synaptic activity, this is likely to trigger the induction of (Na–K) ATPase [7].

To summarise, there is a significant increase in the (Na–K) ATPase activity in homogenates of midbrain regions from spontaneously hypertensive rats. This increase might reflect changes in the permeability of the membranes to sodium or perhaps an increase in the synaptic activity of that region.

Department of Physiology
The London Hospital Medical College
Turner Street
London E1 2AD, U.K.

J. G. LOGAN
M. J. GEORGE

REFERENCES

1. M. B. Pamnani, D. L. Clough, S. J. Huot and F. J. Haddy, *Clin. Sci.* **59**, 179s (1980).
2. H. Losse, H. Wehmeyer and F. Wessels. *Klin. Wschr.* **38**, 393 (1960).
3. Y. V. Postnov, S. N. Orlov, P. Gulak and A. Schevchenko, *Pflügers Arch.* **365**, 257 (1976).
4. Y. V. Postnov, S. N. Orlov, A. Schevchenko and A. M. Adler, *Pflügers Arch.* **371**, 263 (1977).
5. R. P. Garay, G. Dagher and P. Meyer, *Clin. Sci.* **59**, 1915 (1980).
6. T. E. Forrester and G. A. O. Alleyne, *Clin. Sci.* **59**, 199s (1980).
7. S. L. Bonting, in *Membranes and Ion Transport* (Ed. E. E. Brittar) Vol. 1, p.257. John Wiley, New York (1970).
8. E. S. Vizi, *Neuroscience* **3**, 367 (1978).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
10. R. P. Lehr, Jr., R. A. Browning and J. Hurley Myers, *Clin. exp. Hyperten.* **2**, 123 (1980).
11. G. Haeusler, M. Gerold and T. Theonen, *Naunyn Schmiedeberg's Archs Pharmac.* **274**, 211 (1978).

Biochemical Pharmacology, Vol 31, No 6, pp. 1158–1160, 1982
Printed in Great Britain

0006-2952/82/061158-03 $03.00/0

Evidence that prostaglandin endoperoxides can induce platelet aggregation in the absence of thromboxane A_2 production

(*Received* 25 *August* 1981; *accepted* 9 *October* 1981)

Arachidonic acid causes aggregation of platelet rich plasma (PRP). Platelet cyclooygenase converts arachidonic acid to the prostaglandin endoperoxides PGG_2 and PGH_2 which in turn are converted by platelet thromboxane synthetase to thromboxane A_2 (TXA_2), a very potent aggregating agent [1]. Low concentrations of collagen or thrombin release arachidonic acid from platelet phospholipids. The arachidonic acid is converted via the prostaglandin endoperoxides to TXA_2 and aggregation occurs. The addition of either prostaglandin endoperoxides (PGG_2/PGH_2) or TXA_2 to human PRP causes aggregation but TXA_2 is more potent in this respect [2]. It has been assumed that the aggregatory effects of PGG_2/PGH_2 are due mainly to their conversion to TXA_2 but in describing the effects of substituted imidazoles on platelet aggregation induced by arachidonic acid, Yoshimoto *et al.* [3] noted that 1-heptyl imidazole was less effective as an inhibitor of aggregation than as an inhibitor of thromboxane synthetase. Furthermore, Heptinstall *et al.* [4] observed that selective inhibition of platelet thromboxane synthetase with UK 34787 [2-isopropyl-3(1-imidazolyl methyl) indole] led to inhibition of aggregation by arachidonic acid in PRP from some but not all donors. Needleman *et al.* [5] showed that high concentrations of imidazole completely inhibited the formation of TXA_2 from PGH_2 by human washed platelets without abolishing the aggregatory response and demonstrated that imidazole reduced TXA_2 production in response to collagen, thrombin and arachidonic acid without reducing aggregation. Similarly, Blackwell *et al.* [6] during studies of thromboxane synthetase inhibition by 1-*N*-butyl imidazole observed that platelet aggregation induced by PGH_2 could not be completely overcome even by high concentrations of the inhibitor. A stable chemical analogue of the prostaglandin endoperoxides, U 46619 [(15*S*)-hydroxy, 11α, 9α-(epoxymethano) prosta-5Z, 13E-dienioc acid] has recently been described as having a similar pharmacological profile to TXA_2 on isolated tissues. Whilst this compound is more closely related structurally to the endoperoxides, it acts as an agonist at thromboxane receptors [7]. These observations suggest that endoperoxides and TXA_2 may act at the same receptor and therefore that endoperoxides may have significant aggregatory activity in their own right particularly under conditions where conversion to TXA_2 is prevented.

In order to investigate this proposition we have measured concurrently thromboxane B_2 (TXB_2) production and platelet aggregation in response to collagen in human PRP treated with UK 34787, indomethacin and combinations of the two drugs.

Materials and methods

Materials. UK 34787 was generously provided by Pfizer Ltd. (Sandwich, U.K.). The compound was dissolved in 0.1 N HCl and diluted to working concentrations with 0.154 M NaCl. Collagen was purchased as a 1 mg/ml suspension in buffer from Hormon Chemie (Munich, West Germany). Indomethacin was purchased from the Sigma Chemical Co. (London, U.K.). TXB_2 was purchased from the Upjohn Company (Kalamazoo, MI), and [^{3}H]TXB_2 (100 Ci/mM) was purchased from New England Nuclear (Southampton,

U.K.). Lyophilised anti-thromboxane A_2 antisera, raised in rabbits, was purchased from Seragen Inc. (Boston, MA).

Methods. Platelet aggregation was performed as previously described [8]. All donors claimed not to have taken any medicine for over a week. Concentration effect curves of aggregation and TXB_2 production were prepared using collagen in a range of concentrations from 0.1 to 10 μg/ml. In a second series of experiments, UK 34787 and indomethacin (10^{-9}–10^{-4} M) were incubated jointly or separately, at 37° for 5 min with PRP prior to the addition of collagen (1 μg/ml). Aggregation was recorded until 5 min after collagen addition when an equal volume (0.25 ml) of buffer (citric acid/sodium citrate, 1M, pH 3.0), was added to the PRP in the aggregometer cuvette. After 1 min the contents of each cuvette were extracted twice with two volumes of ice cold ethyl acetate and the extract was blown dry under N_2. An aliquot of PRP containing a known quantity of [^{3}H]TXB_2 was extracted in parallel to determine the efficiency of extraction. Platelet number in PRP from each donor was determined visually using a haemocytometer.

TXB_2 was determined by radioimmunoassay using a conventional dextran coated charcoal technique to separate bound from free ligand [9]. Each extract was dissolved in buffer (0.01 M Tris/0.154 M NaCl/0.1% gelatin, pH 7.3) and TXB_2 content was determined with respect to a standard curve of percentage binding against known amounts of TXB_2. After correction for extraction losses TXB_2 production was calculated as ng/10^8 platelets/5 min.

Results

Figure 1 shows that a concentration of collagen (1.2 μg/ml) which causes complete aggregation of human platelets stimulates the production of TXB_2 submaximally. Even at 10 μg/ml collagen TXB_2 production does not appear to be maximal as the concentration effect curve has not reached a plateau.

Figure 2 shows the relationship between aggregation and thromboxane production at a single concentration of collagen (1 μg/ml) in the presence of the cyclooxygenase inhibitor indomethacin and the thromboxane synthetase inhibitor UK 34787. Both inhibitors reduce the synthesis of TXB_2 by 50% before there is significant inhibition of aggregation. However, although indomethacin at 10^{-5} M reduces both aggregation and thromboxane production to insignificant levels UK 34787 (10^{-5} M) fails to reduce aggregation by more than 50% even though the production of TXB_2 is reduced to 15% (2.25 ng/10^8 cells/5 min) of the control value (15 ± 3 ng/10^8 platelets/5 min).

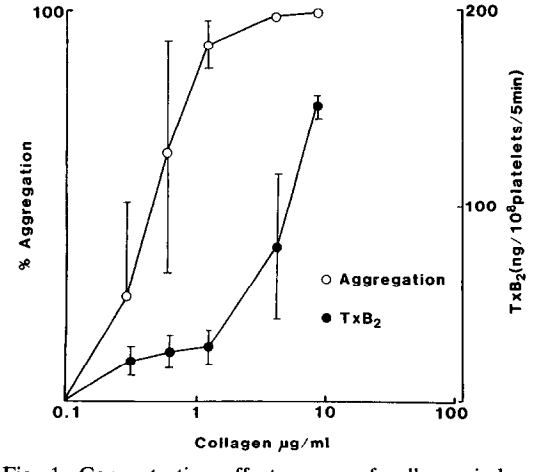

Fig. 1. Concentration effect curves of collagen induced platelet aggregation and TXB_2 production in human PRP. Values are means ± S.E.M. ($n = 3$).

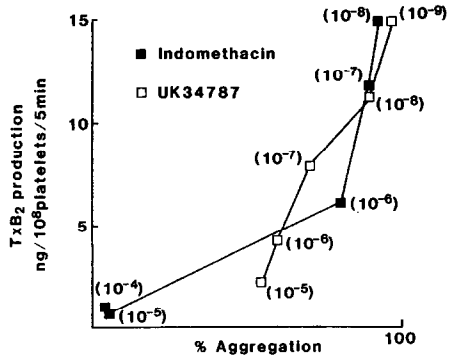

Fig. 2. The relationship between aggregation and TXB_2 production in human PRP in response to collagen (1 μg/ml). Comparison of the effects of increasing concentrations of indomethacin or UK 34787. Values are means of three experiments. Figures in brackets represent molarity.

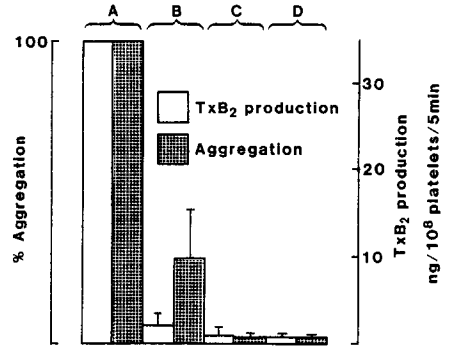

Fig. 3. The effect of single concentrations of UK 34787 and indomethacin on collagen induced platelet aggregation and TXB_2 production. Values are means ± S.E.M. ($n = 4$). (A) Controls (collagen 1 μg/ml), (B) UK 34787 10^{-4} M, (C) indomethacin 10^{-4} M, and (D) UK 34787 (10^{-4} M) + indomethacin 10^{-4} M.

Figure 3 shows that the residual aggregation produced by 1 μg/ml collagen in the presence of 10^{-4} M UK 34787 is abolished by 10^{-4} M indomethacin. Thromboxane production, already reduced by UK 34787 from a control value of 35.2 ± 9.1 ng to 1.7 ± 1.2 ng/10^8 platelets/5 min is only reduced further to 0.4 ± 0.45 ng/10^8 platelets/5 min in the presence of UK 34787 plus indomethacin.

Discussion

The capacity of human platelets to produce thromboxane B_2 and hence thromboxane A_2 in response to an aggregatory stimulus clearly exceeds that required to produce complete aggregation.

Thus sufficient TXA_2 may be produced by stimulation of a given number of platelets to trigger the TXA_2 induced aggregation of many more platelets. This supports the proposal that TXA_2 may recruit platelets to a site of arterial injury [10].

Unlike indomethacin, which inhibits cyclooxygenase, UK 34787 is a selective inhibitor of thromboxane synthetase with little or no activity against cyclooygenase or prostacyclin synthetase [11]. Despite its ability to inhibit thromboxane production in platelets stimulated with collagen UK 34787 even at concentrations as high as 10^{-4} M failed to inhibit aggregation completely. A lack of correlation between ability to inhibit thromboxane synthetase in bro-

ken cell preparations and ability to inhibit aggregation has previously been noted by Yoshimoto *et al.* [3] and in intact platelets by Needleman *et al.* [5] and Blackwell *et al.* [6].

Several groups have shown that high concentrations of collagen can induce aggregation by a mechanism which does not involve the generation of prostanoids [12, 13] but the low concentration of collagen used in the present experiments (1 μg/ml) and the abolition of aggregation by indomethacin suggests that the aggregation observed both in control plasma and in plasma treated with UK 34787 is caused by a prostanoid aggregating agent. The only candidates are TXA_2 and the endoperoxides. Although it is possible that the residual 25% aggregation produced by 1 μg/ml collagen in the presence of 10^{-4} M UK 34787 is due to the residual 5% of thromboxane produced, inspection of the concentration–response curves for aggregation and thromboxane production suggests this is unlikely. Thus, the abolition of residual aggregation by indomethacin does not appear to be related to the further reduction in thromboxane production from 5 to 2% of control. In our opinion, it is more likely that the residual aggregation seen in the presence of selective inhibition of thromboxane synthetase and abolished by indomethacin is due to accumulation of prostaglandin endoperoxides which may act at the same receptor as TXA_2 [7]. This view is supported by the observation that imidazole-resistant aggregation to exogenous arachidonic acid is due to accumulation of prostaglandin endoperoxides [5].

During the preparation of this paper, Bertele *et al.* [14] showed that another inhibitor of thromboxane synthesis, UK 37,248-01 (4-[2-(1H-imidazol-1-yl)-ethoxy] benzoic acid hydrochloride) at concentrations which abolished thromboxane synthesis failed to inhibit the aggregation of human PRP from one of three subjects in response to exogenous arachidonic acid. The use of arachidonic acid as the aggregating agent provides potential substrate for extensive synthesis of prostaglandin endoperoxides in the absence of thromboxane synthesis. Similarly the use of exogenous endoperoxides [5, 6], may provide unrealistically high concentrations of aggregating agent. With the low concentrations of collagen used in the present study the generation of endogenous arachidonic acid and thus the potential for endoperoxide synthesis and accumulation may more nearly reflect the situation *in vivo* when aggregation is stimulated by vascular damage although PRP contains no source of prostacyclin synthetase which might generate PGI_2.

Whether endoperoxide-mediated aggregation in the presence of an inhibitor of thromboxane synthesis can be a significant thrombotic mechanism *in vivo* remains an open question. Endoperoxides generated by the platelet *in vivo* may, in the presence of an inhibitor of thromboxane synthesis, be diverted to the production of anti-aggregatory prostaglandins PGD_2 and PGI_2 [14, 15]. However, the potential importance of prostaglandin endoperoxides as aggregating agents in their own right should not be underestimated.

Department of Biochemistry EDWARD J. HORNBY
Glaxo Group Research Ltd. IAN F. SKIDMORE
Ware
Hertfordshire, U.K.

REFERENCES

1. M. Hamberg, J. Svensson and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **72**, 2994 (1975).
2. J. Svensson, M. Hamberg and B. Samuelsson, *Acta physiol. scand.* **98**, 285 (1976).
3. T. Yoshimoto, S. Yamamoto and O. Hayaishi, *Prostaglandins* **16**, 529 (1978).
4. S. Heptinstall, J. Bevan, S. R. Cockbill, S. P. Hanley and M. J. Parry, *Thromb. Res.* **20**, 219 (1980).
5. P. Needleman, A. Raz, A. Ferrendelli and M. Minkes, *Proc. natn. Acad. Sci. U.S.A.* **74**, 1716 (1977).
6. G. J. Blackwell, R. J. Flower, N. Russel-Smith, J. A. Salmon, P. B. Thorogood and J. R. Vane, *Br. J. Pharmac.* **62**, 435P (1978).
7. R. A. Coleman, P. P. A. Humphrey, I. Kennedy, G. P. Levy and P. Lumley, *Br. J. Pharmac.* **68**, 127P (1980).
8. H. P. Geisow, E. J. Hornby and P. J. McCabe, *Br. J. Pharmac.* **73**, 219P (1981).
9. B. V. Caldwell, S. Burstein, W. A. Brock and L. Speroff, *J. clin. Endocr. Metab.* **33**, 171 (1971).
10. H. J. Weiss, A. L. Willis, D. Kuhn and H. Bland, *Br. J. Haemat.* **32** 257 (1976).
11. P. E. Cross, R. P. Dickinson, M. J. Parry and M. J. Randall, *Agents Actions* **11**, 274 (1981).
12. M. B. Zucker and J. Peterson, *J. Lab. clin. Med.* **76**, 65 (1970).
13. H. J. Weiss, L. M. Aledort and S. Kochwa, *J. clin. Invest.* **47**, 2169 (1968).
14. V. Bertele, C. Cerletti, A. Schiepatti, G. diMinno and G. deGaetano, *Lancet* **i**, 1057 (1981).
15. J. B. Smith, C. M. Ingerman and M. J. Silver, *Thromb. Res.* **9**, 413 (1976).
16. G. J. Blackwell, R. J. Flower, N. Russel-Smith, J. A. Salmon, P. B. Thorogood and J. R. Vane, *Br. J. Pharmac.* **62**, 436P (1978).

Biochemical Pharmacology, Vol 31, No 6, pp 1160–1163, 1982.
Printed in Great Britain.

0006-2952/82/061160-04 $03 00/0

Prednisolone-mediated alterations in ribosomal RNA turnover in rat liver

(*Received* 20 *July* 1981; *accepted* 9 *October* 1981)

Prednisolone (Δ′ hydrocortisone) has found extensive applications in diverse areas of medical therapeutics [1–4]. Unfortunately, prolonged or excessive usage of this drug results in several 'side-effects' including muscle wasting and liver enlargement, to mention but a few. Since the liver is a target organ for prednisolone [5–7] and since all phenotypic response require the generation of RNA [8, 9], it seemed appropriate, in unravelling more clues to the mechanism of action of prednisolone, to investigate the effect of this drug on ribosomal RNA turnover in this organ.