REFERENCES

1. R. C. Peterson, *Cocaine: Natn. Inst. Drug Abuse Res. Monogr.* No. 13 (1977).
2. A. Groppetti and A. M. DiGiulio, in *Cocaine: Chemical, Biological, Clinical, Social and Treatment Aspects*, pp. 93–102. CRC Press, Boca Raton, FL (1974).
3. R. B. Smith, *Archs Otolar.* **98**, 139 (1973).
4. L. L. Iversen, *Biochem. Pharmac.* **23**, 1927 (1974).
5. L. L. Iversen, *Br. med. Bull.* **29**, 130 (1973).
6. L. L. Iversen, *Br. J. Pharmac. Chemother.* **25**, 18 (1965).
7. L. L. Iversen, *J. Pharm. Pharmac.* **17**, 62 (1965).
8. U. S. Von Euler and F. Lishajko, *Biochem. Pharmac.* **9**, 77 (1962).
9. I. Hanbauer, D. G. Johnson, S. D. Silberstein and I. J. Kopin, *Neuropharmacology* **11**, 857 (1972).
10. J. E. Fisher and S. H. Snyder, *J. Pharmac. exp. Ther.* **150**, 190 (1965).
11. H. D. Taube, H. Montel, G. Hau and K. Starke, *Naunyn-Schmiedeberg's Archs Pharmac.* **291**, 47 (1975).
12. R. A. Maxwell, R. M. Ferris and J. E. Burcsu, in *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines* (Ed. D. M. Paton), pp. 95–154. Raven Press, New York (1976).
13. F. J. Carmichael and Y. Israel, *J. Pharmac. exp. ther.* **186**, 253 (1973).
14. H. S. Dengler, H. E. Spiegel and E. O. Titus, *Nature, Lond.* **191**, 816 (1961).
15. S. B. Ross and A. L. Renyi, *Eur. J. Pharmac.* **2**, 181 (1967).
16. C. O. Rutledge, A. J. Azzaro and R. J. Ziance, in *Frontiers in Catecholamine Research* (Eds. E. Usdin and S. Snyder), pp. 973–6. Pergamon Press, New York (1973).
17. R. W. Holz, T. Deguchi and J. Axelrod, *J. Neurochem.* **22**, 205 (1974).
18. R. L. Clarke, S. J. Daum, A. J. Gambino, M. D. Aceto, J. Pearl, M. Levitt, W. R. Cumiskey and E. F. Bogado, *J. med. Chem.* **16**, 1260 (1973).
19. J. Glowinski and J. Axelrod, *J. Pharmac. exp. Ther.* **149**, 43 (1965).
20. E. D. Hendley, G. H. Burrows, E. S. Robinson, K. A. Heidenreich and C. A. Bulman, *Pharmac. Biochem. Behav.* **6**, 197 (1977).
21. A. J. Barr, J. H. Goodnight, J. P. Sall and J. T. Helwig, *A User's Guide to SAS* 76. SAS Institute Raleigh, NC (1976).
22. E. Costa, A. Gropetti and M. K. Naimzada, *Br. J. Pharmac.* **44**, 742 (1972).
23. J. Bralet and A. M. Lallemant, *Archs int. Pharmacodyn. Thér.* **217**, 332 (1975).
24. M. Fekete and J. Borsey, *Eur. J. Pharmac.* **16**, 171 (1971).
25. J. Scheel-Kruger, *Eur. J. Pharmac.* **18**, 63 (1972).
26. R. G. Heath and D. M. Gallant, in *The Role of Pleasure in Behavior* (Ed. R. G. Heath), pp. 83–106. Harper & Row, New York (1964).
27. L. M. Gunne and J. Jonsson, *Psychopharmacologia* **6**, 125 (1964).

Biochemical Pharmacology, Vol. 29, pp. 1863–1865.

0006-2952/80/0615–1863 $02.00/0

The influence of three prostaglandin biosynthesis stimulators on carrageenin-induced edema of rat paw

(*Received* 26 *July* 1979; *accepted* 3 *January* 1980)

Paracetamol [1] and four pyrazol–piridine derivatives [2] were found to stimulate prostaglandin biosynthesis. Phenol [3] and its iododerivative [4] possess the same property, which is supposed to be correlated with anti-inflammatory activity [3]. In contrast inhibition of prostaglandin biosynthesis has been accepted as the mode of action of aspirin-like anti-inflammatory drugs [5].

We compared the effect of 6-(beta-pyridyl)-3-hydroxy-pyrazol (3, 4b)-piridine (PPD) with that of 2-aminomethyl-4-*t*-butyl-6-iodophenol (MK-447) and paracetamol on cyclo-oxygenase and lipoxidase activity *in vitro* as well as on experimental inflammation *in vivo*.

The influence of tested compounds on cyclo-oxygenase activity was tested using solubilized enzyme from ram seminal vesicle microsomes. Microsomes were prepared according to the method of Smith [6], except that the concentration of diethyldithiocarbamate used was 5 mM. Then they were solubilized by the same medium as in the original paper [6]. The solubilized enzyme was diluted 15-fold with 0.1 M phosphate buffer, pH 7, and oxygen consumption and malondialdehyde generation was measured as previously described [2]. The results were expressed in μmoles of oxygen consumed by 1 mg of protein during 1 min. The initial reaction velocity was calculated from the slope of the line obtained on the oxygen monitor recorder. Malondialdehyde was estimated after 2 min incubation and its amount was expressed in nmoles produced during 1 min by 1 mg of protein. The protein was determined by the method of Lowry *et al.* [7].

Crystalline soybean lipoxidase was dissolved in 0.1 M phosphate buffer, pH 7, at a concentration of 5 μg/ml. Arachidonic acid (100 μM) was used as the substrate. Samples were incubated at 25°. The enzymic activity was measured as μmoles of oxygen consumed by 1 mg of enzyme during 1 min. Initial reaction velocity was calculated.

Carrageenin edema of hind paw in rats was produced by the method of Winter *et al.* [8]. Tested compounds were given s.c. 1 hr before the carrageenin injection. The increase in foot volume was expressed as a percentage of the volume before the carrageenin injection. In some experiments PPD was injected in a volume of 0.2 ml at a concentration of 5% and foot volume was measured every hour until the fourth hour. Wistar rats weighing 150–200 g were used.

The reagents used were lipoxidase from soybean (Sigma), carrageenin (Marine Colloids), paracetamol ("Polfa", Poland), diethydithiocarbamic acid (Sigma), flufenamic acid (Parke Davis), MK-447 (Merck, Sharp & Dohme). PPD was synthesized in The Department of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland.

The influence on cyclo-oxygenase activity in ram seminal

Table 1. The influence of 1000 μM of MK-447, PPD and paracetamol on oxygen consumption and malodialdehyde generation by solubilized enzyme from ram seminal vesicle microsomes*

Arachidonic acid concentration (μM)	Control (μmoles/mg/min)		Percentage of control after MK-447		PPD		Paracetamol	
	O_2	MDA	O_2	MDA	O_2	MDA	O_2	MDA
30	2.08 ± 0.16 (5)	0.025 ± 0.003 (6)	127	70	228	90	204	90
100	1.86 ± 0.15 (29)	0.025 ± 0.001 (27)	292	178	429	230	435	198
300	1.30 ± 0.29 (9)	0.022 ± 0.002 (10)	465	234	519	295	610	290

* The composition of the incubation mixture is described in the text. In the case of oxygen consumption, initial reaction velocity is calculated, and in the case of malondialdehyde, the amount of the product formed during 2 min is taken as the basis for calculation of the reaction velocity. Each percentage of control values for samples incubated with tested compounds is the mean of three experiments. Number of experiments is given in parentheses.

vesicle microsomes of the three compounds tested in the same concentration is shown in Table 1. All the compounds tested stimulated oxygen consumption, especially in higher substrate concentrations (100 and 300 μM). The stimulation of malondialdehyde generation was observed only in the concentrations 100 and 300 μM of arachidonic acid. None of the compounds tested stimulated soybean lipoxidase activity. This activity was 8.3 ± 0.52 (N = 12) μmoles of oxygen/min/mg of enzyme in control samples and 6.9 ± 0.44 (N = 3), 9.1 ± 1.6 (N = 3) and 9.2 ± 0.84 (N = 3) in the presence of 1000 μM MK-447, PPD and paracetamol, respectively. The influence of the tested compounds on carrageenin induced inflammation is shown in Table 2. Paracetamol diminished slightly carrageenin-induced inflammation three and four hours after carrageenin injection. MK-447 prevented carrageenin edema in the doses 5, 15 and 45 mg/kg over the whole range of investigation time. In contrast, PPD had no anti-inflammatory activity in the same doses and even potentiated carrageenin-induced inflammation. PPD produced foot edema on its own. With intraplant injection it caused about 20 per cent increase of foot volume at the first, second and third hours after the injection (N = 5). Saline injected in the same volume did not change the foot volume (N = 3).

The three compounds tested (MK-447, PPD and paracetamol), when used *in vitro* at a concentration of 1 mM, stimulated cyclo-oxygenase but not lipoxidase activities. The stimulation of cyclo-oxygenase was dependent on the substrate concentration. It seems that these compounds prevent inhibition of the enzymic activity by excess of substrate. Our unpublished results showed that MK-447 and paracetamol enhanced conversion of the labelled arachidonic acid mainly to PGE_2, similarly to the results found previously for a compound possessing a chemical structure very close to PPD [2]. Kuehl *et al.* [4] found that MK-447 facilitates the interconversion of PGG_2 and PGH_2. Paracetamol is probably a stimulator of the same reaction because it also possesses a phenolic group. Phenols were suggested to facilitate prostaglandin biosynthesis by removing free radicals [4]. PPD does not possess a phenolic group in its molecule, but the hydroxyl group in the pyrazol ring might have a similar physico-chemical function.

The role of prostaglandins in inflammation is controversial. Increase of PGE_2 and $PGF_{2\alpha}$ levels were found

Table 2. The influence of paracetamol, MK-447 and PPD on carrageenin-induced inflammation

	Per cent increase of foot volume after carrageenin			
	1st hr	2nd hr	3rd hr	4th hr
Control	22.4 ± 1.7 (51)	47.9 ± 3.4 (51)	62.2 ± 2.8 (51)	57.4 ± 2.7 (24)
Paracetamol				
5 mg/kg	19.7 ± 5.5 (6)	46.2 ± 3.1 (6)	45.8 ± 2.6 (6)§	39.2 ± 2.6 (6)§
15 mg/kg	16.7 ± 2.7 (6)	44.0 ± 6.0 (6)	49.6 ± 4.1 (6)†	36.7 ± 3.2 (6)§
45 mg/kg	17.4 ± 3.6 (6)	53.8 ± 6.8 (6)	49.4 ± 3.7 (6)‡	41.8 ± 4.0 (6)‡
MK-447				
5 mg/kg	11.4 ± 3.7 (6)‡	19.4 ± 4.4 (6)§	27.4 ± 2.5 (6)§	20.7 ± 2.9 (6)§
15 mg/kg	7.3 ± 2.8 (6)§	13.0 ± 3.6 (6)§	24.0 ± 4.1 (6)§	17.2 ± 3.5 (6)§
45 mg/kg	5.6 ± 1.0 (6)§	11.8 ± 1.8 (6)§	24.8 ± 2.4 (6)§	18.8 ± 1.4 (6)§
PPD				
5 mg/kg	31.4 ± 4.5 (27)	47.9 ± 4.5 (27)	61.2 ± 5.0 (27)	—
15 mg/kg	26.3 ± 4.2 (6)	51.9 ± 6.1 (6)	75.5 ± 4.5 (6)†	62.6 ± 6.3 (6)
45 mg/kg	23.5 ± 2.0 (6)	59.8 ± 3.4 (6)†	79.6 ± 4.2 (6)§	63.2 ± 3.3 (6)

* The increase of the foot volume is expressed as a percentage of the volume before carrageenin injection. The results were analysed using Student's *t*-test, and are expressed as means ± S.E.M. Number of experiments is given in parentheses.

† $P < 0.05$.

‡ $P < 0.01$.

§ $P < 0.001$.

during carrageenin-induced inflammation [9]. Therefore prostaglandin biosynthesis stimulators should potentiate carrageenin-induced edema. This conclusion is not validated by our results. Three stimulators of prostaglandin biosynthesis tested by us had various effects on carrageenin-induced inflammation. MK-447 suppressed both phases of rat paw edema. Paracetamol was a weak anti-inflammatory agent and inhibited only the second phase of inflammation which is thought to be mediated by prostaglandins [8]. The inhibition of this phase of edema by paracetamol resembles classical anti-inflammatory drugs [10], although the effect of this compound *in vitro* is opposed to those drugs which inhibit prostaglandin biosynthesis.

PPD had no anti-inflammatory activity. On the contrary, it potentiated carrageenin-induced edema and had pro-inflammatory action of its own. The discrepancy between the effects of tested compounds *in vitro* and *in vivo* may be explained in two ways:

(1) Tested compounds may stimulate prostaglandin biosynthesis only *in vitro*. It was reported that cofactors (e.g. hydroquinone) added to the incubation mixture reversed a stimulatory effect of paracetamol [1] and pyrazol–piridine derivatives [2]. It may be that *in vivo* in the presence of tissue cofactors (e.g. noradrenaline and tryptophane) MK-447 and paracetamol do not stimulate but inhibit prostaglandin biosynthesis. In fact, paracetamol was found to lower prostaglandin level during experimental inflammation *in vivo* [11].

(2) Another possibility has been suggested by Kuehl *et al.* [3]. They suggest that the real pro-inflammatory prostaglandin is PGG_2 or free radicals which are formed during prostaglandin biosynthesis. Therefore compounds which accelerate the transformation of PGG_2 into PGH_2 are anti-inflammatory. If this concept is correct, then phenolic compounds such as paracetamol and MK-447, being radical scavengers, would be expected to stimulate transformation of PGG_2 into PGH_2 and to exert an anti-inflammatory action. Assuming this concept for paracetamol and MK-447, we must reject such a mechanism of action for PPD which is pro-inflammatory.

Previously, colchicine [11] was found to be anti-inflammatory, although it stimulated prostaglandin biosynthesis *in vitro* and enhanced prostaglandin levels *in vivo*. On the basis of these observations, we conclude that neither the anti-inflammatory effect of colchicine not the pro-inflammatory effect of PPD are connected with prostaglandin biosynthesis.

Our final conclusion is that on the basis of influence of a chemical compound on prostaglandin generation *in vitro* or even *in vivo*, it is impossible to forecast its mode of action on inflammation *in vivo*.

Acknowledgements—We thank Professor P. Nantka-Namirski for supplying of PPD and Dr. Alexander Scriabine for MK-447. We also thank Mrs. J. Reyman for her skilful technical assistance.

Department of Pharmacology, N. Copernicus Academy of Medicine, Grzegórzecka 16, Cracow, Poland.

JADWIGA ROBAK
ELZBIETA KOSTKA-TRĄBKA
ZOFIA DUNIEC

REFERENCES

1. J. Robak and A. Więckowski, *Biochem. Pharmac.* **27**, 351 (1978).
2. H. Kasperczyk and J. Robak, *Biochem. Pharmac.* **27**, 2157 (1978).
3. R. W. Egan, J. Paxton and F. A. Kuehl, Jr., *J. biol. Chem.* **251**, 7329 (1976).
4. F. A. Kuehl, Jr., J. H. Humes, R. W. Egan, E. A. Ham, G. C. Beveridge and C. G. von Arman, *Nature, Lond.* **265**, 170 (1977).
5. J. R. Vane, *Nature, New Biol.* **231**, 232 (1971).
6. W. L. Smith and G. P. Wilkin, *Prostaglandins* **13**, 873 (1977).
7. O. H. Lowry, N. J. Rosebrough, A. D. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
8. C. A. Winter, E. A. Risley and G. W. Nuss, *Proc. soc. exp. Biol. Med.* **111**, 544 (1962).
9. A. L. Willis, in *Prostaglandins, Peptides and Amines.* (Eds P. Mantegazza and E. W. Horton), p. 31. Academic Press, London (1969).
10. R. Vinegar, W. Schreiber and R. Hugo, *J. Pharmac. exp. Ther.* **166**, 96 (1969).
11. G. A. Higgs, E. A. Harvey, S. H. Ferreira and J. R. Vane, in *Advances in Prostaglandin and Thromboxane Research* (Eds. B. Samuelsson and R. Paoletti), Vol. 1, p. 105. Raven Press, New York (1976).