Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 5320–5324, September 1983
Cell Biology

Removal of extraplatelet Na^+ eliminates indomethacin-sensitive secretion from human platelets stimulated by epinephrine, ADP, and thrombin

(secondary aggregation/Ca^{2+} mobilization/arachidonic acid release/cyclooxygenase/prostanoids)

THOMAS M. CONNOLLY AND LEE E. LIMBIRD

Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Communicated by Sidney P. Colowick, May 20, 1983

ABSTRACT **We have previously observed that removal of extraplatelet Na^+ markedly diminishes human platelet aggregation and secretion in response to epinephrine. The present studies demonstrate that this effect of the removal of extraplatelet Na^+ on platelet function is not unique to activation of platelets by α_2-adrenergic agents but represents a phenomenon also evident for other platelet stimuli. Thus, platelet aggregation and secretion in response to maximal concentrations of ADP and lower concentrations of thrombin (<0.04 unit/ml) were also markedly reduced in platelets in "Na^+-free" medium, suggesting that these agents share an effector mechanism that is similarly inhibited by the removal of extraplatelet Na^+. In contrast, platelet aggregation and secretion in response to higher concentrations of thrombin (≥0.04 unit/ml) and to 0.04–1.0 μM (15*S*)-hydroxy-11α,9α-(epoxymethano)prosta-5*Z*,13*E*-dienoic acid (U46619), an endoperoxide analog, were identical in control platelets and in those suspended in "Na^+-free" medium, indicating that platelets suspended in "Na^+-free" medium are functionally intact, at least in response to some stimuli. Furthermore, the observation that U46619 can elicit platelet aggregation and secretion independently of extraplatelet Na^+ indicates that the loss of platelet responsiveness to epinephrine, ADP, and low concentrations of thrombin cannot be attributed to a loss of sensitivity to the stimulus-provoked secondary mediator(s) of platelet function, endoperoxides or thromboxane A_2. Treatment with indomethacin to block the secondary aggregation and secretion pathways of platelets reduced the aggregatory and secretory responses of control platelets induced by epinephrine, ADP, and low concentrations of thrombin to those characteristic of platelets suspended in "Na^+-free" medium. In contrast, indomethacin did not alter the functional responses induced by these agents in platelets suspended in "Na^+-free" medium, suggesting that "primary" aggregation is intact but that the "secondary" aggregation and secretion mediated by arachidonic acid metabolites are eliminated by removal of extraplatelet Na^+. Consistent with this interpretation is the observation that the indomethacin-insensitive aggregation and secretion induced by U46619 and higher concentrations of thrombin were retained in platelets suspended in "Na^+-free" medium. Thus, the responses eliminated by removal of extraplatelet Na^+ are those eliminated by treating control platelets with indomethacin, suggesting a strong link between the presence of extraplatelet Na^+ and the operation of platelet function mediated by the cyclooxygenase pathway.**

We recently reported that human platelets in "Na^+-free" medium have a diminished rate and extent of aggregation and a markedly reduced release of serotonin in response to epinephrine stimulation (1). Despite the effects of the removal of extraplatelet Na^+ on epinephrine-induced aggregation and secretion, α_2-adrenergic receptor-mediated attenuation of prostaglandin E_1 (PGE_1)-stimulated cAMP accumulation in intact human platelets was independent of extraplatelet Na^+ (1). The difference in the effect of reducing extraplatelet Na^+ concentration on α_2-adrenergic receptor-mediated functional responses versus that on lowering of cAMP levels suggests that these two α_2-adrenergic receptor-mediated processes can be dissociated, a result previously shown by using other manipulations (2).

The present studies were undertaken to gain further insight into the possible mechanism(s) by which α_2-adrenergic agents stimulate platelet aggregation and secretion. Two questions were addressed. The first is, what is the locus at which Na^+ acts to modulate α_2-adrenergic receptor-stimulated function? Platelet aggregation and secretion may be divided into two phases, primary and secondary aggregation. During primary aggregation the platelets begin to adhere to each other in a reversible manner, whereas secondary aggregation involves a more rigorous, irreversible clumping phenomenon accompanied by secretion of the contents of many intraplatelet granules (3). Thromboxane A_2, a very potent aggregatory stimulus that is synthesized from membrane-liberated arachidonic acid via the cyclooxygenase pathway (4), is also released during secondary aggregation. We used indomethacin, a cyclooxygenase inhibitor that eliminates secondary aggregation, as a tool to dissect the various phases of platelet functional responses to determine whether removal of extraplatelet Na^+ diminishes epinephrine-induced primary or secondary aggregation, or both. The second question addressed in these studies is whether or not alterations of platelet functional sensitivity after removal of extraplatelet Na^+ might be a generalized phenomenon evident for platelet activation by other stimuli. Thus, we evaluated the ability of two physiologically relevant stimuli of platelet function, ADP and thrombin, and the endoperoxide analog (15*S*)-hydroxy-11α,9α-(epoxymethano)prosta-5*Z*,13*E*-dienoic acid (U46619) to elicit platelet aggregation and secretion in "Na^+-free" media.

MATERIALS AND METHODS

Indomethacin was purchased from Sigma. All other materials were obtained from the sources previously reported and prepared so as not to contain Na^+ (1).

Details of the platelet preparation have been described (1) and when relevant appear in the figure legends. Briefly, blood was drawn from healthy male donors, caffeine-free that day and free of other drugs for at least 10 days. Platelet-rich plasma was obtained by centrifuging blood at 120 × *g* for 15 min and was incubated with 6 nM [^{3}H]serotonin for 30–45 min at room tem-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NMDG, *N*-methyl-D-glucamine; PGG_2, PGH_2, PGE_2, prostaglandins G_2, H_2, and E_2; U46619, (15*S*)-hydroxy-11α,9α-(epoxymethano)prosta-5*Z*,13*E*-dienoic acid; U, unit.

perature to label the contents of the dense granules. Platelet [^{3}H]serotonin release in response to a stimulus was used as a monitor of secretion. The platelets were isolated by sequential albumin gradient centrifugation and Sepharose 2B gel filtration into Na^+-containing or "Na^+-free" buffers (1). Na^+ was replaced by the inert cation *N*-methyl-D-glucamine (NMDG, added as the hydrochloride), and its removal was confirmed by atomic absorption spectrophotometry. Previous studies demonstrated that NMDG is an inert substitute for Na^+ in human platelet studies, as assessed by its lack of deleterious effects on platelet morphology, functional integrity, α_2-adrenergic receptor binding, and adenylate cyclase activity (1, 5). Earlier studies have shown that replacing Na^+ with Li^+ or Cs^+ also results in a loss of epinephrine-induced secretion. Choline chloride (140 mM) interferes with epinephrine binding to the α_2-adrenergic receptor and could not be used as a Na^+ substitute for studies on α_2-adrenergic receptor-mediated functional responses. However, isolation of platelets in choline-containing medium resulted in a loss of platelet secretion indistinguishable from that observed for platelets suspended in NMDG-containing buffers. Taken together, these results suggest that NMDG behaves as an "inert" substitute for Na^+ in the human platelet system described.

RESULTS

The response of the platelets to a maximal concentration of epinephrine, 10 μM, is shown in Fig. 1. The left side of Fig. 1*A* demonstrates the diminished rate and extent of aggregation observed for platelets suspended in "Na^+-free" buffer compared to that of control platelets. A similarly diminished function is shown by the decreased release of [^{3}H]serotonin from platelets in "Na^+-free" buffer compared to the quantity of [^{3}H]serotonin released from control platelets (Fig. 1*B*). The inclusion of indomethacin in the platelet incubation mixture reduced the rate and extent of aggregation of platelets in Na^+-containing buffer to that characteristic of platelets in "Na^+-free" buffer (Fig. 1*A*). Furthermore, indomethacin-treated control platelets released very little [^{3}H]serotonin, again an observation comparable to that for platelets in "Na^+-free" buffer in the absence of indomethacin (Fig. 1*B*). Incubation of the platelets in "Na^+-free" medium with indomethacin did not alter their aggregation or the quantity of [^{3}H]serotonin released in response to epinephrine (Fig. 1). Because indomethacin eliminates secondary aggregation but leaves primary aggregation intact, the similar aggregation tracings observed for indomethacin-treated control platelets and for platelets suspended in "Na^+-free" medium suggest that the initial primary aggregation phase triggered by α_2-adrenergic agents is unaltered when platelets are suspended in "Na^+-free" medium. In contrast, the secondary phase of aggregation and the concomitant [^{3}H]serotonin secretion are eliminated in "Na^+-free" medium.

To determine whether Na^+ modulation of platelet aggregation and secretion was unique to stimulation by α_2-adrenergic agents or represented a more general phenomenon, we evaluated platelet function in response to two other known physiological stimuli, ADP and thrombin. Fig. 2 depicts the ADP-induced aggregation and secretion observed for platelets in the presence and absence of Na^+ and the effect of indomethacin on these responses. Removal of extraplatelet Na^+ eliminated the biphasic aggregation observed in control platelets in response to a maximal concentration of ADP, 5 μM (Fig. 2*A*) and reduced the release of [^{3}H]serotonin (Fig. 2*B*). In the presence of indomethacin, the ADP-induced aggregation response and [^{3}H]serotonin release of the control platelets resembled those of the platelets in "Na^+-free" medium (Fig. 2).

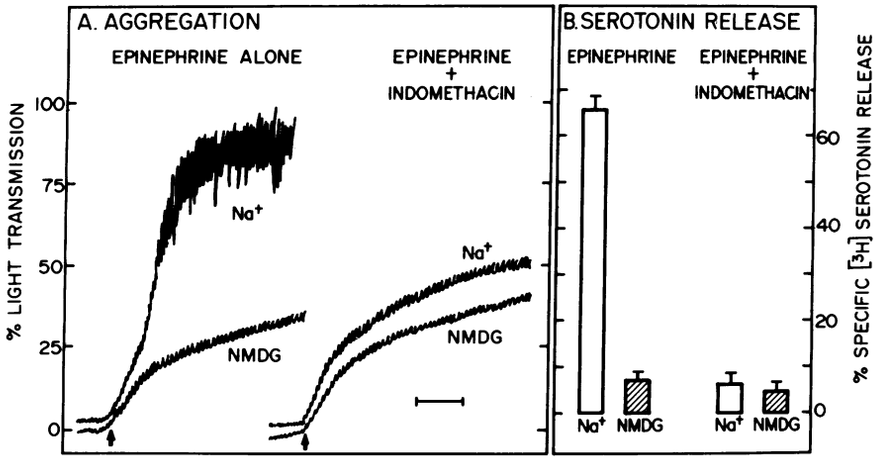

FIG. 1. Effect of removal of extraplatelet Na^+ and the influence of indomethacin on platelet aggregation (*A*) and [^{3}H]serotonin release (*B*) in response to 10 μM epinephrine. Platelets were prepared as described in *Materials and Methods* and ref. 1 and were diluted to $\approx 3.2 \times 10^5$ platelets per mm^3 in Na^+-containing or "Na^+-free" (NMDG) Hepes buffer, pH 7.35, containing 0.1% dextrose and 0.35% bovine serum albumin. The platelet suspensions (0.54 ml) were added to 1-ml aggregometer cuvettes with 0.06 ml of fibrinogen at 1 mg/ml and warmed to 37°C with stirring at 1,000 rpm in a Payton dual-channel aggregometer. After 2 min, a 150-μl aliquot was removed and centrifuged for 30 sec in an Eppendorf microcentrifuge to separate the suspending medium from the platelets and a 125-μl aliquot was removed to monitor "background" [^{3}H]serotonin release (see description for *B* below). Imipramine was added at 47 μM to prevent the reuptake of released serotonin just prior to the addition of 10 μM epinephrine (arrows). Aggregation was monitored as an increase in light transmission (100% = buffer and 0% = platelet suspension at time 0) vs. time. These tracings are from one experiment representative of eight separate experiments. The horizontal bar indicates 1 min of aggregation. (*B*) Quantity of [^{3}H]serotonin released from platelets treated identically as described for experiments in *A*. After 5 min of aggregation, duplicate 150-μl aliquots were removed and centrifuged for 30 sec in an Eppendorf microcentrifuge. Specific [^{3}H]serotonin release was calculated as the radioactivity obtained in 125 μl of the supernatant after centrifugation of samples obtained after the 5-min incubation (total) minus the radioactivity in 125 μl of the sample taken prior to stimulus administration (background). The data are expressed as percent specific [^{3}H]serotonin release, in which 100% equals the total platelet-associated radioactivity in an equal volume of an uncentrifuged platelet suspension, typically 8,000–11,000 cpm per 125 μl. Results shown are the mean ± SEM of eight separate experiments. Indomethacin, when studied, was present at 25 μM final concentration and was added as a 1:100 dilution of a 100 mM K_2CO_3-containing solution 1 min prior to the addition of fibrinogen. K_2CO_3 alone had no effect on platelet aggregation or [^{3}H]serotonin release.

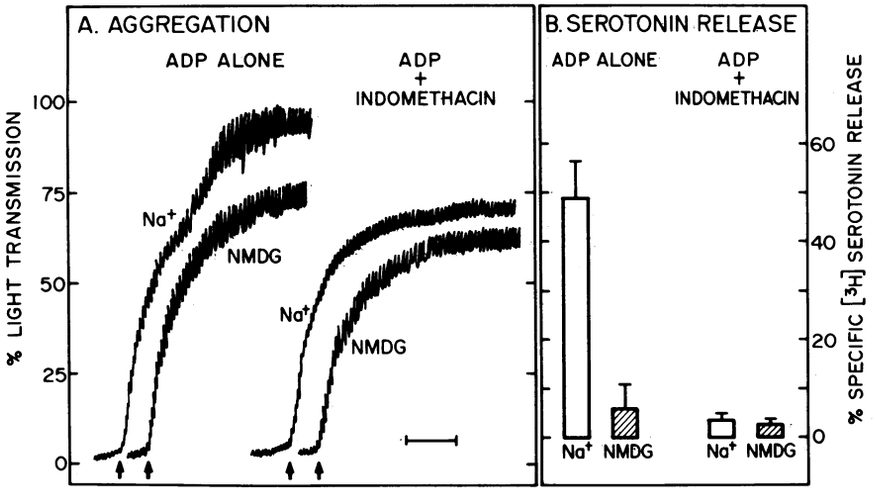

FIG. 2. Effect of removal of extraplatelet Na^+ and the influence of indomethacin on platelet aggregation (*A*) and specific [^{3}H]serotonin release (*B*) in response to 5 μM ADP. Platelets were prepared and experiments were performed as in Fig. 1. *A* is from one experiment representative of four separate experiments. The results in *B* are the mean ± SEM from four separate experiments.

Thus, ADP-induced secondary aggregation and secretion were also eliminated upon removal of extraplatelet Na^+, suggesting that this maneuver alters the platelet responses to a stimulus other than epinephrine.*

We have previously demonstrated that a maximal concentration of thrombin, 0.1 unit (U)/ml, could promote platelet aggregation and serotonin release independently of extraplatelet Na^+ (1). It has been suggested that higher concentrations of thrombin activate platelets via a mechanism that is independent of products of the arachidonic acid pathway (6–8), whereas lower concentrations appear to act via cyclooxygenase products; these effects are prevented by indomethacin (9). Thus, we asked if there is an indomethacin-sensitive component of thrombin-stimulated platelet function under our experimental conditions and whether or not this component would be eliminated by the removal of extraplatelet Na^+, as had been observed above for responses to epinephrine and ADP. Fig. 3 compares the aggregation of human platelets in Na^+-containing and "Na^+-free" medium to low (0.004 U/ml) and high (0.1 U/ml) concentrations of thrombin. Removal of extraplatelet Na^+ reduced the aggregation response to thrombin at 0.004 U/ml (Fig. 3*A*). Furthermore, addition of indomethacin reduced the rate and extent of aggregation of the control platelets in response to thrombin at 0.004 U/ml to that characteristic of the platelets suspended in "Na^+-free" medium, while having no effect on the function of platelets suspended in "Na^+-free" medium. Thus, our data confirm that lower concentrations of thrombin do mediate aggregation via an arachidonic acid metabolite pathway. In contrast, Fig. 3*B* demonstrates that aggregation in response to a high concentration of thrombin (0.1 U/ml) occurred via a mechanism that was insensitive to indomethacin and, furthermore, that the aggregation occurring via this indomethacin-insensitive pathway was not affected by the removal of extraplatelet Na^+.

We examined the release of [^{3}H]serotonin from platelets in Na^+-containing and "Na^+-free" medium in response to increasing concentrations of thrombin. The results presented in Fig. 4 demonstrate that the concentration–response curve for thrombin-stimulated [^{3}H]serotonin release was shifted to the right by almost an order of magnitude for platelets suspended in "Na^+-free" medium. However, the amount of [^{3}H]serotonin released from platelets in "Na^+-free" medium reached the same maximum attained in control platelets in response to higher concentrations of thrombin stimuli ($\geq$0.04 U/ml). The addition of indomethacin to the control platelets shifted the thrombin concentration–response curve to the right to a position comparable to that observed for secretion from platelets in "Na^+-free" medium (Fig. 4). These data are thus consistent with the above findings for epinephrine- and ADP-stimulated platelet function, which indicate that the responses that are eliminated by

* It should be noted that in earlier studies we demonstrated that, although part of the epinephrine-stimulated [^{3}H]serotonin release is secondary to platelet-released dense granule ADP, the use of an "ADP-scavenger" system to eliminate effects of this released ADP demonstrated that a major portion of the epinephrine-induced secretion was attributable to an α-adrenergic response independent of secondary ADP release. Furthermore, this epinephrine-stimulated secretion was markedly diminished in platelets prepared in "Na^+-free" medium (1).

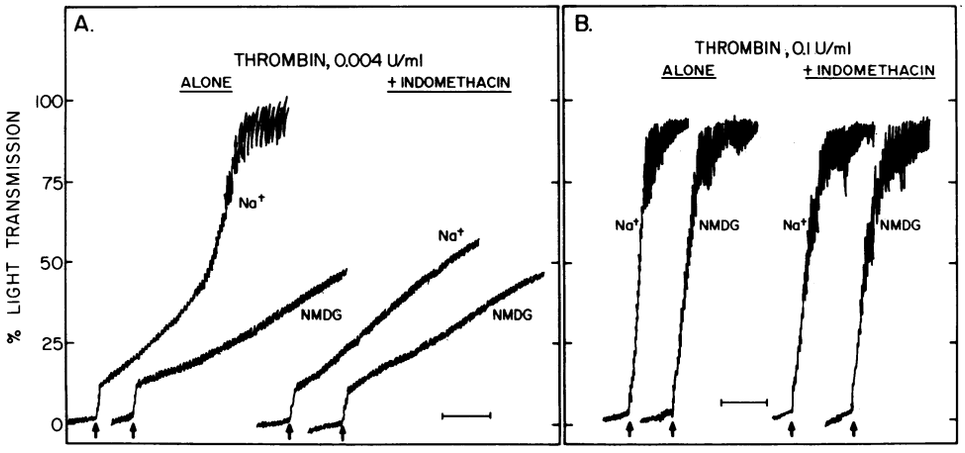

FIG. 3. Effect of removal of extraplatelet Na^+ and the influence of indomethacin on aggregation in response to thrombin. Platelets were prepared and experiments were performed as in Fig. 1. *A* is representative of seven separate experiments in which the effect of thrombin at 0.004 U/ml was evaluated. *B* is representative of seven separate experiments in which thrombin at 0.1 U/ml was used as a stimulus.

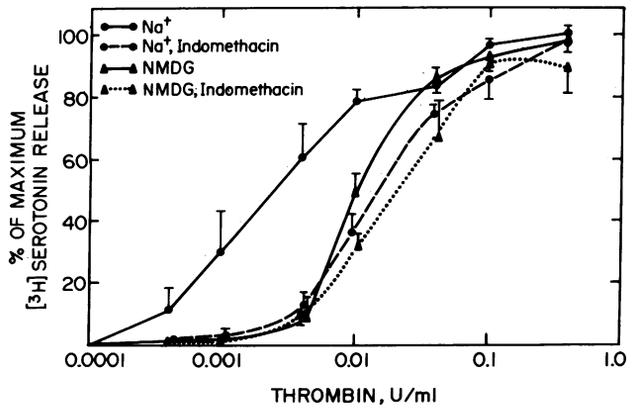

FIG. 4. Effect of removal of extraplatelet Na^+ and the influence of indomethacin on [^{3}H]serotonin release in response to increasing concentrations of thrombin. Platelets were prepared and experiments were performed as in Fig. 1. The data are normalized as a percentage of maximal specific [^{3}H]serotonin release in a particular experiment, which was 73% of total platelet-associated [^{3}H]serotonin for control platelets and 71% of total platelet-associated [^{3}H]serotonin for the "Na^+-free" NMDG platelets. Error bars indicate SEM.

removal of extraplatelet Na^+ are the same ones that are sensitive to inhibition by indomethacin in control platelets. It should be mentioned that the inability of indomethacin to alter the platelet responses to higher concentrations of thrombin was not due to the use of too low a concentration of indomethacin. The concentration used, 25 μM, was intentionally a supramaximal dose for blocking secretion and secondary aggregation. We have observed that the concentration–response curves for indomethacin inhibition of platelet serotonin induced by epinephrine, ADP, and thrombin at 0.004 U/ml are comparable, and that 1 μM can fully inhibit platelet secretion induced by these stimuli.

Because epinephrine-, ADP-, and low concentration thrombin-induced secretion was eliminated by indomethacin and is therefore mediated by cyclooxygenase products, it was of interest to determine if the functional lesion of the platelets in "Na^+-free" medium is an inability to respond to these products. The data shown in Fig. 5 demonstrate that both platelet aggregation and [^{3}H]serotonin release in response to U46619, an endoperoxide analog that acts as a thromboxane A_2 agonist (10), were similar in platelets suspended in Na^+-containing or "Na^+-free" medium. Furthermore, the addition of indomethacin at 1 or 25 μM did not affect the secretion or aggregation induced by U46619 at all concentrations tested (Fig. 5), whereas the thromboxane antagonist (±)-5-*endo*(6′-carboxyhex-2′Z-enyl)-6-*exo*[*N*-(phenylcarbamoyl)hydrazonomethyl]bicyclo[2,2,1]heptane (EP 045) (11) prevented all responses to U46619 (data not shown).

DISCUSSION

The results presented show that removal of extraplatelet Na^+ eliminates secondary aggregation and secretion to a number of platelet stimuli, including epinephrine, ADP, and low concentrations of thrombin. This conclusion is based on the observation that the aggregatory and secretory processes of platelets in "Na^+-free" medium induced by these agents resemble those of control platelets treated with indomethacin to eliminate secondary aggregation and secretion. Thus, the primary aggregation profiles observed after exposure to epinephrine, ADP, or thrombin at 0.004 U/ml of platelets suspended in "Na^+-free" medium, albeit unique for each stimulus, are superimposable on those profiles observed for indomethacin-treated control platelets in response to each stimulus. Furthermore, responses of platelets in "Na^+-free" medium to these three stimuli are not further diminished by indomethacin, a result again consistent with the conclusion that primary aggregation is unaffected by extraplatelet Na^+ removal. The similar effect of removal of extraplatelet Na^+ on secondary aggregation and secretion in response to epinephrine, ADP, and low concentrations of thrombin suggests that the secretory responses induced by these agents may be mediated via a common effector mechanism.

Interestingly, only those platelet responses that are sensitive to indomethacin are eliminated by the removal of extraplatelet Na^+ (cf. Figs. 1–5). This suggests that one or more of the steps in the cyclooxygenase pathway of platelet activation is altered by this maneuver. These steps include mobilization of arachidonic acid from the platelet membrane to provide the cyclooxygenase substrate, cyclooxygenase activity *per se*, synthesis of thromboxane A_2 from the cyclooxygenase products, PGG_2 and PGH_2, and sensitivity to the arachidonic acid metabolites themselves (12). Preliminary studies have demonstrated that cyclooxygenase and thromboxane synthetase activities are unimpaired in platelets suspended in "Na^+-free" medium. This

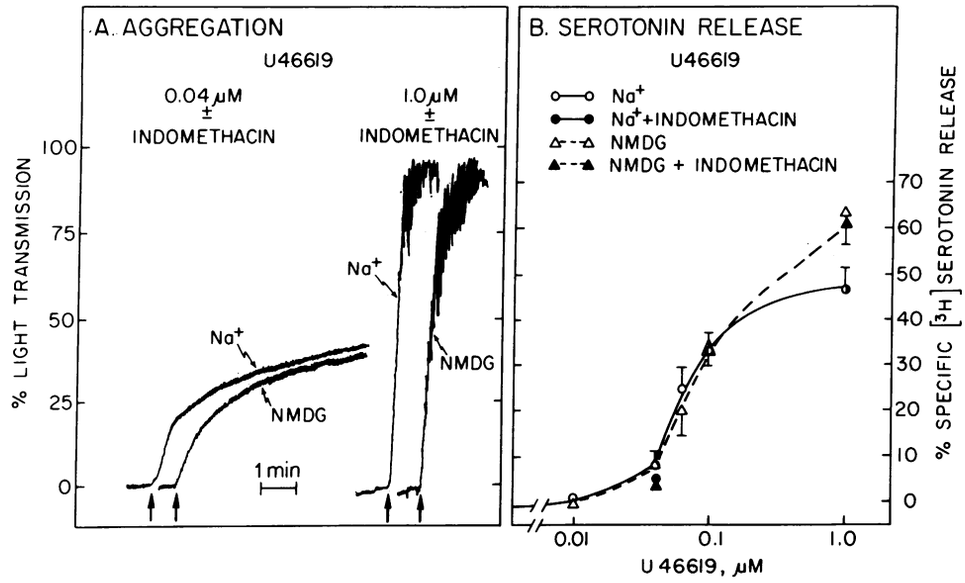

FIG. 5. Effect of removal of extraplatelet Na^+ on platelet aggregation in response to 0.04 and 1.0 μM U46619 (*A*) and [^{3}H]serotonin release in response to increasing concentrations of U46619 (*B*). Platelets were prepared and experiments were performed as in Fig. 1. U46619 was added in 1 μl of ethanol; ethanol addition alone had no effect. The secretion data are the mean ± SEM of five separate experiments in the absence of indomethacin and three experiments in the presence of indomethacin.

conclusion is based on the finding that the platelets in "Na^+-free" medium can synthesize thromboxane A_2 in response to exogenous arachidonic acid (unpublished data), measured by radioimmunoassay of the stable metabolite thromboxane B_2. Furthermore, the observation that the endoperoxide analog U46619 can elicit platelet aggregation and secretion independently of extraplatelet Na^+ indicates that inhibition of platelet secretion mediated by endoperoxides or thomboxane A_2 cannot account for the loss of secretion observed after removal of extraplatelet Na^+. Thus, our data suggest that less arachidonic acid is mobilized by epinephrine, ADP, and low concentrations of thrombin when platelets are suspended in "Na^+-free" media. Our findings are thus consistent with previous observations that synthesis of PGE_2 is attenuated in renal medullary preparations incubated in media containing Na^+ at low concentrations (13).

Although it is argued whether phospholipase A_2 (14–16) or the sequential activity of phospholipase C and diacylglycerol lipase (17–20) is responsible for the release of arachidonic acid from platelet membranes in response to particular stimuli, it is of considerable interest that all three enzymatic activities require Ca^{2+}, although changes in Ca^{2+} levels *per se* may not regulate their function. Recent studies in our laboratory have demonstrated that maximal concentrations of the Ca^{2+} ionophores A23187 and ionomycin can elicit similar secretory responses in platelets suspended in Na^+-containing and "Na^+-free" medium (unpublished data). We have also observed that subthreshold concentrations of these ionophores partially restore platelet secretion in response to epinephrine, ADP, and thrombin at 0.004 U/ml in platelets suspended in "Na^+-free" medium. These results suggest that removal of extraplatelet Na^+ may limit the amount of Ca^{2+} typically mobilized by these stimuli. A relationship between Na^+ and Ca^{2+} availability in the platelet would be analogous to that observed in a number of target tissues (21–24), particularly those involved in secretory phenomena (22–24). Alternatively, removal of extraplatelet Na^+ may otherwise alter the liberation of arachidonic acid from the membrane, such that positive feedback effects of arachidonic acid metabolites on Ca^{2+} availability and platelet secretion do not occur. Whether arachidonic acid release is preceded or followed by Ca^{2+} mobilization is unknown, as is which of these processes is modified by removal of extraplatelet Na^+.

The authors are grateful to Dr. Jacek Hawiger, Departments of Medicine and Pathology, Vanderbilt University, for his helpful discussions; to Dr. Alan Brash and Dr. Joel G. Hardman, Department of Pharmacology, Vanderbilt University, for critically reviewing the manuscript; and to Dr. Howard Uderman for performing the radioimmunoassays for thromboxane B_2. U46619 was obtained from Dr. Robert Gorman, the Upjohn Co., and EP 045 from Dr. Garret FitzGerald, Department of Pharmacology, Vanderbilt University. This work was supported by National Institutes of Health Grants HL 25182 and GM 07628. L.E.L. is a recipient of Research Career Development Award HL 00648.

1. Connolly, T. M. & Limbird, L. E. (1983) *J. Biol. Chem.* **258,** 3907–3912.
2. Haslam, R. J., Davidson, M. M. L., Davies, T., Lynham, J. A. & McClenagham, M. D. (1978) *Adv. Cyclic Nucleotide Res.* **9,** 533–552.
3. Holmsen, H. (1977) *Thrombos. Haemostas.* **38,** 1030–1041.
4. Hamberg, M., Svensson, J. & Samuelson, B. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 2994–2998.
5. Limbird, L. E., Speck, J. L. & Smith, S. K. (1981) *Mol. Pharmacol.* **21,** 609–617.
6. Packham, M. A., Kinlough-Rathbone, R. L., Reimers, H.-J., Scott, S. & Mustard, J. F. (1977) in *Prostaglandins in Hematology,* eds. Silver, M. J., Smith, J. B. & Kocsis, C. C. (Spectrum, Cochecton, NY), pp. 247–266.
7. Packham, M. A., Guccione, M. A., Greenberg, J. P., Kinlough-Rathbone, R. L. & Mustard, J. F. (1977) *Blood* **50,** 915–926.
8. Lapetina, E. G., Schandrabose, K. A. & Cuatrecasas, P. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 818–822.
9. Kinlough-Rathbone, R. L., Packham, M. A., Reimers, H.-J., Cazenave, J.-P. & Mustard, J. F. (1977) *J. Lab. Clin. Med.* **90,** 707–719.
10. Bundy, G. L. (1975) *Tetrahedron Lett.,* 1957–1960.
11. Jones, R. L., Wilson, N. H., Armstrong, R. A., Peesapati, V. & Smith, G. M. (1983) in *Advances in Prostaglandin, Thromboxane, and Leukotriene Research,* eds. Samuelsson, B., Paoletti, R. & Ramwell, P. (Raven, New York), Vol. 2, pp. 345–350.
12. Marcus, A. J. (1978) *J. Lipid Res.* **19,** 793–826.
13. Knapp, H. R. & Oates, J. A. (1980) *Adv. Prostaglandin Thromboxane Res.* **7,** 1027–1032.
14. Bills, T. K., Smith, J. B. & Silver, M. J. (1977) *J. Clin. Invest.* **60,** 1–6.
15. Broekman, M. J., Ward, J. M. & Marcus, A. J. (1980) *J. Clin. Invest.* **66,** 275–283.
16. Billah, M. M., Lapetina, E. G. & Cuatrecasas, P. (1981) *J. Biol. Chem.* **256,** 5399–5403.
17. Rittenhouse-Simmons, S. (1979) *J. Clin. Invest.* **63,** 580–587.
18. Bell, R. L., Kennerly, D. A., Stanford, N. & Majerus, P. W. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3238–3241.
19. Prescott, S. M. & Majerus, P. W. (1983) *J. Biol. Chem.* **258,** 765–769.
20. Neufeld, E. J. & Majerus, P. W. (1983) *J. Biol. Chem.* **258,** 2461–2467.
21. Reeves, J. P. & Sutko, J. L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 590–594.
22. Siegel, E. G., Wollheim, C. B., Renold, A. E. & Sharp, G. W. G. (1980) *J. Clin. Invest.* **66,** 996–1003.
23. Korchak, H. M. & Weissman, G. (1980) *Biochim. Biophys. Acta* **601,** 180–194.
24. Johnson, J. D., Epel, D. & Paul, M. (1976) *Nature (London)* **262,** 661–664.