are accompanied by an increase in permeability of the platelet membrane to calcium [6].

Prostaglandins play a role in the activation of platelets by thrombin as well as by A23187 [7]. Platelets can synthesize prostaglandins in a calcium-dependent process. Prostaglandins, in turn, liberate calcium from the dense tubular system [8]. Inhibition of prostaglandin synthesis by aspirin or by indomethacin reduces the extent of

* To whom correspondence should be addressed.

Abbreviations: TMB-8, 8-*N*,*N*-diethylamino-octyl-3,4,5-trimethoxybenzoate HCl; 2-PIA, 2-propyl-3-dimethylamino-5,6-methylenedioxyindene HCl.

thrombin-induced release of serotonin, especially at low concentration of thrombin [7].

Calcium channel blockers are known to inhibit the influx of calcium into cells of various origins from media containing this ion. In addition they can also inhibit calcium movement into intracellular organelles [9]. Verapamil and lanthanum block calcium movement in several tissues whereas they show little or no effect on sodium movement [10,11]. On the other hand, certain local anesthetics which are also capable of blocking calcium movement show a much more pronounced effect on sodium movement [12].

The effect of calcium blockers on serotonin release from platelets has been studied by several investigators. TMB-8 (8-*N*,*N*-diethylamino-octyl-3,4,5-trimethoxybenzoate HCl), 2-PIA (2-propyl-3-dimethylamino-5,6-methylenedioxyindene HCl) and chlorotetracycline were shown to inhibit serotonin release mediated by thrombin of A23187 [13,14]. In addition, 2-PIA was shown to cause release in the absence of thrombin or A23187. However, all three compounds caused profound leakage from the platelets [13]. Verapamil has been shown to inhibit the thrombin-induced release from the platelets while its effect on non-activated platelets was not investigated [15]. Local anesthetics have also an inhibitory effect on the release reaction in response to thrombin. This effect is attributed to the inhibition of calcium release from intracellular stores [16]. Lanthanum has also been shown to inhibit thrombin-induced release of serotonin. This effect, which was attributed to lanthanum binding to mucopolysaccharides on the platelet membrane, was proposed not to be related to its calcium blocking properties [17,18]. Local anesthetics were also shown to cause a slight release from the platelets.

The investigation of the effect of calcium blockers on unstimulated as well as thrombin-activated platelets might be of value in understanding the relationship between the release reaction, prostaglandin synthesis and the various storage sites of calcium in the platelet.

Methods

Materials

[^{3}H]Serotonin (16.6 Ci/mmol, 1.0 mCi/ml) was purchased from Amersham Radiochemical Centre. Thrombin, topical, from park Davies (spec. act. 1000 U/ml). Verapamil from Ikapharm, Ltd. Ramat Gan. Local anesthetics: Tetracaine from Winthrop, New York, NY. Benzocaine from Merck, Darmstadt, F.R.G. Lidocaine from Dolder, Switzerland. Procaine from Sigma, St. Louis, MO. $LaCl_3$ from BDH Chemicals, Poole, U.K. Indomethacin was a gift from Assia-Riesel, Tel-Aviv, Pyruvic acid from Sigma, St. Louis, MO. Prostaglandin E_1 was a gift from Dr. J. Pike, The Upjohn Company, Kalamazoo, MI. Theophylline from Sigma, St. Louis, MO.

Three buffers were used in the course of the release experiments. Buffer A: sodium chloride 116 mM; potassium chloride 4 mM; potassium dihydrogen phosphate 1.8 mM; magnesium sulfate 1.1 mM; Tris 25 mM; trisodium citrate 10.9 mM; and glucose 0.9 mM at pH 7.4. Buffer B: sodium chloride 137 mM; potassium chloride 2.7 mM: Tris 25 mM; and glucose 11 mM at pH 7.4. Buffer C: choline chloride 116 mM; potassium chloride 4 mM; potassium dihydrogen phosphate 1.8 mM; magnesium sulfate 1.1 mM; Tris 25 mM; citric acid 10.9 mM; glucose 0.9 mM at pH 7.4. The composition of the washing solution was: sodium chloride 0.9%, EDTA 0.5% at pH 7.0.

Preparation of the platelets

Rabbit blood was collected from the ear artery into plastic tubes containing trisodium citrate dihydrate (final concentration 0.38%). Platelet rich plasma was obtained by centrifugation (4 min at $750 \times g$), and collection of the upper layer. [^{3}H]Serotonin was then added to a final concentration of $6 \cdot 10^{-8}$ M and the platelet rich plasma incubated for 15 min at 37°C. The platelets were then centrifuged for 10 min at $460 \times g$. The pellet was separated and suspended in fresh washing solution and recentrifuged. The washing procedure was repeated twice and the pellet was finally suspended in buffer. The final platelet concentration was 300 to $500 \cdot 10^3$ cells/mm^3.

Serotonin release reaction

The release reaction was carried out in Beckman microfuge test tubes at 37°C as follows: 450 µl of platelet suspension was brought to 37°C in a water bath. Buffer, drug vehicle, drug or thrombin

were added in 50 μl aliquots. The reaction was terminated by the addition of 20 μl glutaraldehyde (final concentration 0.4%) and quick chilling. The platelets were spun in a Beckman microfuge and aliquots of the supernatant were counted in a Packard Tri-Carb Scintillation Spectrometer, in a toluene-Triton based scintillation fluid. The radioactivity released was compared to the total radioactivity in the aliquot, which served as reference (100%).

Determination of lactate dehydrogenase activity in the extracellular environment was carried out in the following manner: The release reaction was conducted as above, exept for the addition of glutaraldehyde. The supernatants obtained after centrifugation were collected and enzyme activity was assayed according to Bergmeyer [19] using a Cary 14 Spectrophotometer. The assay is based upon the decrease in the concentration of NADH which is followed at 340 nm. This activity was compared to the activity in an identical platelet incubate extract prepared by seven cycles of freezing and thawing. Protein determination were done according to the method of Lowry et al. [20].

Results

The effect of verapamil on serotonin release from washed rabbit platelets was studied in the presence and absence of thrombin. Fig. 1A shows that verapamil caused a significant release of serotonin from the platelets in the absence of any other stimuli. A close inspection of Fig. 1A reveals some interesting features: The release of serotonin had a time lag which decreased with increasing concentrations of verapamil, and this time lag was not detected at verapamil concentrations of ≥ 0.5 mM. The concentration dependence of the release reaction at different incubation times is depicted in Fig. 1B. Serotonin release over 10 min was linear with verapamil concentration. This was not the case for shorter incubation times with verapamil. For instance, after 1 min of incubation no release was caused by verapamil at concentrations below 0.5 mM, whereas at 1 mM a substantial fraction of serotonin was released.

The effect of verapamil on thrombin-induced release of serotonin is shown in Fig. 2. Preincubation for 30 s with various concentrations of

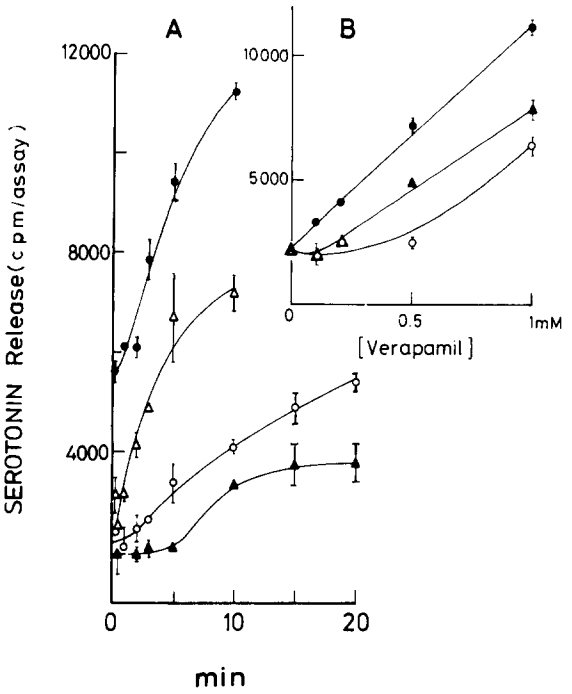

Fig. 1. (A). Verapamil-induced release of serotonin from washed rabbit platelets. Platelets were suspended in buffer A (see Materials and Methods). Final concentrations of verapamil: ▲, 0.1 mM; ○, 0.2 mM; △, 0.5 mM; ●, 1.0 mM. In (B) the same data are plotted as a function of verapamil concentration. Time of incubation: ○, 1 min; ▲, 3 min; ●, 10 min; Total content in the aliquot, 16000 cpm/assay.

verapamil followed by an incubation of 2 min with thrombin caused a significant inhibition of the thrombin-induced release (Fig. 2A). Fig. 2B shows the net release of serotonin by thrombin, i.e. the release after subtraction of the control values (the serotonin released by verapamil alone under identical experimental conditions). An important feature of the inhibitory effect of verapamil should be considered: Whereas the verapamil-induced release was negligible at concentrations up to 0.5 mM, the inhibition of the thrombin-induced release was quite significant at verapamil concentrations as low as 0.2 mM, in the same experiment.

The effect of extracellular calcium on serotonin release is shown in Tables I and II. Various concentrations of $CaCl_2$ (0.3 to 5.28 mM) were tested for their effect on the verapamil-induced and the thrombin-induced release reactions and on the inhibition of thrombin-induced release by verapamil. Calcium had no effect on either spontaneous or on

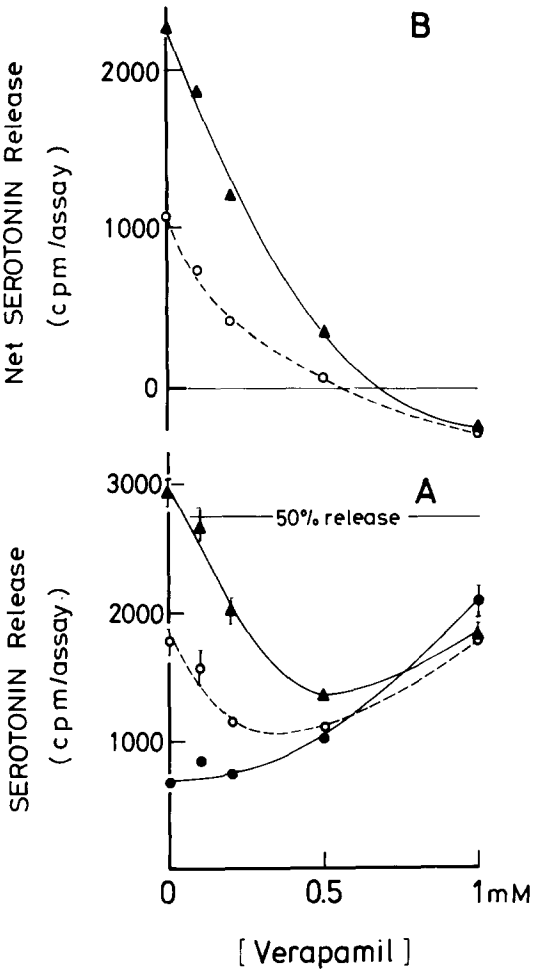

Fig. 2. The effect of verapamil on thrombin-induced release of serotonin. The platelets were suspended in buffer A, and incubated for 0.5 min in the presence of various concentrations of verapamil. Thrombin was then added and the incubation was carried on for another 2 min. (A) ●, saline added at 0.5 min; ○, thrombin, 1 U/ml added at 0.5 min. ▲, Thrombin, 3 U/ml added at 0.5 min. (B) The same data after subtraction of the control (saline) values for each verapamil concentration.

verapamil-induced release (Table I). The effect of calcium on thrombin-induced release was stimulatory at low concentrations of calcium and less stimulatory at higher, perhaps toxic, concentrations. The inhibitory effect of verapamil on thrombin-induced release was reduced by calcium, but not abolished at physiological concentrations (Table II).

Of all local anesthetic agents tested, tetracaine alone released an appreciable amount of serotonin

TABLE I

THE EFFECT OF EXTRACELLULAR CALCIUM ON SEROTONIN RELEASE INDUCED BY VERAPAMIL

The platelets were suspended in buffer B into which $CaCl_2$ (0.33 to 5.0 mM) was added. Verapamil (0.2 mM) was added at time zero and the reaction was terminated after 10 min. At time zero the buffer contained 700 cpm/assay. Total radioactivity in the assay was 14480 cpm/assay. Saline was added as control for verapamil. All values are average of triplicates ±S.D.

$CaCl_2$ (mM)	Buffer (cpm/assay)	Verapamil (cpm/assay)
0	770±124	2014±94
0.3	707± 21	2036± 3
1.25	863± 58	2080±25
5.0	804± 92	2142±44

from the platelets (Table III), and therefore was further investigated. Tetracaine-induced serotonin release was qualitatively similar to the verapamil-induced release (Fig. 3). In addition, we confirmed the finding by Feinstein et al. [16] that tetracaine inhibited thrombin-induced serotonin release. The experiments were done under the same conditions as those with verapamil and the curves obtained were similar to those shown in Fig. 2. 50% inhibition of the thrombin-induced release was obtained at 2 mM of tetracaine. Serotonin release and inhibition of thrombin-induced release by tetracaine were detected in the same concentration range while verapamil inhibited the thrombin-induced release of serotonin at concentrations at which this drug by itself did not stimulate release (Figs. 1 and 2).

The effect of a different calcium blocker, lanthanum, was studied in the presence and in the absence of thrombin. The results of two such experiments are presented in Table IV. 1 mM lanthanum had no effect on the spontaneous release of serotonin even at very long incubation periods, but significantly inhibited the thrombin-induced release. The degree of inhibition was not dependent on the duration of the preincubation at least up to 15 min.

The effect of indomethacin on thrombin-induced release and on verapamil-induced release of serotonin was investigated. Indomethacin was dissolved in saline + propane-1,3-diol (up to 1.1%

TABLE II

THE EFFECT OF EXTRACELLULAR CALCIUM ON SEROTONIN RELEASE INDUCED BY THROMBIN AND ON THE INHIBITION OF THROMBIN-INDUCED RELEASE BY VERAPAMIL

The platelets were suspended in buffer B into which $CaCl_2$ (0.33 to 5.28 mM) was added. Verapamil (0.5 mM) was added at time zero. Thrombin (0.1 U/ml) was added at 0.5 min. The reaction was terminated at 3 min. Saline was added as control for either verapamil or thrombin. The control release was 2044 ± 59 cpm/assay. Verapamil-induced release was 2988 ± 370 cpm/assay. (All values include the zero time values.) The results are average of triplicates ± S.D. The net release by thrombin (column C) was calculated as the difference between each value in the 'thrombin-induced release' (column A) and the average release by buffer (2044 cpm/assay). The net release by thrombin in the presence of verapamil (column D) was calculated as the difference between each value of thrombin-induced release in the presence of verapamil (column B) and the average release by verapamil (2988 cpm/assay). % inhibition is calculated as 1 − (D/C) for each calcium concentration.

$CaCl_2$ (mM)	cpm per assay				% inhibition
	Thrombin-induced release (A)	Thrombin-induced release in the presence of verapamil (B)	Net thrombin-induced release (C)	Net thrombin-induced release in the presence of verapamil (D)	
0	4714 ± 408	3680 ± 320	2670	692	74
0.33	9575 ± 713	7883 ± 857	7531	4895	35
1.32	10614 ± 685	9444 ± 970	8570	6456	25
5.28	6836 ± 195	7169 ± 219	4792	4181	13

v/v) and the control tubes contained the same concentration of propane-1,3-diol. The platelets were incubated for 15 min with either indomethacin (10^{-5} to 10^{-4} M) or vehicle prior to the release reaction (2.5 min with thrombin 0.5 U/ml or 10 min with verapamil 0.5 mM). The drug vehicle had no effect on either thrombin-induced or verapamil-induced release, both of which released 25% of the total serotonin content. Whereas indomethacin

TABLE III

SEROTONIN RELEASE BY LOCAL ANESTHETICS AND VERAPAMIL

The platelets were suspended in buffer A. The concentration of all drugs was 1 mM. Incubation time was 15 min. 100% radioactivity in the assay was 18300 cpm/assay. The values are averages of triplicates ± S.D. Net release is the difference between the release in the absence and in the presence of the drug. All values include the time zero value (2100 cpm/assay).

Drug	Release (cpm/assay)	Net release (cpm/assay)
None	2443 ± 102	0
Benzocaine	2801 ± 113	358
Lidocaine	3766 ± 360	1323
Procaine	3827 ± 230	1384
Tetracaine	7286 ± 305	4843
Verapamil	8639 ± 618	6196

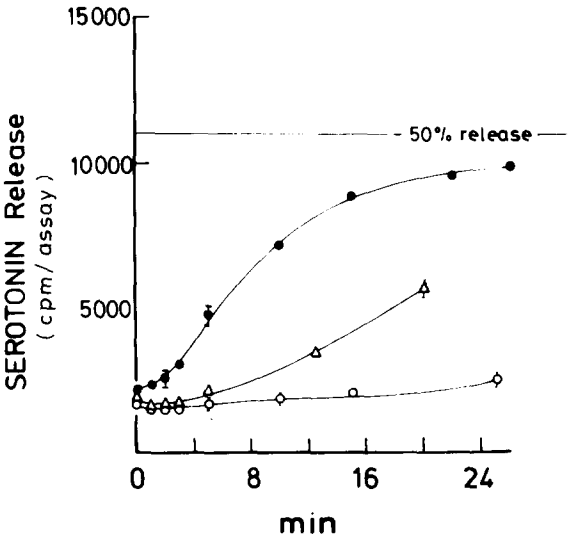

Fig. 3. Tetracaine-induced release of serotonin from washed rabbit platelets. Platelets were suspended in buffer A (see Materials and Methods). Final concentrations of tetracaine: ○, none; △, 0.5 mM; ●, 2 mM.

TABLE IV

THE EFFECT OF LANTHANUM ON SPONTANEOUS AND THROMBIN-INDUCED RELEASE

The platelets were suspended in buffer A. Lanthanum concentration was 1 mM. Thrombin concentration was 1 U/ml. Preincubation for 15 min with lanthanum or saline preceeded a 5 min incubation with thrombin or saline. 100% radioactivity in the aliquot was 13000 cpm/assay and 16000 cpm/assay in experiments 1 and 2, respectively. At time zero the buffer contained 1250 cpm/assay and 1570 cpm/assay, in experiments 1 and 2, respectively. The values are the average of triplicates ± S.D. Net thrombin-induced release is calculated as the difference between the thrombin-induced release and the respective buffer value.

Expt.	cpm per assay					
	Control			Lanthanum		
	Saline	Thrombin	Net thrombin	Saline	Thrombin	Net thrombin
1	1180 ± 79	10506 ± 503	9326	1357 ± 150	3943 ± 90	2586
2	1776 ± 47	6530 ± 143	4754	2013 ± 110	2955 ± 82	942

TABLE V

THE EFFECT OF EXTRACELLULAR SODIUM ON VERAPAMIL-, TETRACAINE- AND THROMBIN-INDUCED RELEASE

The platelets were suspended in buffer A (149 mM sodium) or C (sodium free). Verapamil or tetracaine were added at time zero. Incubation time was 15 min for verapamil or tetracaine and 5 min for thrombin. 100% radioactivity in the aliquots were 18300, 16000 and 13300 cpm/assay, in experiments 1, 2 and 3, respectively. Drugs and thrombin were dissolved in the appropriate buffer. Values are averages of triplicates ± S.D.

Expt.	Drug (mM)	Buffer A	Buffer C
1	None	2443 ± 102	3588 ± 45
	Verapamil, 1	8639 ± 618	9413 ± 230
	Tetracaine, 1	7286 ± 305	9174 ± 68
2	None	3366 ± 357	4445 ± 297
	Verapamil, 0.5	8254 ± 1206	8099 ± 27
	Tetracain, 1	9167 ± 256	9587 ± 231
3	None	1175 ± 45	1311 ± 127
	Thrombin, 1 U/ml	9087 ± 440	6143 ± 500

inhibited the thrombin-induced release by 30%, no effect of indomethacin on verapamil-induced release was observed.

The effect of prostaglandin E_1 and theophylline on verapamil-induced release and on thrombin-induced release was investigated. 15 min of incubation of the platelet suspension in the presence of prostaglandin E_1 ($8.3 \cdot 10^{-6}$ M) and theophylline ($8.3 \cdot 10^{-3}$ M) preceeded incubation of 10 min with verapamil (0.2 to 1 mM) or 2.5 min with thrombin (0.5 U/ml). Prostaglandin E_1 and theophylline supressed the verapamil-induced release by 30%, at all concentrations of verapamil, whereas the thrombin-induced release was reduced by 97%.

The effect of extracellular sodium on the verapamil-induced and on the tetracaine-induced release is shown in Table V. Neither verapamil-induced nor tetracaine-induced release were significantly affected by replacing the extracellular sodium by choline. Under the same conditions, thrombin-induced release was reduced by approx. 30%.

In order to find out whether verapamil and tetracaine cause damage to the cell membrane, lactate dehydrogenase release into the extracellular space was measured. Lactate dehydrogenase activ-

ity in the supernatant after 10 min of incubation with saline was 6.5% of the activity in the whole incubate, which was 2.8 U/mg protein. A further 2.5 min incubation with 10 U/ml thrombin or 10 min incubation with either 1 mM verapamil or 2 mM tetracaine caused no further release of lactate dehydrogenase.

Discussion

The increase in cytosolic calcium concentration is thought to be of crucial importance in the exocytotic reactions of many cells. Serotonin release from platelets is such an exocytotic process [1]. In a calcium-free medium, thrombin and some other activators of serotonin release from platelets, e.g. the ionophore A23187, are thought to exert their effect by mobilizing calcium from intracellular stores [4,5]. The experiments presented in this paper show that various classes of calcium blockers efficiently inhibit the thrombin-induced release even in the presence of the calcium-chelating agent, citrate. Therefore, the effect of calcium blockers should be attributed either to their effect on intracellular calcium or on the movement of other ions, or to a nonspecific mechanism.

Both verapamil and tetracaine cause serotonin release from the platelets. The release by lanthanum is negligible. These results are in agreement with previously reported findings regarding tetracaine [16] and 2-PIA, which is another calcium blocker [13].

The calcium blocker-induced release may be of clinical as well as mechanistic interest and thus warrants detailed study. As can be seen in Figs. 1 and 3, at low concentrations of the drugs the curves describing the time-course of the release reaction are sigmoid, suggesting a cooperative phenomenon. This time lag is not a fundamental property of the release reaction since at higher concentrations of the drug it disappears. The same data can be used to show a sigmoid concentration dependence at short incubation times. The 'concentration lag' thus observed disappears when serotonin release is studied over longer incubation times.

Our finding that thrombin-induced release is inhibited by verapamil is in agreement with recently reported results [15]. Our kinetic studies show that the inhibition curve has a non-sigmoid shape, in contrast to the verapamil-induced release curve. At high concentrations of verapamil, a negative value for the 'net thrombin-induced serotonin release' was obtained. This was a very small but consistent effect. A similar finding was observed with tetracaine as well. The negative value was obtained by subtracting the release in the presence and in the absence of thrombin. Thus, it represents an arithmetical value and not an actual measurement. One possible explanation may be that the verapamil-induced release is inhibited by thrombin. The inhibition of thrombin-induced release by verapamil is reversed by the addition of calcium to the extracellular environment (Table II). In agreement with the findings of Feinstein et al. [16], we show here that the thrombin-induced serotonin release is inhibited by tetracaine. This effect of tetracaine, as well as that of verapamil on thrombin-induced release, has a non-sigmoid concentration dependence, in contrast to the tetracaine-induced release curve and the verapamil-induced release curve. Feinstein et al. showed that the inhibition of thrombin-induced release by tetracaine could be reversed by extracellular calcium. It is, therefore, probable that the effect of both tetracaine and verapamil on thrombin-induced release occur by a similar mechanism. The difference between the shape of the curves for the inhibition and for induction of serotonin release by both drugs suggests that these are two separate phenomena, occurring at two different sites.

A dual effect of tetracaine has been observed in mast cells by Kazimierczak et al. [21]. Tetracaine induced release of histamine from mast cells and inhibited the release induced by 48/80. The tetracaine-induced release had a sigmoid shape.

Several other characteristics distinguish between the verapamil-induced release and the thrombin-induced release, suggesting that the mechanism of the release may be different: (1) Whereas under our experimental conditions thrombin-induced release is reduced by inhibitors of prostaglandin synthesis, the verapamil-induced release was not affected by preincubation of the platelets with indomethacin, although the extent of the release was similar for both activators in the absence of indomethacin. (2) Verapamil-induced

release cannot be due to increased membrane permeability to calcium, since the release was unaffected by extracellular calcium (Table I). (3) The thrombin-induced release involves increased uptake of sodium, as indicated by the inhibition of the platelet response to thrombin in the presence of amiloride [22]. Table V shows that replacing the sodium by choline partially reduces the thrombin-induced release. However, under the same conditions, the verapamil-induced release and the tetracaine-induced release were unaffected. Therefore, it seems that the presence of extracellular sodium is not required for the calcium blocker-induced release reaction. The question then arises, whether the calcium-blocker-induced release of serotonin may be non-specific, i.e., damage to the cell membrane leading to leakage from the cell, as was suggested by Mürer et al. [13] for other blockers, e.g. chlorotetracycline. However, our findings do not support the assumption that verapamil and tetracaine cause extensive cell damage to the platelets, since there was no leakage of the cytosolic enzyme, lactate dehydrogenase. In addition, simple leakage of serotonin from the platelets would probably not show a sigmoid curve of time and concentration dependence. Morever, the verapamil-induced release was partially inhibited by the combined effects of prostaglandin E_1 and theophylline. These compounds can elevate cAMP level in platelets, and thereby lower free calcium in the cytosol [23,24] and thus inhibit exocytotic processes. Thrombin-induced release is, however, more sensitive to prostaglandin E_1 and theophilline.

The kinetic data obtained in this study may suggest that some factor has to accumulate to a certain threshold before serotonin release can take place (see lag period in Figs. 1 and 3). The rate of accumulation of this unknown factor may depend upon the concentration of the drug and result in the different dose-response curves obtained at different incubation times. At low drug concentration, the slow accumulation rate will result in an appreciable lag before a significant release of serotonin is observed. Keeping in mind the role of calcium in exocytotic secretion in general and the known effects of verapamil on calcium distribution, cytosolic calcium accumulation may be suggested as the rate limiting factor in the verapamil- and tetracaine-induced release. The accumulation of calcium ions in the cytosol may be due either to release from intracellular stores or to inhibition of accumulation in these stores. Our kinetic data are compatible with either possibility. An effect of verapamil on intracellular calcium distribution has been suggested previously for cells of different origins [25,26]. In addition, verapamil and local anesthetics have been shown to inhibit calcium uptake by isolated organelles [9]. Another possibility of explaining the time lag of verapamil action is that compounds, other than calcium, released slowly by verapamil, can further enhance the release process, in an autocatalytic manner; or that the drug accumulation in the cell has to reach a certain threshold before release of serotonin can take place. The finding that verapamil-induced release is not dependent on synthesis of prostaglandins may be related to the findings of Knight and Scrutton [27], who showed that once calcium ions have gained access to the interior of the cell, by way of exposure to high-voltage electric field, there is no need of prostaglandin synthesis for the release reaction to proceed.

The inhibitory effect of tetracaine on the thrombin-induced release has been suggested by Feinstein et al. [16] to result from increased binding of calcium to intracelullar storage pools. This explanation seems to be in contrast to the finding that tetracaine and verapamil themselves induced release of serotonin. Since increased extracellular calcium reduced the inhibitory effect of tetracaine and verapamil on thrombin action, it is probable that at least part of the inhibitory effect is due to inhibition of calcium movement across the plasma membrane, as previously shown by Owen and LeBreton [28] for verapamil inhibition of the effect of epinephrine. In the absence of extracellular calcium, this inhibition may be due to impairment of intracellular release of membrane-bound calcium.

References

1 Stormoken, H. (1972) in The Role of Platelets in Hemostatis and Thrombosis, (Pudlack, P., Libansky, J., Hermansky, F., eds.), Acta Universitatis Carolinae, Medical Monographia LIII-IV

2 Mürer, E.H. and Holme, R. (1970) Biochim. Biophys. Acta 222, 197–205

3 Mürer, E.H., Stewart, G.J., Raush, M.A. and Day, H.J. (1975) Thromb. Diath. Haemorrh. 34, 72–82
4 Massini, P. and Lüscher, E.F. (1974) Biochim. Biophys. Acta 372, 109–121
5 Detwiler, T.C. and Feinman, R.D. (1973) Biochemistry 12, 282–289
6 Massini, P., Käser-Glanzmann, R. and Lüscher, E.F. (1978) Thromb. Haemost. 40, 212–218
7 Packham, M.A., Guccione, M.A., Greenberg, J.P., Kinlough-Rathbone, R.L. and Mustard, J.F. (1977) Blood 50, 915–926
8 Gerrard, J.M., White, J.G. and Peterson, D.A. (1978) Thromb. Haemost. 40, 224–231
9 Kohda, C. and Gemba, M. (1979) Jap. J. Pharmacol. 29, 745
10 Kohlhardt, M., Bauer, B., Krause, H. and Fleckenstein, A. (1972) Pfluegers Arch. 335, 309–322
11 Weiss, G.B. (1974) Annu. Rev. Pharmacol. 14, 343–354
12 Feinstein, M.B. and Paimre, M. (1969) Fed. Proc. 28, 1643–1648
13 Mürer, E.H., Stewart, G.J., Davenport, K. and Siojo, E. (1981) Biochem. Pharmacol. 30, 523–530
14 Charo, I.F., Feinman, R.D. and Detwiler, T.C. (1976) Biochem. Biophys. Res. Commun. 72, 1462–1467
15 Ikeda, Y., Kikuchi, M., Toyama, K., Watanabe, K. and Ando, Y. (1981) Thromb. Haemost. 45, 158–161
16 Feinstein, M.B., Fiekers, J. and Frazer, C. (1976) J. Pharmacol. Exp. Ther. 197, 215–228
17 Holmsen, H., Whaun, J. and Day, H.J. (1971) Experientia 27, 451–453
18 O'Brien, J.R. (1970) J. Clin. Pathol. 23, 784–788
19 Bergmeyer, H.U. and Bernt, E. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), pp. 574–576, Verlag Chemie, Weinheim, New York
20 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
21 Kazimierczak, W., Peret, M. and Maslinski, C. (1976) Biochem. Pharmacol. 25, 1747–1750
22 Horne, W.C. and Simons, E.R. (1978) Thromb. Res. 13, 599–607
23 Käser-Glanzmann, R., Jakábová, M., George, J.N. and Lüscher, E.F. (1977) Biochim. Biophys. Acta 466, 429–440
24 Feinstein, M.B. and Frazer, C. (1975) J. Gen. Physiol. 66, 561–581
25 Publicover, S.J. and Duncan, C.J. (1979) Eur. J. Pharmacol. 54, 119–127
26 Gotgil, I.M. and Magazanik, L.G. (1978) Neurophysiology 9, 320–325
27 Knight, D.E. and Scrutton, M.C. (1980) Thromb. Res. 20, 437–446
28 Owen, N.E. and LeBreton, G.C. (1980) Thromb. Res. 17, 855–863