Platelet Inhibition by Sodium Nitroprusside, a Smooth Muscle Inhibitor

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The effects of sodium nitroprusside (N.P.), a pure smooth muscle inhibitor, on platelet function were studied. Platelet-rich plasmas (PRP) from normal controls and from patients receiving N.P. were studied in vitro for aggregation in response to adenosine diphosphate (ADP), epinephrine, and collagen. Platelet ADP release (release reaction) was also investigated. Normal platelets demonstrated marked inhibition of aggregation when incubated with N.P. for 3 min. Prolonging the incubation was without additional effect. ADP and ATP release from platelets in response to collagen was also inhibited. PRP from patients receiving nitroprusside at concentrations between 25 μg/min and 165 μg/min showed inhibition of aggregation when compared to findings prior to the administration of N.P. N.P. acts by inhibiting contractile proteins and thus platelet ADP release and aggregation may depend on contraction of platelet smooth muscle-like protein, thrombosthenin.

Irreversible platelet aggregation results from platelet shape change, primary aggregation, adenosine diphosphate (ADP) release, and secondary aggregation. Sodium nitroprusside (N.P.) is a potent smooth muscle inhibitor recently reintroduced into clinical use. This drug is far more active against vascular type smooth muscle than against uterine or intestinal smooth muscle and is reported to inhibit platelet aggregation. We have examined N.P.’s effect on platelet aggregation induced by ADP, epinephrine, and collagen, platelet ADP release (release reaction) in response to collagen, and N.P.’s effect on platelets when infused into humans.

MATERIALS AND METHODS

Sodium nitroprusside (1 μg/ml = 3.8 μM), obtained as 50 mg of anhydrous powder (Roche Labs, Nutley, N.J.) was diluted with 5% dextrose in water (D5W) to the desired concentrations, protected from light, and used within 4 hr. ADP and epinephrine (Sigma Chemical Co., St. Louis, Mo.) were dissolved in 0.15 M saline. Bovine tendon collagen (Sigma Chemical Co.) was prepared as a stock solution in weak acid by the method of Holmson et al. at a concentration of 1 mg/ml and stored at −60°C.

To obtain platelet-rich plasma (PRP), blood from normal volunteers and patients receiving intravenous infusions of N.P. was anticoagulated with a volume of 3.1% sodium citrate and centrifuged at 150 g in an Adams Dyncel centrifuge for 20 min at room temperature. All equipment used to handle the blood or PRP was plastic except for the aggregometer cuvettes which were untreated glass. Platelets were used at an unadjusted concentration of 350,000-500,000/cu mm. Individual dose response experiments and ADP release experiments were done on aliquots of the same PRP to keep the platelet number constant. Platelets from patients receiving N.P. were tested prior to and during N.P. infusion.

Platelet aggregation was measured in a Chrono-Log Aggregometer (Model 300, Chrono-Log Corp., Broomall, Pa.) attached to a Corning recorder (Corning Scientific Instrument Co., Medfield, Massachusetts).
**RESULTS**

*Effect of Sodium Nitroprusside on Epinephrine-, ADP-, and Collagen-induced Aggregation*

Platelet aggregation in response to $10^{-5} \text{ M}$ epinephrine was markedly depressed in the presence of N.P. (Fig. 1B). At low concentrations of N.P. (0.15 $\mu$g/ml) secondary aggregation was mainly affected, while at higher concentrations the primary wave of epinephrine-induced aggregation was also abolished. Platelet aggregation in response to 4.5 $\mu$M ADP was partially inhibited at low concentrations of N.P. (0.15 $\mu$g/ml). Much higher concentrations (15 $\mu$g/ml) were necessary to inhibit profoundly ADP-induced aggregation (Fig. 1A). Potent inhibition of collagen-activated (0.625 $\mu$g) aggregation was noted, even 1.5 $\mu$g/ml of N.P. completely arresting aggregation (Fig. 1C). N.P.'s effect was complete within 3 min, while inhibition was not increased by incubation of N.P. with PRP for up to 80 min. The effect of increasing the amount of aggregating agent could be blocked by a proportional increase in the amount of N.P.
Effect of Sodium Nitroprusside on Collagen-induced ADP Release

ADP and ATP released from platelets in response to 2.5 μg of collagen were measured to determine whether inhibition by N.P. was due in part to blocking of ADP release from platelets or platelet unresponsiveness to the released ADP. Marked inhibition of ADP and ATP release was found in the presence of N.P. (Table 1). PRP without N.P. averaged 5.4 x 10^-6 M ADP and 2.3 x 10^-6 M ATP released, while PRP with N.P. averaged 0.8 x 10^-6 M ADP and 0.5 x 10^-6 M ATP released. N.P. had no effect on the ADP and ATP assay itself as shown by the fact that ADP and ATP levels were the same in PRP and PRP with N.P. at 15 μg/ml (results not shown).

Effect of Sodium Nitroprusside In Vivo

Three patients were studied who were given N.P. for hypertension in the face of acute myocardial infarction. Infusion rates of N.P. were 25, 75, and 165 μg/min. In each case, platelet aggregation in response to ADP, epinephrine, and collagen was depressed while the patients were receiving N.P. (Fig. 2). The higher the rate of N.P. infusion, the greater the inhibition of induced aggregation in vitro. No patient had evidence of abnormal bleeding in vivo. Template bleeding times would not be justified in these patients. Serum N.P. levels have never been measured. However, the concentrations achieved in vivo should fall within the range of concentrations of N.P. used in vitro (0.015–15.0 μg/ml).

DISCUSSION

Previous investigators have shown that N.P. inhibits ADP-, epinephrine-, and collagen-induced aggregation.5,6 Our data confirmed these reports, and the concentration of N.P. causing 50% inhibition of aggregation for each aggregating agent correlated well with the published data.

ADP and epinephrine-induced aggregation of platelets results from both a direct effect and by causing platelets to release ADP.9,10 N.P. in low concentrations does not prevent primary aggregation (direct effect) while it inhibits the secondary wave of epinephrine-induced aggregation. Only at higher concentrations of N.P. is the direct effect of epinephrine or the effect of exogenous ADP effectively blocked. Collagen stimulates platelets to react by initiating ADP release from the platelets,12 and collagen-induced aggregation is most sensitive to N.P. These data suggest that the release reaction is most sensitive to N.P. Our investigations also have shown that N.P. is indeed capable of preventing
ADP and ATP release. Previously, N.P. has been shown to block the release of serotonin from platelets.

The mechanisms of ADP release and platelet shape change are unknown, but they are energy requiring processes. Platelet shape change may be a consequence of the contraction of thrombosthenin with the resultant deformation of the platelet's skeleton of microtubules. The release of ADP from platelets may also depend on thrombosthenin contraction. Since N.P. has its primary mode of action as a direct inhibitor of vascular-type smooth muscle, its depression of platelet aggregation and ADP and ATP release suggests that contractile processes are necessary early events in the release reaction and aggregation processes.

Various drugs such as aspirin and dipyridamole have been used to inhibit platelet aggregation in humans. Sodium nitroprusside appears to be a class of platelet inhibitor which has its effect through direct inhibition of platelet smooth muscle-like protein, thrombosthenin. It exerts its effect in vitro in concentrations similar to those used in clinical medicine. Platelets from patients receiving N.P. also demonstrate inhibition of aggregation, and the effect is proportional to the amount of N.P. being infused. N.P. infused at 165 μg/min causes a markedly impaired response to ADP, epinephrine, and collagen, while doses up to 400 μg/min are used in clinical practice. A similar effect has been reported in rabbits where it is dose dependent and dissipates within 20 min.
PLATELET INHIBITION BY N.P.

upon ending the N.P. infusion. Sodium nitroprusside has gained increasing popularity in the treatment of hypertension and congestive heart failure accompanying acute myocardial infarction. Inhibition of platelet aggregation in this situation may be an unappreciated benefit. Other direct smooth muscle relaxants in clinical use, such as Diazoxide, Hydralazine, and Minoxidil, may well have similar effects on platelet aggregation.

REFERENCES

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