The Effect of Pyrazole Compounds on Thrombin-Induced Platelet Aggregation* (33490)

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A number of studies showed that collagen, antigen–antibody complexes, or gamma-globulin coated surfaces appear to induce platelet aggregation by causing the release of platelet ADP(1, 2). Platelet aggregation by these agents can be inhibited by a number of compounds such as the pyrazole drugs (2–4) and acetylsalicylic acid (5). These compounds diminish the amount of adenine nucleotides released from platelets by these stimuli and part of their inhibitory effect on platelet aggregation may be attributable to this.

Thrombin also is believed to cause platelet aggregation through the release of platelet ADP (6). We have recently found that acetylsalicylic acid inhibits thrombin-induced platelet aggregation (7), but in our original studies with the pyrazole compounds (2–4) we did not demonstrate any effect of these agents on thrombin-induced platelet aggregation. We have now reexamined the effect of the pyrazole compounds on thrombin-induced platelet aggregation.

Materials and methods. Solutions. The Tyrode's solutions used in this study were prepared as described in a previous publication (3). Modified Tyrode's solution contained no calcium or magnesium; Tyrode's-gelatin solution contained 0.25% gelatin.

Platelet-rich plasma (PRP). Blood was taken from either rabbits or pigs through a carotid artery cannula, mixed with either 3.8% trisodium citrate or 2% EDTA–0.33% saline (1 part anticoagulant to 9 parts of blood) and centrifuged at 77*g* for 15 min at room temperature.

Platelet suspensions. The PRP from EDTA blood was centrifuged at 650*g* for 15 min at 4°. The platelets were resuspended and washed as described previously (3). The final platelet suspensions were made up in Tyrode's-gelatin solution to a concentration of 500,000 to 600,000 platelets/mm^3.

Platelet counts. These were done with a Coulter counter (8).

Platelet aggregation. This was studied by a turbidimetric method (9).

In vitro *serotonin-^{14}C labeling of platelets.* The platelets were labeled with 5-hydroxytryptamine-3^1-creatinine-^{14}C sulfate (Radiochemical Centre, Amersham, England) as previously described (7).

Release of AMP, ADP, and serotonin-^{14}C. One ml of platelet suspension was mixed with 0.1 ml of test solution, prior to the addition of 0.1 ml of thrombin solution. The experimental procedure and methods used for measuring AMP, ADP, and serotonin-^{14}C have been described (3).

Thrombin. Crude bovine thrombin (Parke Davis and Co., Detroit, Mich.) was diluted with modified Tyrode's solution to the desired concentrations.

Fibrinogen. Human fibrinogen (lyophilized Cohn Fraction I, Connaught Medical Research Laboratories, Toronto) was dissolved in modified Tyrode's solution (500 mg/100 ml and adsorbed with Al(OH)$_3$ (1 part Al(OH)$_3$ moist gel [British Drug Houses, Toronto] to 5 parts fibrinogen solution) for 10 min at 37°. Following removal of the gel by centrifugation, the fibrinogen solution was dialyzed for 12 hr against modified Tyrode's solution.

Phenylbutazone. (Butazolidin, G-15137, Geigy Pharmaceuticals, Montreal, Quebec). Phenylbutazone was solubilized in water with NaOH in equimolar amounts and diluted, as required, with modified Tyrode's solution.

Fibrinogen clotting time. To 0.2 ml of the fibrogen solution in a glass tube at 37° was added 0.1 ml of phenylbutazone solution (or

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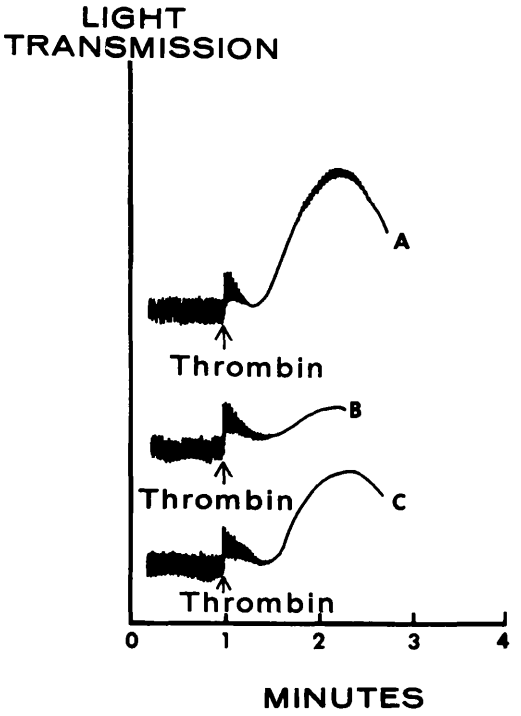

FIG. 1. The effect of phenylbutazone on thrombin-induced platelet aggregation in rabbit citrated platelet-rich plasma (PRP). Increased light transmission indicates platelet aggregation. One ml of PRP was mixed with either 0.1 ml of modified Tyrode's solution, (A); 0.1 ml of phenylbutazone solution (10 mg/ml, (B); or 0.1 ml of phenylbutazone solution (1 mg/ml), (C); 1 min before adding thrombin. The thrombin solution (0.1 ml of 1 unit/ml) was added at the points indicated by the arrows.

modified Tyrode's solution), followed by 0.02 ml of 0.25 *M* $CaCl_2$ solution. Thrombin solution (0.2 ml) was added and the clotting time was determined.

Results. The addition of phenylbutazone to rabbit citrated platelet-rich plasma inhibited thrombin-induced platelet aggregation when low concentrations of thrombin were used (Fig. 1). The addition of phenylbutazone to a suspension of washed pig platelets also inhibited thrombin-induced platelet aggregation (Fig. 2).

The effect of phenylbutazone on thrombin-induced release of platelet constituents was studied using a suspension of washed pig platelets. Table I shows that phenylbutazone depressed thrombin-induced release of radioactivity from serotonin-^{14}C labeled platelets. The effect was most marked with the high concentrations of the drug but with the lower concentration of thrombin the effect was evident with smaller concentrations of phenylbutazone. The effect on serotonin release was paralleled by inhibition of the release of ADP and the preservation of the platelet nucleotide levels in the form of ATP, ADP, and AMP.

Although phenylbutazone inhibited platelet aggregation induced by small concentrations of thrombin it did not appear to affect the rate of clotting of fibrinogen by thrombin (Table II).

Discussion. In our original study of the action of the pyrazole drugs (2–4) we concluded that they did not inhibit thrombin-induced release of platelet constituents or platelet aggregation. The results from the present study indicate that phenylbutazone

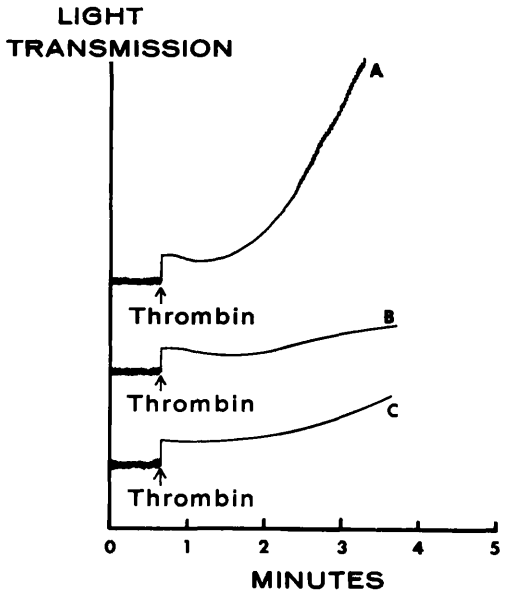

FIG. 2. This shows the eflect of phenylbutazone on thrombin-induced platelet aggregation of washed pig platelets (suspended in Tyrode's-gelatin solution). One ml of platelet suspension was mixed with either 0.1 ml of modified Tyrode's solution, (A); 0.1 ml of phenylbutazone solution (10 mg/ml), (B); or 0.1 ml of phenylbutazone solution (1 mg/ml), (C); 1 min before adding the thrombin. The thrombin solution (0.1 ml of 2 units/ml) was added at the points indicated by the arrows.

TABLE I. The Effect of Phenylbutazone on the Release of Radioactivity, ADP, and AMP from Serotonin-^{14}C Labeled Pig Platelets Exposed to Thrombin.

Material[a] added to platelet suspension	Final concentration of phenylbutazone (μg/ml)	^{14}C		Nucleotides				
		Platelets (cpm/10^6 platelets)	Suspending fluid (cpm /0.1 ml)	Platelets (nmoles/10^9 platelets)			Suspending fluid (nmoles/ml of platelets)	
				ATP	ADP	AMP	ADP	AMP
Thrombin (final conc 0.17 units/ml)	0	11	8891	37	41	11	65	14
	1000	93	612	89	32	15	53	17
	500	70	2777	69	65	39	39	12
	100	42	5882	70	51	11	54	12
	50	38	6455	42	49	42	51	12
Thrombin (final conc 0.08 units/ml)	0	39	5358	69	60	10	49	8
	1000	114	235	96	44	19	28	10
	500	95	101	74	92	33	33	22
	100	94	545	62	73	27	28	6
	50	61	3342	73	56	16	49	8
Thrombin (final conc 0.04 units/ml)	0	102	164	82	53	29	34	5
	1000	109	180	80	79	29	23	9
	500	101	80	82	55	46	22	3
	100	97	128	79	77	29	29	2
	50	99	175	79	53	43	32	4
Modified Tyrode's solution	0	93	117	73	81	41	24	7
	1000	96	210	85	83	24	24	9
	500	102	148	82	61	14	22	7
	100	99	76	75	71	39	24	3
	50	100	56	85	78	22	27	5

[a] In these experiments 0.1 ml of phenylbutazone solution or modified Tyrode's solution was added to the suspension followed by 0.1 ml of thrombin solution. The mixture was incubated at 37° for 10 min with gentle agitation.

inhibits the effect of small amounts of thrombin on platelet aggregation and the release of platelet constituents. The effect is difficult to show unless minimum concentrations of thrombin are used. It appears that this is the reason why this was not observed in our earlier study.

Phenylbutazone inhibits the release of platelet constitutents induced by thrombin but does not affect the action of thrombin on fibrinogen. This may indicate that these effects of thrombin are mediated through different mechanisms. Since phenylbutazone and acetylsalicylic acid (7) inhibit the release of platelet constituents induced by collagen, antigen–antibody complexes, and gamma-globulin coated particles (2, 3), as well as by

TABLE II. The Effect of Phenylbutazone on the Clotting Time of a Fibrinogen Solution.

Expt.	Thrombin (final conc) (units/ml)	Final concentration of phenylbutazone in fibrinogen solution (μg/ml)	Clotting time of fibrinogen solution (sec)
1	1.4	0	26
	1.4	4000	27
	1.4	2000	28
	1.4	0	28
2	0.7	0	38
	0.7	4000	32
3	0.23	0	54
	0.23	290	54
	0.23	1450	56

thrombin, a common pathway may be involved in the release reaction caused by all these stimuli.

Summary. Phenylbutazone inhibits thrombin-induced platelet aggregation and the release of platelet constituents but this effect can only be demonstrated with low concentrations of thrombin. The action of thrombin on fibrinogen is not affected by phenylbutazone.

1. Hovig, T., Thromb. Diath. Haemorrthag. **9**, 264 (1963).

2. Mustard, J. F., Glynn, M. F., Nishizawa, E. E., and Packham, M. A., Federation Proc. **26**, 106 (1967).

3. Packham, M. A., Warrior, E. S., Glynn, M. F., Senyi, A, S., and Mustard, J, F., J. Exptl. Med. **126**, 171 (1967).

4. Packham, M. A., Nishizawa, E. E., and Mustard, J. F., Biochem. Pharmacol. Suppl. **171** (1968).

5. Evans, G., Nishizawa, E. E., Packham, M. A., and Mustard, J. F., Blood **30**, 550 (1967).

6. Haslam, R. J., Nature **202**, 765 (1964).

7. Evans, G., Packham, M. A., Nishizawa, E. E., Mustard, J. F., and Murphy, E. A., J. Exptl. Med. **128**, 877 (1968).

8. Glynn, M. F., Movat, H. Z., Murphy, E. A., and Mustard, J. F., J. Lab. Clin. Med. **65**, 179 (1965).

9. Mustard J. F., Hegardt, B., Rowsell, H. C., and MacMillan, R. L., J. Lab. Clin. Med. **64**, 548 (1964).

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Effects of Subtotal Nephrectomy on Renal Hypertension* (33491)

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On the assumption that renal hypertension is due to relative renal ischemia and increased renin release, it has been suggested that reduction of renal mass—by restoring a proper balance between renal blood flow and renal mass and by reducing the number of cellular complexes responsible for renin formation—might reverse the course of hypertension (1, 2). Results have been inconclusive, however (1–6). In some experiments, remission of hypertension was attributed to establishment of a secondary circulation (3). On the other hand, in those in which hypertension persisted (4, 5), it was not proven to be still dependent on renal mechanisms. The purpose of the present investigation was to study sequentially the effects of partial and total resection of clipped kidneys on blood pressure and to correlate the data obtained with renal renin.

Methods. Female Sprague-Dawley rats weighing 155–165 g were made hypertensive by unilateral clipping of the renal artery with and without contralateral nephrectomy. In experimental series 1, one kidney was clipped and the other was left untouched. After stabilization of pressure, hypertensive animals were evenly divided according to blood pressure levels into 3 groups. Part of the clipped kidney was surgically removed in group 1 and excluded by a ligature in group 2. Animals of group 3 were sham-operated. After 35 days, all animals of group 2 and 6 of group 1 were killed; clipped kidneys were resected in the remaining animals of group 1 and in those of group 3. The experiment was terminated on day 42. The number of surviving animals in each group was 14, 6, and 8, respectively.

In experimental series 2, the contralateral kidney was removed at the time of renal artery clipping. Hypertensive animals were also divided into 3 groups and treated as in the first series. Twenty-six days later, kidneys were unclipped in all groups, but not removed, so as to avoid renoprival hypertension. At the end of the experiment, on day 49 there were 6, 8, and 6 rats in each group, respectively.

All operations were performed under ether

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