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Depression of prostaglandin synthetase activity in kidney medulla by *Shigella* endotoxin injected intravenously

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Prostaglandin synthetase activity in ocular tissues increases in experimental ocular inflammation produced by intravitreal injection of *Shigella* endotoxin [1, 2]. Similar alteration of the enzyme activity in ocular tissues was also observed during immune-complex reactions [3]. These findings are contrary to the well established facts that lipopolysaccharides inhibit various enzyme systems including those involved in carbohydrate metabolism [4, 5] and tryptophan oxygenase and tyrosine α-ketoglutarate transaminase [6, 7]. The inhibitory effect of endotoxin on enzyme activity is probably due to the cell damage caused by acidosis, reduced mitochondrial respiration and breakdown of homoestasis in response to bacteral lipopolysaccharides [8] and recently, inhibition of hepatic microsomal drug metabolising enzymes in endotoxin injected rats has been reported [9]. The purpose of the present study was to examine the effect of intravenously administered *Shigella* endotoxin on kidney microsomal prostaglandin (PG) synthetase activity.

Four groups of adult albino rabbits weighing between 2.5–3.5 kg, each group consisting of three animals, were used, one group serving as a control received saline, the second and third group were injected with 10 μg/kg and the fourth group with 50 μg/kg *Shigella* endotoxin dissolved in saline. Four and 24 hr later the animals were killed with sodium pentobarbitone (60–70 mg/kg). One kidney from each animal and iris-ciliary processes were dissected out. One gramme of kidney medulla was chopped, washed and homogenised in 8 ml of phosphate buffer pH 7.5 at 4° and microsomes were prepared as described previously [1]. Microsomal suspension equal to 250–300 μg of protein [10] were incubated in 1 ml of phosphate buffer containing 1 mM adrenaline bitartrate, 1 mM glutathione and arachidonic acid at concentrations ranging from 1.03 to 33 μM for 15 min at 37° in a shaking water bath (Unitemp, Baird and Tatlock Ltd), frequency of shakings being 172/min. At each substrate concentration microsomal fraction from each kidney medulla in duplicate was incubated. At the end of the incubation the reaction was terminated by adding absolute alcohol. Intact iris-ciliary processes were incubated in 1 ml phosphate buffer for 30 min at 37° without added substrate and cofactors. Basal levels of prostaglandin were determined in iris ciliary processes and aliquots of microsomal suspension prior to incubation. Also substrate and enzyme blanks were included in each set of samples. Prostaglandin values yielded by these blanks were subtracted from the total PGs synthesized by each sample of microsomes. Prostaglandins were extracted [11] and assayed in terms of authentic PGE_2 using rat fundus strip [12] suspended in 10 ml of Krebs solution at 37° containing antagonists [13] and gasses with 5% CO_2 in O_2. Prostaglandins were tentatively identified by thin layer chromatography [14]. As expected more than 80 per cent of PG-like substances formed were of E-type as determined by assaying zones corresponding with authentic PGs.

The results are presented graphically as the velocity of reaction as a function of the substrate concentration and also as Lineweaver–Burk plots. Data obtained with microsomes from kidneys 4 hr after 10 μg/kg *Shigella* endotoxin

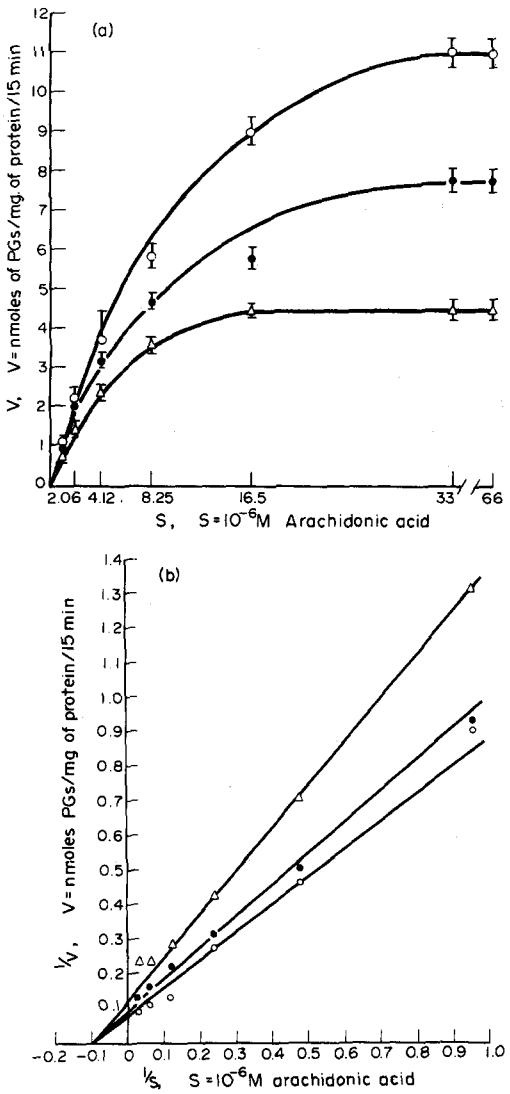

Fig. 1. The effect of intravenous injection of *Shigella* endotoxin on the kinetics of prostaglandin synthetase in kidney microsomes. (a) Velocity of reactions (ordinate) against arachidonic acid concentrations (abscissa). (b) Lineweaver–Burk plot relating arachidonic acid concentration and velocity. Abscissa, reciprocal of arachidonic acid concentration in 10^{-6} M; Ordinate, reciprocal of velocity expressed as nmoles of PGs/mg of protein/15 min. Normal microsomes O——O $K_m = 10.5 \times 10^{-6}$ M; $V_{max} = 13.0$ nmoles/mg of protein/15 min. Microsomes, 4 hr after 50 μg/Kg *Shigella* endotoxin ●——● $K_m = 10.0 \times 10^{-6}$ M; $V_{max} = 11.0$ nmoles/mg of protein/15 min. Microsomes, 24 hr after 10 μg/Kg *Shigella* endotoxin: Δ——Δ $K_m = 10.0$; $V_{max} = 8.3$ nmoles/mg of protein/15 min.

are not shown since they do not differ from those of normal microsomes. It can be seen from Fig. 1a that PG synthetase activity in kidney microsomes prepared from animals injected with endotoxin is significantly depressed, the depression being greatest at 24 hr. At an arachidonic acid concentration of 16.5 μM (5 μg/ml), PGs synthesized with microsomes 4 and 24 hr after endotoxin were 5.8 $\pm$ 0.28 and 4.52 $\pm$ 0.14 nmoles (Mean $\pm$ S.E.M.) per mg of protein per 15 min compared with 9.0 $\pm$ 0.35 nmoles (Mean $\pm$ S.E.M.) produced by normal microsomes. This inhibitory effect is more pronounced at higher substrate concentrations. For example, PGs produced by microsomes 4 hr after endotoxin at a substrate concentration of 8.25 μM was 80 per cent of the control compared with 96 per cent at the substrate concentration of 1.03 μM. Apparent K_m values of PG synthetase from normal and endotoxin injected kidneys for arachidonic acid calculated from Lineweaver–Burk plots (Fig. 1b) do not show any significant difference, but the effect of endotoxin on relative V_{max} was very marked. Prostaglandin-like substances produced (3.75 $\pm$ 0.65 μg) by iris-ciliary processes from endotoxin injected rabbits do not differ significantly from those (4.50 $\pm$ 0.75 μg) formed by normal tissues ($P > 0.2$ by Student's 't' test).

It is obvious from the results that *Shigella* endotoxin administered intravenously suppressed the PG synthetase activity in the kidney medulla. The precise mechanism by which *Shigella* endotoxin exerts an inhibitory effect on this and other enzyme systems is yet to be elucidated. The fact that endotoxin added directly to tissue homogenates does not alter the enzyme activity [15] and does not form a complex with the enzyme [16] because of its large particle size rules out the possibility of a direct effect. Inhibition of *de novo* synthesis of protein does not seem to be the cause of decrease in the enzyme activity since it has been shown [17] that cycloheximide while inhibiting protein synthesis did not affect prostaglandin synthetase activity. Probably the metabolic changes *in vivo* such as increased glycolysis [18], enhanced formation of lactic acid and subsequent acidosis [19], decreased mitochondrial respiration [20] and breakdown of homeostasis [21] following endotoxin shock cause widespread cellular damage. Inhibition of PG synthetase activity in the kidney may be the consequence of such damage.

In contrast to the above findings, we previously reported [2] that injection of *Shigella* endotoxin into the vitreous body of the rabbit eye stimulates PG synthetase activity by several folds in the iris-ciliary processes. Since both the microsomal preparation and whole tissue homogenates produced a greater amount of PGs from exogenous and endogenous substrate respectively, we concluded that in ocular inflammation the release of PGs is due to the stimulation of the enzyme and increased availability of substrates. In the present study, PG synthesis in the iris-ciliary processes obtained from rabbits injected with *Shigella* endotoxin intravenously remained unchanged. The failure of PG synthetase activity of iris-ciliary processes to decrease in parallel with that in the kidney may be due to the inadequate amount of endotoxin reaching the ocular tissues from circulation, which does not cause severe cellular damage. However, a comparison of effects of endotoxin administered intravenously and by local routes is probably not justified since intraocular administration involves injection into avascular vitreous body and probably factors operating in response to endotoxin administered by two different routes are not similar. Furthermore, metabolic responses evoked by intravenously injected endotoxin are not expected to operate following local injection and even if it does, may not be very intense.

Whatever the mechanism of inhibition of PG synthetase activity is, it seems certain that the affinity of the enzyme for the substrate has not altered as indicated by almost identical K_m values of PG synthetase systems of normal and endotoxin injected kidney medulla. Since the relative V_{max} of the endotoxin injected kidney microsomes is substantially lower than the normal values it is most likely that the reduced enzyme activity observed is due to the decreased rate of breakdown of the enzyme-substrate complex.

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REFERENCES

1. P. Bhattacherjee, *Exp. Eye Res.* **24**, 215 (1977).
2. P. Bhattacherjee and A. Phylactos, *Eur. J. Pharmac.* **44**, 75 (1977).
3. P. Bhattacherjee, A. H. Rahi and R. N. Misra, *Br. J. Ophthal.* (in press) (1977).
4. G. Weber, *Adv. Enzyme Reg.* **1**, 1 (1963).
5. G. Weber, S. K. Srivastava and R. L. Singhal, *J. biol. Chem.* **240**, 750 (1965).
6. W. E. Knox and V. H. Auerbach, *J. biol. Chem.* **214**, 307 (1955).
7. F. T. Kenney and R. W. Flora, *J. biol. Chem.* **236**, 2699 (1961).
8. L. J. Berry, in *Microbial Toxins* (Eds S. Kadis, G. Weinbaum and A. L. Ajl), Vol. 5, p. 165. Academic Press, N.Y. (1971).
9. R. Gorodischer, J. Krasner, J. J. McDivitt, J. P. Nolan and S. J. Yaffe, *Biochem. Pharmac.* **25**, 351 (1976).
10. O. H. Lowry, N. J. Roseborough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
11. W. G. Unger, I. Stamford and A. Bennett, *Nature, New Biol.* **233**, 236 (1971).
12. J. R. Vane, *Br. J. Pharmac.* **12**, 344 (1957).
13. N. Gilmore, J. R. Vane and J. H. Wyllie, *Nature, Lond.* **218**, 1135 (1968).
14. H. C. Kiefer, C. R. Johnson, K. L. Arora and H. S. Kantor, *Analyt. Biochem.* **68**, 336 (1975).
15. J. L. Berry, D. S. Smythe and I. S. Colwell, *J. Bact.* **92**, 107 (1966).
16. Y. Cho-Chung and H. C. Pitot, *J. Biochem.* **242**, 1192 (1967).
17. G. J. Blackwell, R. J. Flower, M. F. Parsons and J. R. Vane, *Br. J. Pharmac.* **53**, 467p (1975).
18. T. Fakuda, *Jap. J. Physiol.* **13**, 155 (1963).
19. M. H. Weil and B. S. Miller. *J. Lab. clin. Med.* **57**, 683 (1961).
20. W. Schumer, T. K. Dasgupta, G. S. Moss and L. M. Nyhus, *Ann. Surg.* **171**, 875 (1970).
21. T. S. Zimmerman and J. H. Muller-Eberhard, *J. exp. Med.* **134**, 1601 (1971).