Br. J. Pharmac. (1983), **78**, 287–292

Responsiveness of platelets and coronary arteries from different species to synthetic thromboxane and prostaglandin endoperoxide analogues

Sandra E. Burke, Allan M. Lefer, Kyriacos C. Nicolaou*, Gordon M. Smith & J. Bryan Smith**

Departments of Physiology and Pharmacology and The Cardeza Foundation of Jefferson Medical College**, Thomas Jefferson University, Philadelphia, PA 19107, and Department of Chemistry*, University of Pennsylvania, Philadelphia, PA 19104, U.S.A

1 Platelet-rich plasma (PRP) from humans, cats, dogs (after addition of 10 μM adrenaline), rabbits and guinea-pigs aggregated in response to sodium arachidonate or 9,11-azo-prostaglandin H_2, while PRP obtained from sheep was unresponsive to either agent.

2 The stable thromboxane (Tx) analogues, carbocyclic TxA_2 (CTA_2) and pinane TxA_2 (PTA_2) significantly inhibited these aggregatory responses in platelets from humans, dogs and guinea-pigs, while PTA_2 but not CTA_2 produced significant inhibition in cat platelets. The aggregatory response of PRP from rabbits was not significantly blocked by either analogue.

3 CTA_2 and the endoperoxide analogue 9,11-methanoepoxy PGH_2 (U-46619) constricted coronary arteries from cats, dogs, rabbits and guinea-pigs, while sheep vessels were unresponsive to either analogue.

4 Vasoconstrictor responses to U-46619 were significantly attenuated by PTA_2 in vessels from all species. However, constriction produced by CTA_2 was blocked significantly only in vessels from cats, dogs and guinea-pigs.

5 These results demonstrate the species differences which exist in the responsiveness of platelets and coronary arteries to thromboxane and endoperoxide analogues. Furthermore, the results illustrate the importance of species selection in the study of thromboxane antagonists for potential therapeutic use.

Introduction

Thromboxane A_2 (TxA_2), a vasoactive metabolite of arachidonic acid, has recently been implicated in the spread of myocardial ischaemic damage (Morooka, Kobayashi, Takahashi, Takashima, Sakamoto & Shimamoto, 1979) and as a mediator of sudden death in rabbits after arachidonic acid injection (Smith, Araki & Lefer, 1980). A potent vasoconstrictor and inducer of platelet aggregation, TxA_2 is generated in relatively large amounts by circulating platelets. However, recent studies have indicated that TxA_2 also can be produced by endothelial cells in the vasculature (Salzman, Salmon & Moncada, 1980; Ingerman-Wojenski, Silver & Smith, 1981). It has been suggested that TxA_2 promotes the extension of ischaemic damage by constricting coronary arteries and by inducing the formation of platelet aggregates and microthrombi (Lewy, Smith, Silver, Saia, Walinsky & Wiener, 1979).

Although TxA_2 has not yet been isolated or synthesized, stable analogues have been developed and used as tools to study its effects. These include 9,11-methano-epoxy PGH_2 (Bundy, 1978) and 9,11-azo-PGH_2 (Corey, Nicolaou, Machida, Malsten & Samuelsson, 1975), endoperoxide analogues that may act as thromboxane receptor agonists, particularly in vascular smooth muscle (Lefer, Smith, Araki, Smith, Aharony, Claremon, Magolda & Nicolaou, 1980; Coleman, Humphrey, Kennedy, Levy & Lumley, 1981). Pinane thromboxane A_2 (PTA_2), another stable thromboxane analogue antagonizes the effects of these endoperoxide analogues on smooth muscle and platelets (Nicoloau, Magolda, Smith, Aharony, Smith & Lefer, 1979). Recently, carbocyclic thromboxane A_2 (CTA_2) was described as a thromboxane analogue that constricts cat coronary arteries and aggravates myocardial ischaemia (Lefer *et al.*, 1980;

0007-1188/83/020287-06 $01.00

Smith, Lefer, Aharony, Smith, Magolda, Claremon & Nicolaou, 1981). However, CTA_2 is only a weak inducer of human platelet aggregation. These results would indicate that the vasoconstrictor effect of TxA_2 is the major reason for the spread of ischaemic damage in myocardial ischaemia. However, species variation may be an important factor in analyzing the effects of these analogues. The primary purpose of this study was to compare the responses of coronary arteries and platelets in a variety of mammalian species to thromboxane-like agents and the inhibition of these responses by the thromboxane receptor antagonist, PTA_2.

Methods

Blood was collected by venipuncture from cats, dogs, humans, rabbits and sheep, and either by carotid artery cannulation or by cardiac puncture from guinea-pigs, into one-tenth volume of 3.8% trisodium citrate and centrifuged at 180 *g* for 15 min to prepare platelet-rich plasma (PRP). Platelet aggregation was studied in a Payton Dual-Channel aggregometer, at 37°C with continuous stirring at 1000 rev/min by measuring the increase in light transmission as the platelets clumped together. Aggregating agents employed were sodium arachidonate (50–500 μM final concentration) and 9,11-azo-prostaglandin H_2 (1 μM final concentration). Carbocyclic thromboxane A_2 and pinane thromboxane A_2 were stored in 100% ethanol and added in a 2 μl volume to achieve a concentration of 20 μM, and unless otherwise stated were added to PRP 60 s before the aggregating agent being studied. Ethanol alone did not alter platelet function. Adrenaline HCl was obtained from Sigma Chemical Co., St. Louis, MO, and arachidonic acid from Nuchek Prep, Elysian, MN.

Isolated coronary arteries

Hearts were obtained from adult guinea-pigs, rabbits, cats, dogs and sheep anaesthetized with sodium pentobarbitone (30 mg/kg, i.v.). Immediately after excision, each heart was placed in ice-cold Krebs-Henseleit solution oxygenated with 95% O_2 plus 5% CO_2 until isolation of the coronary arteries 15–30 min later. To obtain vessels from the hearts of guinea-pigs and rabbits, the coronary ostium was exposed and a 23 gauge stainless-steel adapter was inserted. In cats, a 20 gauge adapter was inserted into the right coronary artery and into the left circumflex (LCX) or left anterior descending (LAD) coronary arteries via the coronary ostia. In dogs and sheep, 18–20 gauge adapters were inserted into branches of the LCX or LAD. In all species, the cannulae were tied in place with 3-0 suture silk and surrounding fat and myocardial tissue were carefully removed. A final length of approximately 1.5 cm of each vessel was immediately placed in a 10 ml bath containing Krebs-Henseleit solution. Arteries were oxygenated with 95% O_2 plus 5% CO_2 and perfusate temperature was maintained at 37°C.

All vessels were perfused at constant flow with a Harvard peristaltic pump so that changes in perfusion pressure reflected changes in vascular resistance. Initially, flow of the perfusate was adjusted so that arterial pressure was between 65–85 mmHg. The flow rate was then maintained at this level throughout the experiment. Perfusion pressure was measured with Statham P23Db pressure transducers and recorded continuously on a Grass Model 7 oscillographic recorder.

After an equilibration period of 45–60 min, each vessel was tested for responsiveness to 25–50 mM KCl. After replacing with fresh perfusate, the arteries were used to determine the responses to CTA_2 (15 nM) and 9,11-methano-epoxy PGH_2 (U-46619), at 50 or 100 nM before and after PTA_2 (1.0 μM). All test agents were made as stock solutions in 100% ethanol and added to the recirculating perfusate in volumes of 10 to 20 μl.

Data in the text and figures are presented as means ± s.e.mean. Data were analyzed using Student's *t* test for paired comparisons. *P* values less than 0.05 were considered statistically significant.

Results

Platelets in plasma prepared from the blood of 6 cats, 4 guinea-pigs, 4 humans and 5 rabbits responded to 1 μM 9,11-azo-PGH_2 with pronounced aggregation which did not reverse during the 3 min of observation. Irreversible aggregation also was induced in PRP from these species by sodium arachidonate (in a concentration range of 50 to 200 μM in PRP from cat, guinea-pig and rabbit and 400 to 800 μM in PRP from humans). In PRP prepared from 5 dogs, the addition of 1 mM sodium arachidonate produced little or no aggregation. However, the addition of 10 μM adrenaline, which itself produced no detectable aggregation, prior to the addition of 9,11-azo-PGH_2 or sodium arachidonate, converted the response to these agents to a pattern identical to that seen with the PRP from the other species. In contrast, PRP prepared from the blood of 6 different sheep responded poorly or not at all to either 1 μM 9,11-azo-PGH_2 or 50 to 500 μM sodium arachidonate. Also, adrenaline did not make sheep PRP responsive to sodium arachidonate or 9,11-azo-PGH_2.

The effects of the thromboxane analogues of CTA_2 and PTA_2 were studied directly on PRP prepared from cat, guinea-pig, human and rabbit blood and

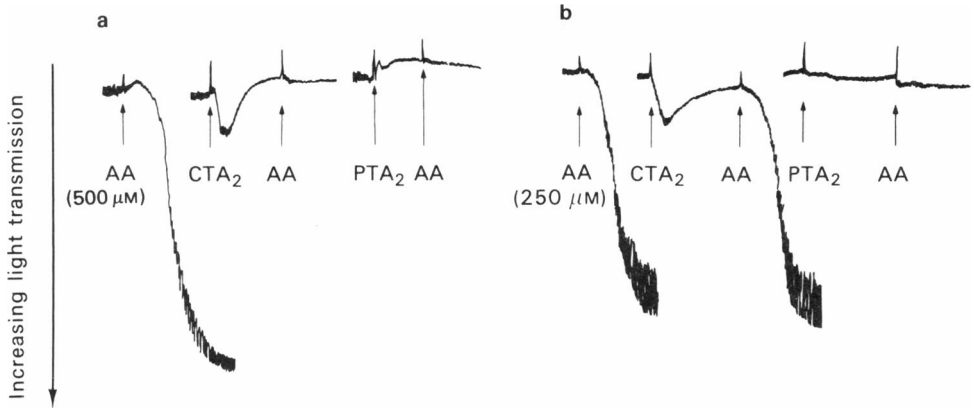

Figure 1 Typical records of platelet-rich plasma from human (a) and cat (b) blood. AA = arachidonic acid, CTA_2 = carbocyclic thromboxane A_2 at 20 μM, PTA_2 = pinane thromboxane A_2 at 20 μM.

after the addition of 10 μM adrenaline to PRP prepared from dogs. As reported previously and shown in Figure 1, both CTA_2 and PTA_2 completely abolished the aggregation response of human platelets to either 9,11-azo-PGH_2 or sodium arachidonate. PTA_2 has been reported to be a weak agonist (Nicolaou, Magolda, Smith, Aharony, Smith & Lefer, 1979) and induced a small reversible aggregation in the present study. Similar results to those with human PRP were obtained in PRP prepared from guinea-pigs as shown in Figure 2 and dogs (containing 10 μM adrenaline). A slightly different response was obtained with CTA_2 and PTA_2 in PRP prepared from cats. In the PRP of this species, PTA_2 itself caused little or no aggregation but completely abolished aggregation in response to 9,11-azo-PGH_2 or arachidonic acid. CTA_2 caused the platelets to aggregate to a greater extent than PTA_2, but after the platelets had disaggregated, essentially no inhibition of aggregation in response to 9,11-azo-PGH_2 or sodium arachidonate was observed.

The greatest differences in the effects of CTA_2 and

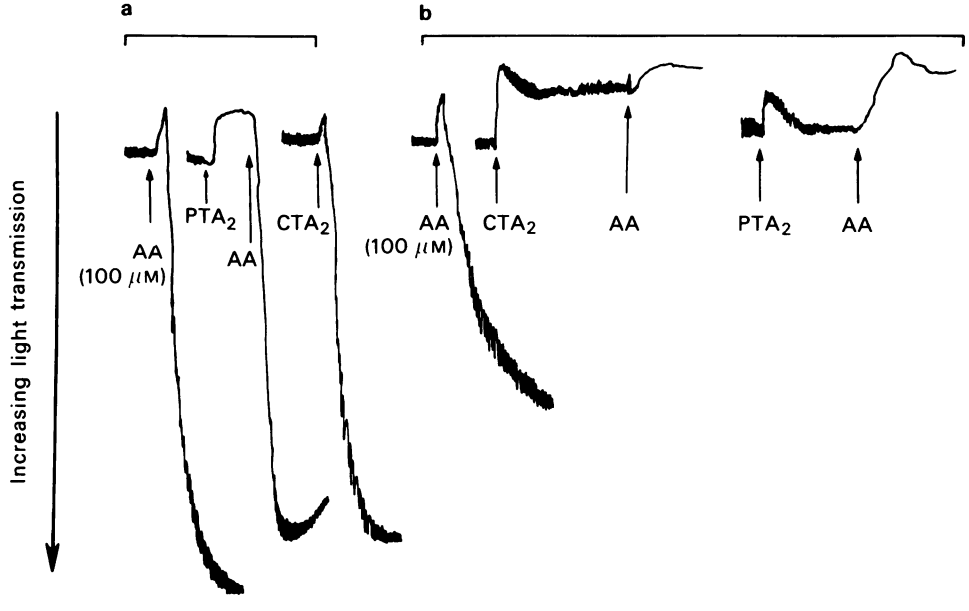

Figure 2 Typical records of platelet-rich plasma from rabbit (a) and guinea-pig blood (b). Arrow indicates increasing light transmittance. AA = arachidonic acid. CTA_2 = carbocyclic thromboxane A_2 at 20 μM. PTA_2 = pinane thromboxane A_2 at 20 μM.

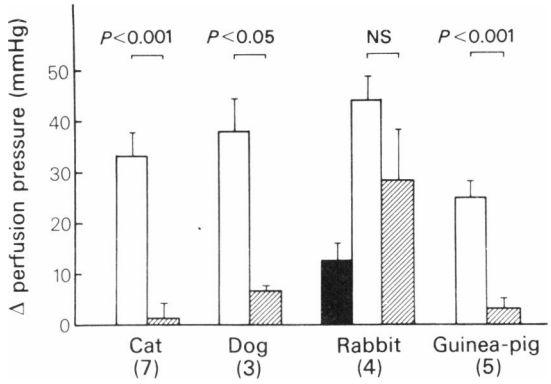

Figure 3 Change in perfusion pressure in response to carbocyclic thromboxane A_2 (CTA_2, 15 nM) before (open columns) and after (hatched columns), pinane thromboxane A_2 (PTA_2, 1.0 μM) in cat, dog, rabbit and guinea-pig coronary arteries. The solid column represents the direct effect of PTA_2 on rabbit vessels. Numbers in parentheses indicate number of vessels studied in each species.

PTA_2 from those seen with human PRP were obtained with PRP prepared from the blood of the 5 rabbits studied. In this species, CTA_2 produced fairly extensive aggregation (Figure 2) and little or no inhibition of aggregation in response to sodium arachidonate was observed after either PTA_2 or CTA_2. However, PTA_2 inhibited the response to 9,11-azo-PGH_2 to a small extent while CTA_2 was inactive.

Isolated coronary arteries

CTA_2 (15 nM) constricted coronary arteries of cats, dogs, rabbits and guinea-pigs as shown in Figure 3.

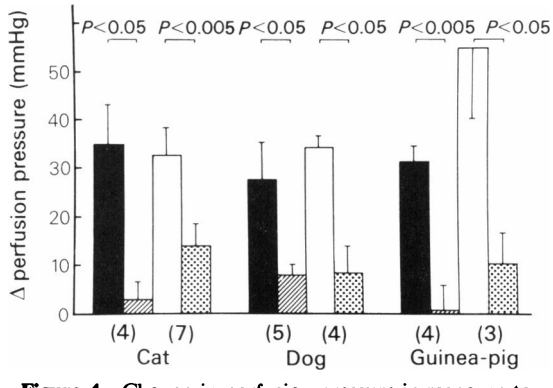

Figure 4 Change in perfusion pressure in response to U-46619 (50 and 100 nM) before (■,□) and after (▨,⊠) pinane thromboxane A_2 (1.0 μM) in cat, dog and guinea-pig coronary arteries. Numbers in parentheses indicate number of vessels studied in each species.

The constrictor effect was significantly inhibited by 1.0 μM PTA_2 in all species except the rabbit. Rabbit coronary arteries responded to CTA_2 with a change in perfusion pressure of 44.3 ± 4.3 mmHg. This response was attenuated to 28.3 ± 9.9 mmHg (NS) after PTA_2. Also, CTA_2 produced a significantly greater constriction of rabbit vessels than the same concentration in guinea-pigs ($P < 0.01$). There were no significant differences in the responses to CTA_2 when comparisons were made among other species listed in Figure 3. Responses to KCl were obtained in six sheep coronary arteries. However, none of these vessels was responsive to CTA_2.

The effects of U-46619 (50 and 100 nM) on cat, dog and guinea-pig coronary arteries before and after PTA_2 (1.0 μM) are shown in Figure 4. The response to either concentration of U-46619 was significantly inhibited by PTA_2 in all three species.

Guinea-pig vessels were constricted to a greater degree by the higher concentration of U-46619. However, cat and dog coronary arteries were constricted to a similar extent by both concentrations of the endoperoxide analogue. Also, the degree of vasoconstriction produced by U-46619 was similar to that produced by CTA_2 in cats and dogs, while guinea-pig vessels were more responsive to U-46619 than to CTA_2.

Responses were obtained to 50 and 100 nM U-46619 in three rabbit coronary arteries. The lower concentration of U-46619 increased perfusion pressure by 26 ± 4 mmHg compared with 38 ± 6 mmHg for the 100 nM concentration. In the presence of 1.0 μM PTA_2, the increase in perfusion pressure was 11 ± 2 mmHg for the 50 nM concentration and 15 ± 3 for the 100 nM concentration of U-46619. PTA_2 alone at 1.0 μM increased perfusion pressure 7 ± 2 mmHg in these experiments, partially accounting for the lack of a complete antagonism.

As mentioned above, significant constrictor responses to KCl were obtained in six sheep coronary arteries. However, only one of these arteries responded to 100 nM U-46619 with an increase of at least 7 mmHg, and none of these arteries responded to CTA_2.

Discussion

The demonstration that differences exist in the responsiveness of platelets from different species to agonists and antagonists is not new. For example, adrenaline induces the aggregation of platelets in PRP obtained from humans but not from other species such as rat, dog, guinea-pig, rabbit or horse (Mustard & Packham, 1970). However, adrenaline does potentiate aggregation in response to other aggregating agents in PRP of all species, and this

property was taken advantage of in the present investigation to enhance the responsiveness of dog platelets to 9,11-azo-PGH_2 and sodium arachidonate. A more striking example of differences in responsiveness between platelets of different species has been observed with the platelet activating factor (PAF) 1-O-alkyl-2-acetyl-glycero-3-phosphocholine, which is an extremely potent aggregator of platelets from humans, rabbits and guinea-pigs, but has no effect on platelets from mouse or rat (Namm, Tadepalli & High, 1982). The responsiveness of platelets from different species to prostaglandins also is species-dependent based on results of previous studies as well as those described here. In previous studies, for example, prostaglandin D_2 was shown to be a potent inhibitor of the aggregation of human platelets but to have little or no effect on platelets obtained from rabbits or rats (Smith, Silver, Ingerman & Kocsis, 1974).

Our finding that sheep platelets are poorly responsive or unresponsive to sodium arachidonate or to 9,11-azo-PGH_2 is reminiscent of similar observations made with PRP obtained from bovine blood (Meyers, Katz, Clemmons, Smith & Holmsen, 1980). It would appear that ruminant platelets do not possess a significant population of receptors for prostaglandin endoperoxides and thromboxane A_2. Furthermore, sera from sheep or cow blood contains very little thromboxane B_2 or prostaglandin $F_{2\alpha}$ in contrast to the relatively large amount present, as a result of platelet activation by thrombin, in sera obtained from omnivores and carnivores including man (Meyers *et al.*, 1980; Hwang, Carroll, Godke & Ring, 1980). The lack of responsiveness of sheep coronary arteries to either CTA_2 or U-46619 suggests that the sheep is not an appropriate species for the study of thromboxane receptors.

In our previous studies with the thromboxane analogues CTA_2 and PTA_2, we demonstrated that PTA_2 antagonizes the constriction of cat coronary arteries in response to prostaglandin endoperoxide analogues and inhibits the aggregation of human platelets in response to these analogues as well as sodium arachidonate. In contrast, CTA_2 was a potent constrictor of cat coronary arteries without exerting antagonistic properties, but inhibited the aggregation of human platelets in response to endoperoxide analogues and sodium arachidonate (Lefer *et al.*, 1980). The present studies suggest that this dissociation obtained with CTA_2 between platelets and coronary vascular smooth muscle may be related in part to differences between species. In this regard, PTA_2 was consistent in inhibiting the effects of endoperoxides on coronary arteries and platelets from both cat and man. CTA_2 was less consistent in that it strongly inhibited human aggregation, only weakly inhibited cat platelet aggregation and strongly constricted cat coronary arteries. Furthermore, CTA_2 caused little or no aggregation in PRP from cat and man, and a marked aggregation in rabbits.

Of the five species that were studied, platelets from cats and guinea-pigs appeared to resemble most closely those from humans in responsiveness to the agonists and antagonists of the prostaglandin cascade that were tested. Interestingly, platelets of these species more closely resembled those of man than those of two other species in their ability to release adenine nucleotides and platelet factor 4 in response to collagen or thrombin (Thomas, Niewiarowski & Ream, 1970). In contrast, apart from the sheep as discussed above, rabbit platelets were the most dissimilar from human platelets in that induction of aggregation was obtained with CTA_2, and little or no inhibition was obtained with either CTA_2 or PTA_2. Furthermore, only with rabbit coronary arteries was PTA_2 unable to antagonize thromboxane effects.

It would appear that, with the exception of sheep, the differences between species observed in the present studies reflect subtle conformational differences between the receptors for prostaglandin endoperoxides and thromboxane A_2 present on platelets and the coronary arteries of different species. However, while these differences may be subtle from a conformational point of view they undoubtedly present an important consideration for those interested in developing thromboxane antagonists for therapeutic use in man.

With regard to coronary vascular responsiveness, species differences also exist. Previously, Wang, Kulkarni & Eakins (1980) showed that adult dog coronary vessels do not respond to thromboxane A_2 generated biologically, in contrast to puppy coronary vessels that did respond to thromboxane A_2. In the present study, we also observed that sheep coronary arteries were unresponsive to the synthetic thromboxane analogue, CTA_2. Other than in this case, all other mammalian species investigated (i.e., cat, dog, rabbit and guinea-pig) were comparable in their responses to CTA_2. We do not know whether our observation of a marked vasoconstrictor response to CTA_2 in the perfused dog coronary artery represents a peculiarity of our preparation (i.e., large artery vs the entire vascular bed) or whether it reflects the effect of a stable thromboxane analogue. Our findings do not permit us to answer this question at present.

One interesting difference in coronary vascular responsiveness occurred in response to the thromboxane receptor antagonist, PTA_2. All species studied that responded to CTA_2 with a significant vasoconstriction, were antagonized by PTA_2 except the rabbit. No reason for this difference is apparent except that PTA_2 acts as a thromboxane agonist (i.e., induces constriction) in rabbit coronary vessels,

rather than as a thromboxane antagonist.

Similar degrees of coronary vasoconstriction were observed in cat, dog and guinea-pig coronary arteries to the stable endoperoxide analogue, U-46619. In all cases, PTA_2, the thromboxane receptor antagonist, significantly attenuated the coronary constrictor effects of U-46619. Since U-46619 has been previously shown to have essentially the same receptor profile as thromboxane A_2 (Coleman *et al.*, 1981), these findings can be considered as additional evidence that many mammalian species constrict markedly to thromboxane agonists. Thus, with regard to coronary vasoactivity, there are more similarities than differences in thromboxane responsiveness. Cat, dog, rabbit and guinea-pig coronary vessels constrict to thromboxane agonists (e.g., CTA_2, U-46619) and these responses are markedly attenuated by the thromboxane receptor antagonist, PTA_2. Only sheep coronary arteries are essentially unresponsive to thromboxanes. Human coronary tissue was unavailable for use in this study.

We gratefully acknowledge the supply of U-46619 from Dr John Pike of the Upjohn Company, Kalamazoo, Michigan, and of 9,11-azo-PGH_2 from Dr Don N. Harris, Squibb Institute for Medical Research, Princeton, New Jersey. We also thank Dr Marlys Gee of Jefferson Medical College for the sheep blood and tissue. This study was supported in part by Research Grant HL-25575-01A1, National Heart, Lung, and Blood Institute of the National Institutes of Health, Bethesda, Maryland. S.E.B. is a predoctoral Fellow of the Ischemia-Shock Research Center. K.C.N. is a Fellow of the A.P. Sloan Foundation (1979–1983), and a recipient of a Camille and Henry Dreyfus Teacher-Scholar Award (1980–1985). G.M.S. was on leave from Robert Gordon's Institute of Technology, School of Pharmacy, Aberdeen, Scotland.

References

BUNDY, G.L. (1978). The synthesis of prostaglandin endoperoxide analogs. *Tetrahedron Lett.*, **24,** 1957.

COLEMAN, R.A., HUMPHREY, P.P.A., KENNEDY, I., LEVY, G.P. & LUMLEY, P. (1981). Comparison of the actions of U-46619, a prostaglandin H_2-analogue, with those of prostaglandin H_2 and thromboxane A_2 on some isolated smooth muscle preparations. *Br. J. Pharmac.*, **73,** 773–778.

COREY, E.J., NICOLAOU, K.C., MACHIDA, Y., MALSTEN, C.L. & SAMUELSSON, B. (1975). Synthesis and biological properties of a 9,11-azo-prostanoid. *Proc. natn. Acad. Sci. U.S.A.*, **72,** 3355–3358.

HWANG, D.H., CARROLL, A., GODKE, R.E. & RING, R.W. (1980). Species variation in serum levels of prostaglandins (PG's) and their precursor. *Fed Proc.*, **39,** 323.

INGERMAN-WOJENSKI, C., SILVER, M.J. & SMITH, J.B. (1981). Bovine endothelial cells in culture produce thromboxane as well as prostacyclin. *J. clin. Invest.*, **67,** 1292–1296.

LEFER, A.M., SMITH, E.F. III, ARAKI, H., SMITH, J.B., AHARONY, D., CLAREMON, D.A., MAGOLDA, R.L. & NICOLAOU, K.C. (1980). Dissociation of vasoconstrictor and platelet aggregatory activities of thromboxane by carbocyclic thromboxane A_2. *Proc. natn. Acad. Sci. U.S.A.*, **77,** 1706–1710.

LEWY, R.I., SMITH, J.B., SILVER, M.J., SAIA, J., WALINSKY, P. & WIENER, L. (1979). Detection of thromboxane B_2 in peripheral blood of patients with Prinzmetal's angina. *Prostaglandins Med.*, **5,** 243–248.

MEYERS, K.M., KATZ, J.B., CLEMMONS, R.M., SMITH, J.B. & HOLMSEN, H. (1980). An evaluation of the arachidonate pathway of platelets from companion and food-producing animals, mink and man. *Thromb. Res.*, **20,** 13–24.

MOROOKA, S., KOBAYASHI, M., TAKAHASHI, T., TAKASHIMA, Y., SAKAMOTO, M. & SHIMAMOTO, T. (1979). Experimental ischemic heart disease-effects of synthetic thromboxane A_2. *Exp. Molec. Pathol.*, **30,** 449–457.

MUSTARD, J.F. & PACKHAM, M.A. (1970). Factors influencing platelet function: Adhesion, release, and aggregation. *Pharmac. Rev.*, **22,** 97–187.

NAMM, D.H., TADEPALLI, A.S. & HIGH, J.A. (1982). Species specificity of the platelet responses to 1-O-alkyl-2-acetyl-glycero-3-phosphocholine. *Thromb. Res.*, **25,** 341–351.

NICOLAOU, K.C., MAGOLDA, R.L., SMITH, J.B., AHARONY, D., SMITH, E.F. III & LEFER, A.M. (1979). Synthesis and biological properties of pinanethomboxane A_2, a selective inhibitor of coronary artery constriction, platelet aggregation, and thromboxane formation. *Proc. natn. Acad. Sci. U.S.A.*, **76,** 2566–2570.

SALZMAN, P.M., SALMON, J.A., & MONCADA, S. (1980). Prostacyclin and thromboxane A_2 synthesis by rabbit pulmonary artery. *J. Pharmac. exp. Ther.*, **215,** 240–247.

SMITH, E.F. III, LEFER, A.M., AHARONY, D., SMITH, J.B., MAGOLDA, R.L., CLAREMON, D. & NICOLAOU, K.C. (1981). Carbocyclic thromboxane A_2: Aggravation of myocardial ischemia by a new synthetic thromboxane A_2 analog. *Prostaglandins*, **21,** 443–456.

SMITH, J.B., ARAKI, H. & LEFER, A.M. (1980). Thromboxane A_2, prostacyclin and aspirin: Effects on vascular tone and platelet aggregation. *Circulation*, **62,** (Suppl. V): V19–V25.

SMITH. J.B., SILVER, M.J., INGERMAN, C.M. & KOCSIS, J.J. (1974). Prostaglandin D_2 inhibits the aggregation of human platelets. *Thromb. Res.*, **5,** 291–299.

THOMAS, D.P., NIEWIAROWSKI, S. & REAM, V.J. (1970). Release of adenine nucleotides and platelet factor 4 from platelets of man and four other species. *J. Lab. clin. Med.*, **75,** 607–618.

WANG, H.-H., KULKARNI, P.S. & EAKINS, K.E. (1980). Effects of prostaglandins and thromboxane A_2 on the coronary circulation of adult dogs and puppies. *Eur. J. Pharmac.*, **66,** 31–41.

(Received May 19, 1982.
Revised October 13, 1982.)