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EFFECTS OF VASOACTIVE DRUGS ON PLATELET AGGREGATION IN VIVO AND IN VITRO

Mary P. Wiedeman, Ronald F. Tuma and Harvey N. Mayrovitz
Department of Physiology, Temple University School of Medicine
Philadelphia, Pennsylvania and The Miami Heart Institute,
Miami Beach, Florida

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ABSTRACT

Based on the generalization that most vasoconstrictor agents promote platelet aggregation while most vasodilator agents inhibit it, experiments were designed to test this premise in the living animal. Platelet aggregates, induced by a laser beam in arterial vessels of the hamster cheek pouch and bat wing, were observed microscopically to determine changes in growth, embolization, and vessel wall adherence in the presence of epinephrine, norepinephrine, phenylephrine, vasopressin, serotonin, isoproterenol, dipyridamole and sodium nitroprusside. Concentrations of the drugs too weak to alter microvessel diameters were used. In vitro responses were determined by inspection of a platelet-rich plasma-drug mixture in a hemocytometer chamber. It was established that drugs which enhanced platelet aggregation in vivo also produced aggregates in platelet-rich plasma from the same animal except for epinephrine and norepinephrine where the results in vitro were not definitive. Phenylephrine and isoproterenol produced changes contrary to expectation. Phenylephrine, an alpha adrenergic stimulator, caused a reduction in the duration of activity of platelets at the site of a laser-induced aggregate. When mixed with platelet-rich plasma, no platelet aggregates were produced. Isoproterenol, a beta adrenergic stimulator, enhanced the duration of activity of the platelet aggregate. When mixed with PRP, small, evenly dispersed aggregates were seen in the hemocytometer chamber.

INTRODUCTION

There is abundant evidence that drugs which are excitatory to alpha adrenergic receptor sites on the platelet membrane can either potentiate or initiate platelet aggregation in vitro. Drugs or agents stimulatory to beta adrenergic receptor sites have an inhibitory effect. Proof that the response is based on stimulation of receptor sites lies in the fact that platelets

bathed in alpha or beta blocking agents prior to exposure by the agonists are prevented from reacting as before (1, 2).

In assessing the action of any agent it is necessary to be specific about the source of platelets in that human platelets may respond differently from animal platelets (3). Platelets from common laboratory animals may differ in reaction to the same drug (2). Furthermore, the reactions of platelets in vivo have not been compared with the in vitro responses in most instances. While the manipulation of platelets in the test tube has been fruitful in establishing characteristics of aggregation and adherence of these cells, it is equally important to know if similar responses occur in the flowing blood of the living animal, and to what extent stimulation of adrenergic receptor sites on vascular smooth muscle affect or contribute to aggregation and adherence.

The study reported here was designed to compare the effect of selected vasoconstrictor and vasodilator drugs on platelet aggregation in vivo and in vitro. The in vivo studies were done by microscopic observations of the growth and embolization of laser induced platelet aggregates in arterial vessels of the bat wing and the hamster cheek pouch. In vitro studies were conducted by exposing platelet-rich plasma (PRP) from each animal to the vasoactive drugs. In this way, similarities or differences between the in vivo and in vitro responses could be established.

Two classes of drugs were used, those known to affect adrenergic receptor sites and those known to promote constriction or dilation of vascular smooth muscle by some other stimulatory mechanism.

MATERIALS AND METHODS

Sites for in vivo microscopic observation of platelet aggregates consisted of the arterial vessels of the hamster cheek pouch and the bat wing.

The hamster cheek pouch was prepared for observation following the method of Duling (4). A 90-100 gm animal was anesthetized with sodium pentothal (6 mg/100 gm body weight) after which the trachea was cannulated with PE200 tubing to assure an airway and the femoral vein was cannulated with PE10 tubing for the administration of anesthesia and drugs. The cheek pouch was everted and spread over a plastic mount whose center was a thin circular coverslip. An arterial vessel with a diameter of 30 to 50 μ was selected for observation.

Preparation of the bat wing consisted of spreading one wing of an unanesthetized bat across a glass plate, holding the wing in place with spring clips (5). A first order arterial vessel (30 μ) was exposed along its length by teasing away the top layer of epithelial cells. The vessel was covered with Ringer's solution on which a coverslip was floated.

Platelet aggregates were produced by the output from a single pulse ruby red laser which struck the arterial vessel. It was previously established that ADP released from injured red blood cells in the flowing blood was responsible for the formation of an aggregate (6). The growth, embolization and subsequent cessation of any platelet adherence around the cluster of heat-lysed red blood cells was timed and expressed as duration of activity. Generally, three trials were done to establish control values before administration of the drug. All reported values are averages of numerous trials. Vessel diameters were measured with an eyepiece micrometer before and at intervals after drug administration to determine if the drug produced a vasoconstriction or vasodilation.

In vitro tests for platelet aggregation were conducted by mixing platelet-rich plasma with each of the drugs tested in the living animal.

Procurement of PRP was achieved by collecting arterial blood in small plastic tubes containing acid citrate dextrose (ACD) (1 part ACD to 6 parts blood). The collected blood was then centrifuged at 40-50 G for 10 minutes, the plasma was removed immediately by withdrawal into a syringe and mixed with a drug.

Arterial blood of the hamster was collected by inserting a cannula into the carotid artery and letting the blood flow through a siliconized tube into a 2 ml plastic centrifuge tube. Arterial blood of the bat was obtained by cannulation of the median artery that runs parallel to the bone of the forearm.

Two drops of a drug (0.042 ml) of the same concentration as used for the *in vivo* trials were placed in a small tube to which was added two drops of PRP. The solution was mixed at room temperature by stirring with a magnetic rod for three minutes after which it was withdrawn and placed in a hemocytometer chamber. At the same time, PRP and physiological saline were also combined, mixed and placed in the other chamber of the hemocytometer for immediate comparison with the drug. PRP from both the hamster and the bat were tested with ADP (5×10^{-3} M) to demonstrate their responsiveness to this universal aggregator. In instances when the drug under consideration failed to produce aggregation, the reactiveness of the platelets was tested with ADP.

The drugs selected for experimental trials were: norepinephrine (Levophed, Winthrop), epinephrine (Adrenalin Chloride, Parke-Davis), phenylephrine (Neosynephrine, Winthrop), as primary alpha adrenergic agents; Serotonin (Regis) as a vasoconstrictor; isoproterenol (Iprenol, Vitarine Co.), as a beta adrenergic agent; sodium nitroprusside (Nipride, Roche) and dipyridamole (Persantine, Boehringer-Ingelheim).

RESULTS

Effect of drugs used in this study on platelets *in vivo* and *in vitro*.

Epinephrine and norepinephrine. Intra-arterial injections of epinephrine were made as repeated small injections into the bat wing. The epinephrine flowed over the site of a laser-induced platelet aggregate and the length of time was noted during which platelets continued to be attracted to the initial aggregate, as evidenced by increase in size of the aggregate, through the time period that small clumps of the aggregate broke off and no further platelet adherence occurred. This time period is defined as the duration of activity. In the hamster, epinephrine was administered by i.v. infusion. Norepinephrine was given in the same way.

In both animals the time period during which platelets were seen to be active around the initial aggregate was prolonged, expressed as duration of activity, as a result of epinephrine and norepinephrine infusion.

The effect of epinephrine on PRP from both animals tested in the hemocytometer was less definitive. Although small clusters of aggregates were seen occasionally, the response was not consistent and therefore can not be considered strongly positive. The same was true for *in vitro* tests with norepinephrine.

Serotonin. A continuous intra-arterial infusion of serotonin was given to the bat during and after formation of a platelet aggregate by the laser. The

hamster received serotonin as a single i.v. dose.

In both animals, the activity of platelets at the site of the initial aggregate was prolonged.

Platelet rich plasma from both animals yielded aggregates when mixed with serotonin as seen in the hemocytometer chamber.

Isoproterenol. Isoproterenol produced an unexpected response in the bat and hamster in that the duration of platelet activity was increased in treated animals. Isoproterenol was administered by constant intravenous infusion into the tail vein of the bat and the femoral vein of the hamster. The drug did not cause any diameter change in arterial vessels of the bat wing or the hamster cheek pouch. In the bat, platelet activity increased during i.v. infusion of isoproterenol as did the values in the hamster.

The in vitro results were also consistent with this finding in that numerous very small clusters of platelets formed when PRP was mixed with isoproterenol and inspected in the hemocytometer.

Sodium nitroprusside. Sodium nitroprusside was given intravenously in both bat and hamster and was infused continuously. The drug acted as an inhibitor to platelet activity in that duration of growth and embolization of platelets around the cluster of lysed red blood cells was reduced from the experimental period. In the hamster, a reduction was also noted.

hen sodium nitroprusside was mixed with PRP and viewed in the hemocytometer, evidence of platelet aggregation was not seen in either bat or hamster preparations.

Phenylephrine. An intravenous injection of phenylephrine was given to bats and activity of platelets around a laser-induced aggregate was determined in a 15 min period following the injection. Contrary to the expected enhancement of activity resulting from an alpha adrenergic stimulator, an inhibition occurred. Duration of platelet activity was reduced significantly. The same procedure was followed in the hamster and in these animals duration of activity was also reduced in the 15 min following injection of phenylephrine.

Dipyridamole. Intravenous injections of dipyridamole in the tail vein of the bat resulted in a decrease in duration of platelet activity. In the hamster, an intravenous injection in the femoral vein reduced platelet activity from the control values.

The dosages, route of injection, period of activity and in vitro results of the drugs used in this study are given in Table 1.

DISCUSSION

This investigation was designed to compare the effects of selected vasoactive rugs on platelet activity in vivo and in vitro. The results show agreement in he effect on platelets of selected vasoactive drugs when inspected in the living animal or in the hemocytometer chamber. An exception in the degree of response was seen with epinephrine and norepinephrine, both of which caused a prolongation of platelet activity at the site of a laser-induced aggregated on an arterial vessel in the living animal, but neither drug consistently produced

TABLE 1

In Vivo and In Vitro Responses to Vasoactive Drugs in Bat and Hamster Arterial Vessels

	BAT				HAMSTER			
DRUG	Amount of drug administered	DURATION OF ACTIVITY (min) CONTROL	DURATION OF ACTIVITY (min) P=Significance POST-DRUG	IN VITRO	Amount of drug administered	DURATION OF ACTIVITY (min) CONTROL	DURATION OF ACTIVITY (min) P=Significance POST-DRUG	IN VITRO
EPINEPHRINE	1×10^{-6} mg/ml	6.58 (n=9)	P= <.1 9.29 (n=16)	FEW SMALL AGGREGATES	2.5×10^{-3} mgs/min/kg	5.38 (n=14)	P= <.01 11.05 (n=25)	FEW SMALL AGGREGATES
NOREPINEPHRINE	2×10^{-2} mg/min/kg	4.49 (n=9)	P= <.1 9.15 (n=8)	FEW SMALL AGGREGATES	1.25×10^{-2} mgs/min/kg	5.21 (n=10)	P= <.05 11.48 (n=28)	FEW SMALL AGGREGATES
SEROTONIN	0.1 mg/ml	5.17 (n=24)	P= <.001 9.97 (n=46)	AGGREGATES	1.0 mgs/kg	4.48 (n=15)	P= <.05 7.01 (n=15)	AGGREGATES
ISOPROTERENOL	1.2×10^{-3} mgs/min/kg	4.81 (n=8)	P= <.01 9.68 (n=20)	SMALL AGGREGATES	1.2×10^{-3} mgs/min/kg	5.36 (n=16)	P= <.01 8.54 (n=36)	SMALL AGGREGATES
SODIUM NITROPRUSSIDE	3×10^{-3} mgs/min/kg	4.65 (n=7)	P= <.05 2.04 (n=26)	NO AGGREGATES	1.5×10^{-3} mgs/min/kg	4.47 (n=9)	P= <.05 1.61 (n=29)	NO AGGREGATES
PHENYLEPHRINE	4×10^{-2} mg/kg	5.48 (n=10)	P= <.01 2.32 (n=18)	NO AGGREGATES	3×10^{-3} mg/kg	6.80 (n=33)	P= <.01 2.94 (n=45)	NO AGGREGATES
DIPYRIDAMOLE	1.0 mg/kg	5.40 (n=15)	P= <.05 3.53 (n=28)	NO AGGREGATES	0.5 mg/kg	6.22 (n=14)	P= <.01 3.74 (n=19)	NO AGGREGATES

aggregates when mixed with PRP in vitro. All other drugs which enhanced duration of activity in the living animal also produced aggregates when mixed with PRP. This would suggest a potentiating effect with epinephrine and norepinephrine without the ability to initiate an aggregate. The inability of these catecholamines to consistently aggregate bat and hamster platelets in vitro is in agreement with studies using platelets from other animals although a potentiating effect was demonstrated using platelets from rats, rabbits, guinea pigs, horses, and dogs (3, 7, 8). Ahtee and Michal (9) reported causing rabbit platelets to aggregate with both epinephrine and norepinephrine.

The observation that epinephrine and norepinephrine were more effective in enhancing duration of activity of an aggregate on the arterial wall might be indicative of some influence of factors associated with flowing blood, the endothelial cells of the vessel wall, or vascular smooth muscle which contributed to the continuation of aggregation and adhesion. From results of earlier studies, additional support for participation of the vessel is found in the fact that duration of platelet activity was diminished following surgical denervation of vessels of the bat wing and the i.v. administration of phenoxybenzamine (10). Measurements of the velocity of blood flow and calculation of wall shear stress before and after denervation indicated that denervation of the vessels did not increase velocity or wall shear stress. Therefore, it cannot be argued that the platelets were washed away more quickly in the denervated vessels by an alteration in the character of flow.

The unexpected responses of enhancement of the duration of platelet activity by isoproterenol rather than inhibition, and the decrease in duration of activity rather than enhancement caused by phenylephrine are difficult to explain. It is interesting however, that the in vivo response were in agreement with the platelet response as seen in the hemocytometer chamber after mixing the drugs with PRP. The small platelet aggregates produced by the isoproterenol were rather evenly distributed throughout the hemocytometer chamber. These small, evenly dispersed aggregates may have escaped recognition using other techniques. In vitro tests have not yet been done to establish whether phenylephrine actually inhibited ADP aggregation of PRP, but it was demonstrated that when the drug was mixed with PRP, no aggregates were produced.

It would seem, then, that the generalization regarding the enhancement of platelet activity by vasoconstrictor agents and its inhibition by vasodilator agents does not hold for the drugs tested in this study on these animals.

Perhaps of most significance is that in vivo and in vitro responses had the same effect on platelet aggregation (except to a lesser degree with epinephrine and norepinephrine) even though the predicted action of some drugs based on adrenergic receptor stimulation did not follow the general rule.

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