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EFFECTS OF RESERPINE ON RABBIT PLATELET AGGREGATION AND ADHERENCE TO COLLAGEN OR INJURED RABBIT AORTA*

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Abstract—Effects of reserpine *in vivo* and *in vitro* on rabbit platelets in citrated platelet-rich plasma and in suspensions of washed platelets have been studied. Administration of reserpine (5 mg/kg) intraperitoneally 18 hr before platelets were isolated caused inhibition of collagen-induced aggregation but not of aggregation induced by ADP or thrombin. Thrombin-induced aggregation was slightly enhanced. Platelets from reserpine-treated rabbits were less adherent than control platelets to collagen-coated glass surfaces or to the subendothelium of the rabbit thoracic aorta. Similar effects on aggregation were obtained when reserpine (0.2 to 10 μM) was added to suspensions of washed rabbit platelets as little as 2 sec before the addition of collagen. Collagen-induced release of nucleotides and [^{14}C]serotonin from prelabeled washed rabbit platelets was not affected by the presence of reserpine, whereas thrombin-induced release was slightly enhanced. Inhibition by reserpine (2–10 μM) of platelet adherence to a collagen-coated surface or to the subendothelium was also observed within a time interval too short for the reserpine to have caused depletion of platelet granule contents. Thus, reserpine has an immediate effect on the plasma membrane of the platelets which is responsible for inhibition of platelet adherence to collagen and hence of collagen-induced aggregation. This inhibitory effect differs from a much slower effect of reserpine at the granule membrane which results in the depletion of the granule contents of serotonin and adenine nucleotides. The effect of reserpine is not abolished by washing and resuspending platelets that have been exposed to reserpine *in vivo*. By inhibiting the interaction of platelets with collagen, reserpine may interfere with one of the components of hemostatic plug and thrombus formation.

Reserpine is a lipid-soluble drug which is known to affect the membrane of the amine storage organelles of platelets, and to inhibit the transport of serotonin into these organelles [1, 2]. Thus, reserpine depletes platelets of the serotonin that is normally present in their amine storage granules. A lipid-soluble drug would be expected to affect the plasma membrane also, and tritiated reserpine has been shown to bind to the plasma membrane of platelets *in vitro* [3]. It therefore seemed possible that reserpine might affect the response of platelets to some of the aggregating or release-inducing agents. Several investigators have reported that reserpine has little or no effect on platelet aggregation induced by ADP or thrombin *in vitro* [4, 5] or after administration *in vivo* [6]. It has been found [6, 7] to decrease platelet retention in glass bead columns, indicating that reserpine may have an effect on the interaction of platelets with surfaces. Effects on collagen-induced aggregation do not seem to have been investigated previously.

In this paper we report the effect of reserpine on the interaction of rabbit platelets with collagen, ADP and thrombin, and with the subendothelium of the rabbit aorta. In some experiments, reserpine was added to washed platelets *in vitro*. In other experiments, washed platelets were prepared from rabbits given reserpine intraperitoneally 18 hr before collection of the blood. In two experiments, reserpine was given intraarterially.

MATERIALS AND METHODS

Reserpine

Reserpine used for the experiments *in vitro* and *in vivo* was a commercial product (Serpasil, Ciba). This product is solubilized in a diluent that contains a number of components. To ensure that none of these affected platelet function, control studies were done with the diluent obtained from Dr. R. Ellis, Ciba Pharmaceuticals, Dorval, Québec. The diluent was not responsible for the inhibition of collagen-induced platelet aggregation observed with Serpasil. Pure reserpine powder was also obtained from Ciba and dissolved in absolute ethanol at a concentration of 1 mM. When 0.01 ml of this solution was added to 1 ml of platelet suspension, collagen-induced platelet aggregation was inhibited compared with the ethanol control.

For studies *in vitro*, the Serpasil solution was diluted before use with unbuffered 0.85% saline. After addition to the platelet suspension the pH was 7.3. Unbuffered 0.85% saline was added to the control sample. Serpasil was also used for studies *in vivo* and injected intraperitoneally (5 mg/kg) into rabbits. This

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dose was based on that used by Shore *et al.* [1]. Control rabbits received intraperitoneally an equal volume of distilled pyrogen-free water. Blood was collected 18 hr later as described below. Reserpine or Serpasil (0.5 mg/kg) was also injected in the carotid artery of two anesthetized rabbits and blood was collected before injection, at 2 and at 30 min after injection.

Blood

Blood was collected from 2- to 2.5-kg rabbits through a polyethylene cannula in the carotid artery. The rabbits were anesthetized with sodium pentobarbital (30 mg/kg). Platelet-rich plasma (PRP) was prepared from blood collected into 0.1 vol. of 3.8% sodium citrate dihydrate by centrifugation at 77 *g* for 15 min at room temperature.

Preparation of washed rabbit platelets

Suspensions of washed platelets in Tyrode solution containing 0.35 or 4% bovine albumin and apyrase were prepared from blood collected into acid citrate dextrose according to the method of Ardlie *et al.* [8]. Unless otherwise stated, the platelet count was adjusted to 700,000/mm^3.

Platelet aggregation

Platelet aggregation was studied by a turbidimetric method [9] using 1-ml aliquots of PRP or platelet suspension. The solution to be tested (0.1 ml reserpine or saline) was added to a prewarmed (5 min) sample of PRP or suspension and the mixture was incubated for 2 sec–15 min. It was transferred to the aggregometer, stirred at 37° and 0.1 ml of the aggregating agent was added. The aggregating agents used were adenosine diphosphate (ADP, Sigma Chemical Co., St. Louis, Mo.), topical bovine thrombin (Parke, Davis & Co., Detroit, Mich.) or bovine tendon collagen (Sigma Chemical Co.). Collagen suspensions [10] and acid-soluble collagen [11] were prepared as previously described.

Platelet labeling

Platelets were labeled in the first washing fluid as described previously [12] using [^{14}C]serotonin ([^{14}C]5-HT), 5-hydroxytryptamine[3′-^{14}C]creatinine sulfate, 55 μCi/μmole (Amersham/Searle, Arlington Heights, Ill.), or ^{51}Cr (sodium chromate, 1 μCi/μl, Amersham/Searle).

Release of platelet constituents

Platelet constituents were measured in the supernatant fluid obtained by centrifuging the platelet suspension in an Eppendorf centrifuge 4 min after the addition of an aggregating agent. The release of ^{14}C from [^{14}C]5-HT prelabeled platelets was measured as described previously [13]. The release of adenine nucleotides was measured by their light absorbance at 259 nm in a spectrophotometer, as described elsewhere [14]. In some experiments, the released ATP and ADP were measured by the firefly luciferase assay [15]. In both instances, the amount of released material was expressed as a percentage of its total content in the platelets.

Platelet adherence to a collagen-coated surface or to damaged aorta

The adherence of ^{51}Cr-labeled rabbit platelets to collagen-coated glass tubes was examined as previously described [11] or by a modification of the method in which collagen-coated glass rods are rotated at 200 rev/min for 10 min in a platelet suspension. Adherence to damaged everted thoracic aorta segments from rabbits was studied as previously described [16]. The segments were mounted on rods and rotated at 200 rev/min for 10 min in the platelet suspension.

Electron microscopy

Platelets for electron microscopy were fixed in 1% osmium tetroxide (4°, 30 min) and washed in Millonig's buffer. The fixed platelets were dehydrated in increasing concentrations of ethanol, and embedded in Spurr's resin. Sections were cut with an LKB microtome, stained with uranyl acetate and lead citrate, and examined on a Philips 300 electron microscope.

RESULTS

Effects of administration in vivo *of reserpine*

Collagen-induced aggregation. Platelets in citrated platelet-rich plasma from rabbits given reserpine intraperitoneally aggregated less extensively upon the addition of acid-soluble collagen than platelets from control rabbits (Fig. 1). The lag phase before aggregation began was also prolonged by reserpine treatment of the rabbits. The inhibitory effect of reserpine was most apparent when low concentrations of collagen were used. Collagen-induced aggregation of washed platelets also was inhibited by intraperitoneal reserpine treatment of the rabbits (Fig. 2), indicating that separation of the platelets from plasma did not remove the inhibitory effect.

In two experiments, collagen-induced aggregation (collagen dilution 1/1800) of platelets in citrated platelet-rich plasma was inhibited by about 35 per cent when reserpine (0.5 mg/kg) was injected intraarterially 2 or 30 min before collection of blood.

ADP-induced aggregation. In contrast, in citrated platelet-rich plasma prepared from rabbits given reserpine intraperitoneally, both ADP-induced aggregation and the onset of deaggregation were similar to aggregation and deaggregation in citrated platelet-rich plasma from control rabbits. Likewise, ADP-induced aggregation of washed rabbit platelets was similar in platelet suspensions prepared from either control or reserpine-treated rabbits (Fig. 3). However, it was consistently observed that when high concentrations of ADP were used, deaggregation occurred more rapidly with washed platelets from the reserpine-treated rabbits (see Fig. 3).

Thrombin-induced aggregation. Although the extent of thrombin-induced aggregation was similar in platelet suspensions prepared from either control or rabbits given reserpine intraperitoneally, the lag phase before aggregation began was shorter with platelets from reserpine-treated rabbits (Fig. 4).

Platelet adherence to surfaces. Platelets in suspensions prepared from rabbits given reserpine intraperitoneally were less adherent than control platelets to

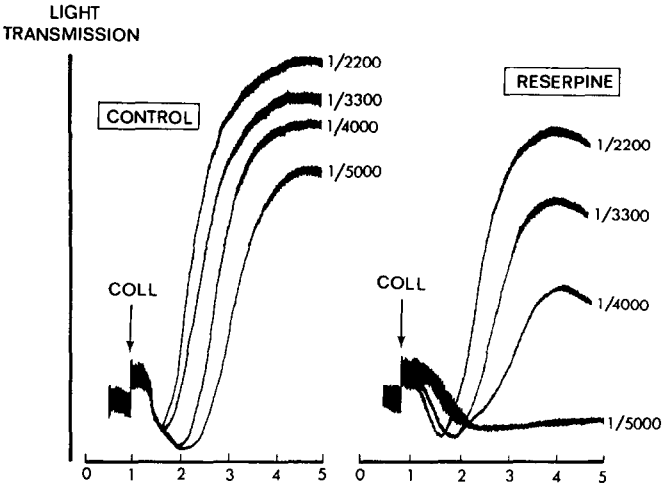

Fig. 1. Effect of intraperitoneal administration of reserpine on collagen-induced aggregation of rabbit platelets in citrated PRP. The rabbits were given 5 mg/kg of reserpine 18 hr before collection of blood; the control rabbits received an equal volume of pyrogen-free water. The four dilutions of acid-soluble collagen used to induce aggregation are indicated beside the aggregation curves. Typical of two experiments.

collagen-coated glass surfaces or to the subendothelium of the rabbit thoracic aorta (Table 1).

Effects of addition in vitro *of reserpine*

Addition of reserpine (0.2 to 10 μM) to suspensions of washed rabbit platelets did not cause a change in the normal disc shape of the platelets (Fig. 5), nor was ^{51}Cr lost from prelabeled platelets, indicating that cytoplasmic constituents were not lost.

Collagen-induced aggregation. The addition of reserpine (10 μM) to a suspension of washed rabbit platelets 2 sec before the addition of acid-soluble collagen completely inhibited collagen-induced aggregation (Fig. 6 and Table 2). The addition of reserpine also inhibited aggregation caused by a suspension of collagen particles (see Table 2). This was more apparent in platelet-rich plasma than in suspensions of washed platelets, with the high concentration of collagen suspension that was used (see Table 2). Reserpine inhibited low concentrations of collagen more effectively than high concentrations of collagen (see Tables 2 and 3). When collagen was used at a concentration which was not completely inhibited by 10 μM reserpine, increasing the time of incubation of the platelets with reserpine before the addition of acid-soluble collagen from 3 to 10 min increased the extent of inhibition of aggregation (Table 3).

Collagen caused the release of [^{14}C]serotonin from prelabeled platelets, and material absorbing at 259 nm also appeared in the suspending fluid of platelets exposed to collagen; this material, when measured by the firefly luciferase assay, is largely released ATP and

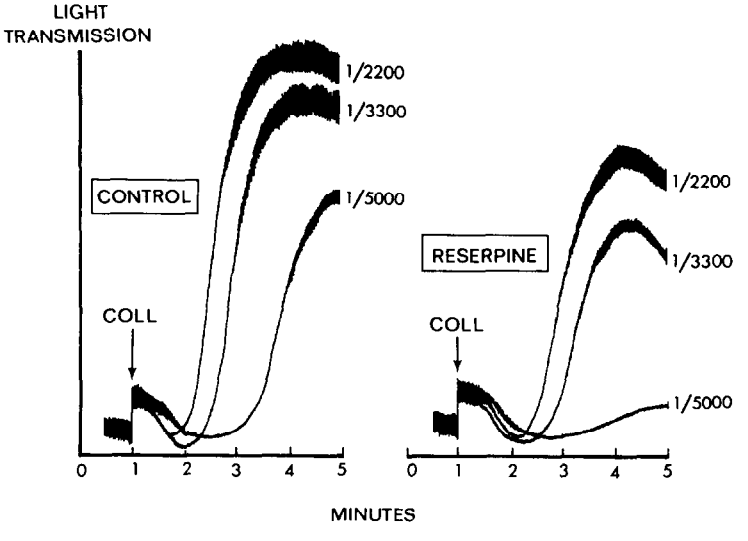

Fig. 2. Effect of intraperitoneal administration of reserpine on collagen-induced aggregation of washed rabbit platelets. See legend of Fig. 1 for further details. Typical of eight experiments with two control rabbits and two reserpine-treated rabbits/experiment.

Table 1. Effect of intraperitoneal administration of reserpine on the adherence of rabbit platelets labeled with ^{51}Cr to collagen-coated glass or everted scraped segments of rabbit aorta.

Expt.	Surface exposed to platelets	Per cent of Albumin in suspending medium*	N	No. of platelets/mm²† Control	No. of platelets/mm²† Reserpine‡	2P§
1	Collagen-coated glass tubes	0.35	5	70,700 ± 3,700	53,500 ± 4,200	< 0.02
2	Collagen-coated glass rods	4	5	30,200 ± 4,400	13,600 ± 1,000	< 0.005
3	Collagen-coated glass rods	4	9	28,900 ± 900	25,900 ± 1,000	< 0.05
4	Everted scraped	4	5	104,500 ± 13,800	67,400 ± 6,800	< 0.05

* Labeled rabbit platelets were washed and resuspended in Tyrode solution containing albumin and apyrase. Final platelet concentration 700,000/mm³.

† Means ± standard errors of the means.

‡ Reserpine (5 mg/kg) was injected intraperitoneally 18 hr prior to collection of blood. Controls were injected similarly with an equal volume of water.

§ Compared with control in each experiment. Unpaired two-tailed *t*-test.

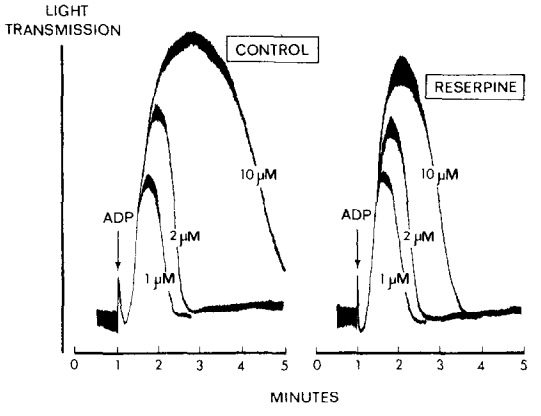

Fig. 3. Effect of intraperitoneal administration of reserpine on ADP-induced aggregation of washed rabbit platelets. See legend of Fig. 1 for further details. The final concentrations of ADP in the platelet suspension are indicated on the aggregation curves. Typical of eight experiments.

ADP (see Table 3). Although reserpine inhibited the extent of aggregation caused by collagen, it did not affect the amounts of [^{14}C]serotonin or adenine nucleotides that appeared in the platelet-suspending fluid upon stimulation with collagen at concentrations that caused less than 25 per cent release of [^{14}C]serotonin.

No loss of [^{14}C]serotonin from prelabeled platelets was detectable within 4.5 min of reserpine addition; about 6 per cent of the platelet [^{14}C]serotonin was lost within 15 min of the addition of reserpine (2 μM). Without reserpine, addition of serotonin (10–100 μM) to the platelet suspension did not inhibit collagen-induced aggregation; some potentiation of collagen-induced aggregation was observed when serotonin was added (130–160 per cent of control).

ADP-induced aggregation. Addition of reserpine (0.2 to 10 μM) 30 sec before ADP (1–5 μM) did not affect the extent of aggregation of washed rabbit platelets. Deaggregation occurred sooner in the presence of reserpine.

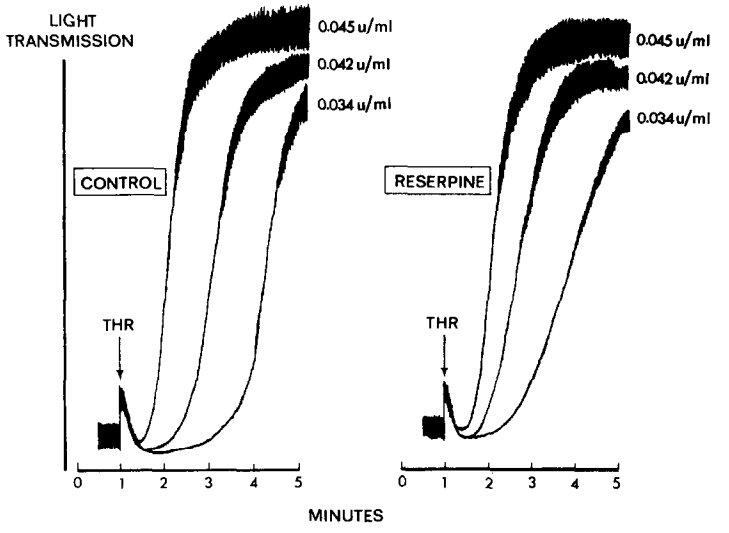

Fig. 4. Effect of intraperitoneal administration of reserpine on thrombin-induced aggregation of washed rabbit platelets. See legend of Fig. 1 for further details. The final concentrations of thrombin in the platelet suspensions are indicated beside the aggregation curves. Typical of eight experiments.

Table 2. Effect of addition *in vitro* of reserpine on collagen-induced aggregation of washed rabbit platelets or rabbit citrated platelet-rich plasma

Expt.	Reserpine* μM	Reserpine* Incubation (sec)	Collagen dilution†	Aggregation at 4 min (% of total)
1‡	0	30	No collagen	0
	10	30	No collagen	0
	0	2	1/2500	100
	10	2	1/2500	0
	0	10	1/2500	100
	10	10	1/2500	0
	0	30	1/2500	100
	0.2	30	1/2500	88
	2	30	1/2500	75
	10	30	1/2500	0
	0	60	1/2500	100
	10	60	1/2500	0
	0	30	1/1666	100
	10	30	1/1666	76
	0	30	1/833	100
	10	30	1/833	95
2§	0	900	1/100	100
	0.2	900	1/100	99
	2	900	1/100	94
	10	900	1/100	85
3‖	0	900	1/100	100
	0.2	900	1/100	84
	2	900	1/100	70
	10	900	1/100	39

* Final concentration of reserpine which was incubated with the platelets before addition of collagen.

† In Expt. 1, acid-soluble collagen was used. In Expts. 2 and 3, a suspension of collagen was used.

‡ Platelet suspension 700,000/mm^3.

§ Platelet suspension 510,000/mm^3.

‖ Citrated platelet-rich plasma 430,000/mm^3.

Thrombin-induced aggregation. Addition of reserpine (0.2 to 10 μM) 30 sec before thrombin shortened the lag phase before aggregation began. The observations were similar to those obtained in the experiments in which the platelet suspension was prepared from the blood of rabbits given reserpine. Reserpine also increased the amount of [^{14}C]serotonin in the suspending medium of the washed rabbit platelets exposed to thrombin (Table 4). When low concentrations of thrombin were used, reserpine also increased the amount of material absorbing at 259 nm in the supernatant (see Table 4).

Platelet adherence to surfaces. The presence of reserpine (0.2 to 10 μM) in the platelet-suspending medium reduced the number of platelets that adhered to collagen-coated glass surfaces or to the subendothelium of the rabbit thoracic aorta (Table 5).

DISCUSSION

Administration of reserpine (5 mg/kg) intraperitoneally to rabbits 18 hr before collecting blood inhibited collagen-induced platelet aggregation, tested *in vitro*. The inhibition was evident in suspensions of washed platelets as well as in citrated platelet-rich plasma, indicating that the reserpine affected the platelets rather than constituents of the plasma and that the effect was not removed by washing the platelets. This inhibitory effect of reserpine may be attributable to the diminished ability of platelets from the reserpine-treated rabbits to adhere to collagen, since fewer platelets from reserpine-treated rabbits than from control rabbits adhered to collagen-coated glass surfaces.

Zweifler [6] found that reserpine inhibited platelet interaction with glass surfaces. He reported that 24 hr (but not 4 hr) after intravenous administration of 0.2 mg/kg of reserpine to rabbits, platelet retention in glass bead columns was reduced.

The ability of the platelets to adhere to subendothelial structures of the damaged thoracic aorta of rabbits was decreased by reserpine treatment of the rabbits from which suspensions of washed platelets were prepared. This effect may be due to inhibition of platelet adherence to collagen in the subendothelial tissue, but inhibition of platelet adherence to other structures, such as the microfibrils around elastin [17], may also play a part.

The inhibition of collagen-induced aggregation was not due to inhibition of the effect of ADP on platelets, since ADP-induced aggregation was now impaired by reserpine treatment of the rabbits. It is likely, however, that less ADP and ATP were present in the amine storage granules of platelets from the reserpine-treated animals [18] and hence less ADP would be released upon addition of collagen. Since ADP and

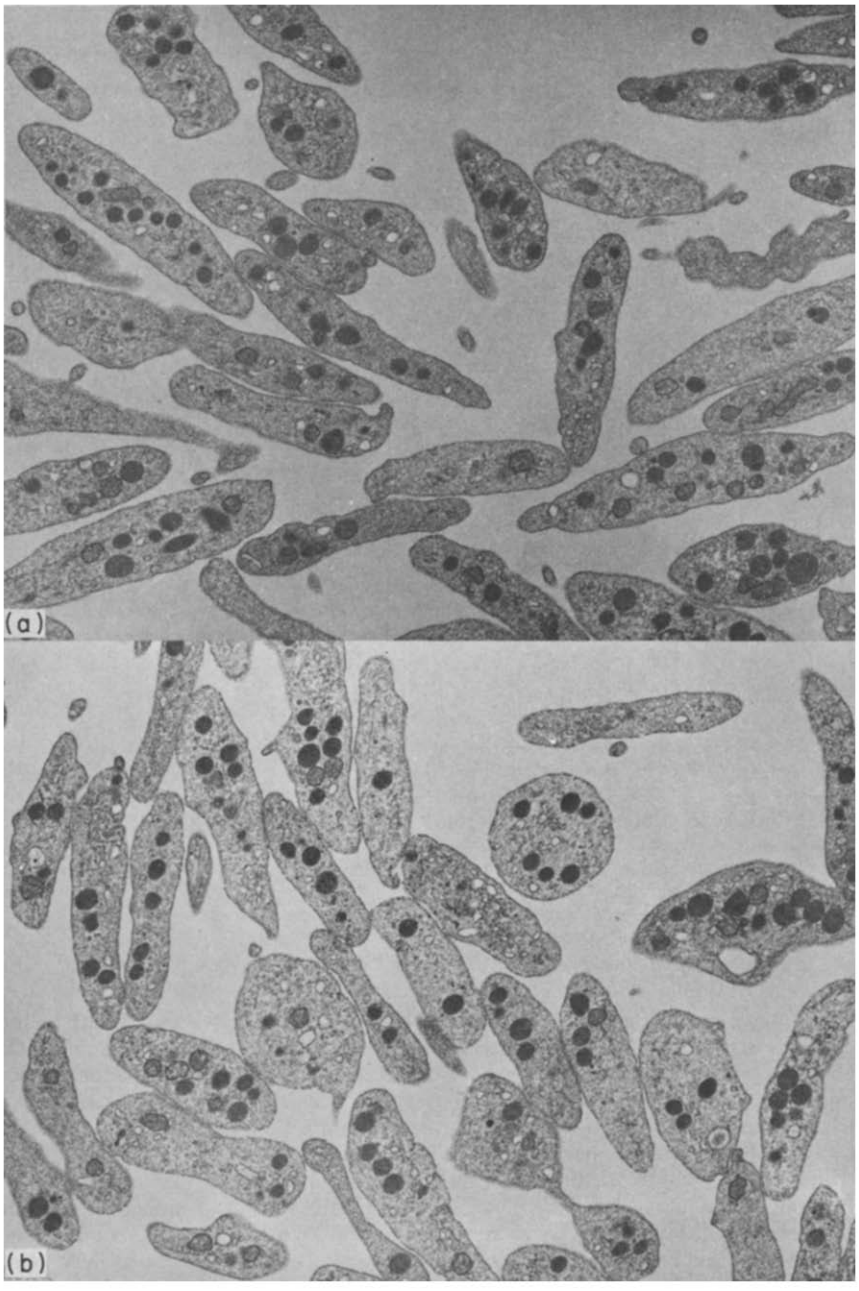

Fig. 5. Electron micrographs of washed rabbit platelets. A, control platelets; B, platelets incubated for 4 hr at 37° with 2 μM reserpine. Magnification: × 15,000.

low concentrations of collagen act synergistically in platelet aggregation [19], diminished release of ADP would also reduce the extent of collagen-induced aggregation.

In the experiments *in vitro*, inhibition of collagen-induced aggregation was not due solely to depletion of releasable ADP. Reserpine inhibited collagen-induced aggregation when added at as short a time as 2 sec before collagen. This is far too short a time for depletion of the storage granules of serotonin or adenine nucleotides. No loss of serotonin was demonstrable at 30 sec, and even at 15 min only a 6 per cent loss was observed after addition of 2 μM reserpine. Depletion of the adenine nucleotides in the storage granules of rabbit platelets requires an even longer exposure to reserpine; 18 hr after administration of 5 mg/kg of reserpine the releasable adenine nucleotides are diminished by 20–30 per cent [18]. *In vitro*, over a 3-hr period, 2 μM reserpine causes the accumulation in the suspending fluid of only 9.5 per cent of the total platelet material absorbing at 259 nm. Since serotonin does not inhibit collagen-induced aggregation (but actually potentiates it), any small amount of serotonin lost from the platelets under the influence of reserpine in the experiments *in vitro* cannot be responsible for inhibition of collagen-

Table 3. Effect of addition *in vitro* of reserpine on collagen-induced aggregation and release of [^{14}C]serotonin or adenine nucleotides from prelabeled washed rabbit platelets*

Expt.	Reserpine†		Collagen dilution‡	Aggregation at 4 min (% of control)	Amount in suspending fluid at 4 min§ (% of total)	
	μM	Incubation (sec)			[^{14}C]serotonin	Material absorbing at 259 nm
1	0	30	1/2500	100	7.8	6.9
	10	30	1/2500	38	6.1	8.7
	0	30	1/1666	100	14	15.1
	10	30	1/1666	82	13.7	13.8
	0	30	1/833	100	23.2	18.7
	10	30	1/833	91	23	18.1
2‖	0	180	1/2400	100		6.4
	10	180	1/2400	77		4.8
	0	300	1/2400	100		5.2
	10	300	1/2400	40		5.9
	0	600	1/2400	100		5.8
	10	600	1/2400	14		5.9
						ATP + ADP
3¶	0	30	1/1666	100	13.1	4.6
	10	30	1/1666	56	14.3	4.3

* Platelet count 700,000/mm^3.

† Final concentration of reserpine which was incubated with the platelets before the addition of collagen.

‡ Acid-soluble collagen was used to induce aggregation. This batch of collagen was not the same as was used for the experiment in Table 1; it had a stronger aggregating effect at the same dilution.

§ Rabbit platelets were prelabeled with [^{14}C]serotonin, washed and resuspended in Tyrode solution containing 0.35% albumin and apyrase. The amount of ^{14}C was measured in the supernatant fluid prepared from the platelet suspension 4 min after the addition of collagen. Light absorbance at 259 nm of the supernatant fluid is mainly due to adenine nucleotides released from the platelets.

‖ One of four experiments that gave similar results.

¶ Rabbit platelets were washed and resuspended at a platelet count of 1,000,000/mm^3 in Tyrode solution containing 0.35% albumin and in the absence of apyrase. The amount of ATP and ADP released in the supernatant fluid prepared from the platelet suspension 2 min after the addition of collagen was measured by the firefly luciferase assay [15]. Mean values of four determinations in two experiments.

Table 4. Effect of addition *in vitro* of reserpine on thrombin-induced aggregation and release of [^{14}C]serotonin and adenine nucleotides from washed rabbit platelets*

Expt.	Reserpine		Thrombin (units/ml)	Lag phase† (sec)	Amount in suspending fluid at 4 min‡ (% of total)	
	μM	Incubation (sec)			[^{14}C]-serotonin	Material absorbing at 259 nm
1	0	30	0.013	123	0.1	
	0.2	30	0.013	99	9.2	
	2	30	0.013	71	14	
	10	30	0.013	71	15.5	
2	0	900	0.03	∞	0.2	0
	4	900	0.03	186	4.2	5.4
	0	900	0.04	162	0.7	2.5
	4	900	0.04	129	12.4	6.9
	0	900	0.05	71	8.7	7.4
	4	900	0.05	66	21	10.3
	0	900	0.07	77	12.2	15.6
	4	900	0.07	54	26.6	10.3
	0	900	0.1	33	35.1	28
	4	900	0.1	29	44	26.2
	0	900	0.5	18	85	54.4
	4	900	0.5	9	78	54.6

* Rabbit platelets were prelabeled with [^{14}C]serotonin, washed and resuspended in Tyrode solution containing 0.35% albumin and apyrase. Final platelet concentration 700,000/mm^3.

† Lag phase was expressed as the number of seconds, after addition of thrombin, for the aggregation tracing to return to the baseline level.

‡ See footnote § of Table 3.

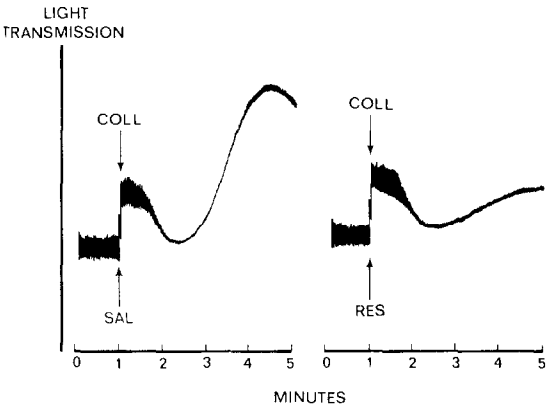

Fig. 6. Effect of addition of reserpine (RES, final concentration 10 μM) to a suspension of washed rabbit platelets on aggregation induced by acid-soluble collagen diluted 1/3600. Reserpine was added 2 sec before collagen. An equal volume of saline (SAL) was added to the control tube. Typical of eight experiments.

induced aggregation. In the experiments *in vitro*, reserpine inhibited collagen-induced aggregation without detectable inhibition of adenine nucleotide release. We have shown previously [20] that collagen can induce platelet aggregation without the participation of ADP. Since reserpine does not inhibit ADP-induced aggregation, it seems most likely that reserpine inhibits some other mechanism involved in collagen-induced aggregation. *In vitro*, reserpine also caused immediate inhibition of platelet adherence to collagen.

We have concluded that reserpine has a rapid effect on the plasma membrane of the platelets which is responsible for inhibition of platelet adherence to collagen and hence of collagen-induced platelet aggregation. This effect is apparent within seconds, in contrast to the much slower effect of reserpine at the granule membrane, which results in the depletion of the granule contents of serotonin and adenine nucleotides.

The finding that collagen-induced aggregation (but not aggregation induced by ADP or thombin) was inhibited by reserpine *in vitro* and *in vivo* either 2 or 30 min after intraarterial injection or 18 hr after intraperitoneal injection raises the possibility that the same mechanism is responsible for the observed effects. This could involve an effect of reserpine on the platelet plasma membrane. This is in accord with the findings that reserpine is readily taken up by platelets [21] and apparently remains in the platelets throughout their lifetime in the circulation. It has been shown recently [3] that tritiated reserpine gives the same platelet survival curves as ^{51}Cr. However, it is also possible that the mechanism of inhibition of collagen-induced aggregation observed after administration *in vivo* of reserpine differs from that observed *in vitro*. Reserpine could exert its effect *in vivo* indirectly by influencing secretion of hormones or liberation of biogenic amines from other cells. In addition, biotransformation of reserpine may give rise to metabolites which inhibit the interaction of platelets with collagen.

Reserpine has no demonstrable effect on blood coagulation [22, 23] nor on the bleeding time of normal rabbits [1, 23]. However, combinations of reserpine

Table 5. Effect of addition *in vitro* of reserpine on the adherence of rabbit platelets labeled with ^{51}Cr to collagen-coated glass or everted scraped segments of rabbit aorta

Expt.	Surface exposed to platelets	Per cent of albumin in suspending medium*	N	Reserpine† (μM)	No. of platelets/mm^2‡	2P§
1	Collagen-coated glass tubes	0.35	5	0	67,800 ± 5,900	
			5	0.2	18,500 ± 3,100	< 0.001
			5	2	20,400 ± 1,400	< 0.001
			5	10	3,600 ± 300	< 0.001
2	Collagen-coated glass tubes	0.35	5	0	112,700 ± 1,000	
			5	10	68,200 ± 250	< 0.02
	Everted scraped aorta	0.35	10	0	538,500 ± 104,500	
			10	10	75,000 ± 12,400	< 0.001
3	Collagen-coated glass rods	4	5	0	33,500 ± 1,000	
			5	0.2	35,000 ± 3,500	< 0.80
			5	2	26,600 ± 1,600	< 0.01
			5	10	19,000 ± 2,800	< 0.005
4	Everted scraped aorta	4	5	0	40,300 ± 3,300	
			5	0.2	31,500 ± 6,500	< 0.30
			5	2	16,700 ± 5,100	< 0.01
			5	10	20,300 ± 5,300	< 0.02

* Labeled rabbit platelets were washed and resuspended in Tyrode solution containing albumin and apyrase. Final platelet concentration 700,000/mm^3.

† Final concentration of reserpine, which has been incubated with the platelet suspension (Expts. 1, 3 and 4: 10 min of incubation. Expt 2.: 30 min of incubation).

‡ Means ± standard errors of the means.

§ Compared with control in each experiment. Unpaired two-tailed *t*-test.

and heparin or reserpine and other anticoagulants do prolong the bleeding time, undoubtedly because these combinations interfere with two of the aspects of hemostatic plug formation [22, 23]. Similar observations have been made in hemophilic dogs given phenylbutazone, which inhibits platelet interaction with collagen [24], and the difficulties experienced by hemophilic patients upon aspirin ingestion are well recognized [25]. It seems possible that reserpine, like aspirin or phenylbutazone, may inhibit platelet interaction with subendothelial structures and thus could interfere with one of the components of hemostatic plug formation.

However, since interaction of platelets with the subendothelium is also thought to be one of the first steps in thrombus formation, reserpine may have some inhibitory effect on arterial thrombosis. It has been shown to inhibit thrombus growth in rabbit carotid arteries [6] but Baumgartner *et al.* [26] reported that it had no effect on thrombus formation in injured ear veins of rabbits. It has been suggested that release of materials from platelets during their interaction with damaged vessel walls may contribute to the development of atherosclerotic lesions [27, 28]. Reserpine has been reported [29, 30] to inhibit the development of cholesterol-induced atheromatous lesions in rabbits; although this may be partly attributable to the ability of reserpine to interfere with collagen synthesis in the arterial wall by inhibiting prolyl hydroxylase activity [31], it could also be related to an effect of reserpine on platelets.

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