J. Pharm. Pharmac., 1976, **28**, 683–686 *Received April* 21, 1976

Influence of various substances on prostaglandin biosynthesis by guinea-pig chopped lung*

B. FJALLAND

The Department of Pharmacology and Toxicology, H. Lundbeck & Co. A/S, Ottiliavej 7–9, DK-2500 Copenhagen-Valby, Denmark

Guinea-pig chopped lung tissue was used to investigate the inhibitory effect of various steroidal and non-steroidal anti-inflammatory agents, antipyretics, analgesics, local anaesthetics and psychotropic drugs on mechanically induced release of prostaglandin-like material. Low concentrations of non-steroidal anti-inflammatory agents inhibited synthesis, but other antipyretics and analgesics, and the local anaesthetics had little effect. Thymoleptics, neuroleptics and monoamine oxidase inhibitors except phenelzine exhibited weak activity. It is concluded that the method is a useful pharmacological model to study prostaglandin biosynthesis. The weak effects of the psychotropic drugs suggest that they do not exert their clinical effect by inhibiting PG biosynthesis.

Since the proposal that some therapeutic effects of aspirin-like drugs are due to inhibition of the synthesis of prostaglandins (PGs) (Vane, 1971), various substances have been studied on PG-synthetase. Drugs from different pharmacological groups including steroidal and non-steroidal anti-inflammatory agents, antipyretics, local anaesthetics and different psychotropic drugs, are described as inhibitors of the PG-synthetase in different tissues (Greaves & McDonald-Gibson, 1972; Flower & Vane, 1974; Ham, Cirillo & others, 1972; Kunze, Bohn & Vogt, 1974; Kunze, Bohn & Bahrke, 1975; Lee, 1974; Krupp & Wesp, 1975). However, other investigations have not been able to show any effect of e.g. steroids and antipyretics (Flower, Gryglewski & others, 1972; Flower, Cheung & Cushman, 1973). From the relative potency between various non-steroidal anti-inflammatory agents (NAIAs), Flower & Vane (1974) concluded that there are multiple forms of PG-synthetase within an organism, each having its own drug specificity. This might explain some of the contradictory results published, using different enzyme systems. I have therefore evaluated the specificity of a simple *in vitro* model to assess inhibition of PG synthesis by groups of pharmacologically different substances.

MATERIALS AND METHODS

The method described by Fjalland (1974) was used. Chopped lung tissue from unsensitized guinea-pigs (Palmer, Piper & Vane, 1973) was superfused at 5 ml min^{-1} with Krebs (NaCl 118, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 0·57, KH_2PO_4 1·2, $NaHCO_3$ 25, Glucose 10·1 mM) solution containing atropine sulphate 100 ng ml^{-1}, mepyramine maleate 100 ng ml^{-1}, methysergid bimaleate 200 ng ml^{-1}, phenoxybenzamine hydrochloride 100 ng ml^{-1} and propranolol hydrochloride 2 μg ml^{-1} (Gilmore, Vane & Willie, 1968). It was manually agitated for 45 s with a blunt rod to release a PG-like material which was assayed biologically by superfusing rat stomach strips (Vane, 1957). Test drugs were incubated for 40 min and the inhibition of a PG release was calculated as the percentage of the pre-drug release. The concentration producing 50% inhibition (IC50) was determined by probit analysis (Finney, 1952) based on 3–5 concentrations of each substance, and 3–12 estimations at each concentration.

RESULTS

The ability of some standard NAIAs to inhibit the release of PG-like material is shown in Table 1. Indomethacin was most potent, 50% reduction of spasmogenic activity in the effluent from the lung tissue was observed with 10^{-7} M. Potency decreased in the order flufenamic acid, naproxen, ibuprofen, phenylbutazone, oxyphenbutazone, acetylsalicylic acid and sodium salicylate.

The effects of various non-acidic anti-inflammatory, antipyretic, analgesic and local anaesthetic agents are given in Table 2. Flazalone, benzydamine and paracetamol inhibited PG-release only in high concentrations, whereas penicillamine, the immunosuppressant cyclophosphamide, the gold compound sodium aurothiosulphate, hydrocortisone and the other drugs tested (anti-

* Part of this work was presented at the VI International Pharmacology Congress, Helsinki, July 1975.

Table 1. *Inhibition by non-steroidal anti-inflammatory agents of mechanically induced prostaglandin biosynthesis.*

Substance	IC50 (μM)*
Indomethacin	0·10 (0·03–0·33)
Flufenamic acid	0·17 (0·04–0·80)
Naproxen	0·27 (0·02–4·3)
Ibuprofen	0·62 (0·15–2·5)
Phenylbutazone	2·8 (0·65–12·2)
Acetylsalicylic acid	11 (2·4–47)
Oxyphenbutazone	19 (3·9–90)
Sodium salicylate	595 (113–3112)

* 95% confidence limits in brackets. n = 4–12.

pyretics, centrally active analgesics, local anaesthetics) seemed inactive. With benzydamine (100–200 μM) a release of PG-like material was registered. This was not observed with other substances.

All the thymoleptics tested (Table 3) inhibited synthesis of PG-like material but were about 250–750 times less potent than indomethacin. The benzodiazepines and the neuroleptics were also weakly active. Tetrabenazine was practically inactive, whereas reserpine seemed relatively potent. Among the monoamine oxidase inhibitors tested tranylcypromine was inactive and iproniazide very weak. Nialamide was as active as the neuroleptics and thymoleptics, whereas phenelzine which possess a free hydrazine group was much more potent.

DISCUSSION

The present experiments have confirmed the finding in earlier investigations (Ham & others, 1972; Flower, 1974; Ferreira & Vane, 1974) that the NAIAs are potent inhibitors of PG biosynthesis. The data for indomethacin, flufenamic acid, naproxen, ibuprofen, phenylbutazone and acetylsalicylic acid correlate significantly with data by Takeguchi & Sih (1972), Flower & others (1973) and Ham & others (1972) (Table 4). This indicates that the present model is an appropriate pharmacological model to study drugs which inhibit PG-biosynthesis, and seems at least as sensitive to the NAIAs as are different enzyme system models (Flower, 1974; Flower & Vane, 1974; Ferreira & Vane, 1974). The mechanism of PG release by the mechanical agitation of chopped lung tissue is not clear. Piper & Vane (1971) suggested that mechanical, physiological and pathological stimuli may disturb the cell membrane, leading to generation and release of PGs.

The lack of effect in the present model of the local anaesthetics and the weak activity of most of the psychotropic drugs tested are more or less in contrast to results described by Kunze & others (1974) and Lee (1974) using bovine seminal vesicle and cell-free homogenates of guinea-pig lung as sources of enzymes, respectively. Perhaps this is explained by the finding (Flower & Vane, 1974) that PG-synthetases in different tissues differ in their responses to inhibitory drugs. The present data do not support the suggestion (Krupp & Wesp, 1975) that inhibition of PG-synthesis is important in the action of antidepressant drugs. If this were so one might expect a

Table 2. *Inhibition by various anti-inflammatory-, antipyretic, analgesic and local anaesthetic agents of mechanically induced prostaglandin biosynthesis.*

Substance	IC50 (μM)*
Flazalone	31 (7·1–137)
Benzydamine	65 (36–120)
Penicillamine	>860
Cyclophosphamide	>100
Sodium aurothiosulphate	>500
Hydrocortisone	>100
Antipyrine	>500
Paracetamol	207 (32–1356)
Morphine	>500
Ketobemidone	> 56
Dextropropoxyphene	>100
Tetracaine	>100
Cocaine	>190
Procaine	>230

* 95% confidence limits in brackets. n = 4.

Table 3. *Inhibition by various psychotropic drugs of mechanically induced prostaglandin biosynthesis.*

Substance	IC50 (μM)*
Imipramine	35 (4 – 319)
Desipramine	76 (21 – 275)
Chlorimipramine	51 (15 – 173)
Amitriptyline	41 (14 – 124)
Nortriptyline	58 (7 – 476)
Protriptyline	27 (6 – 128)
Benzoctamine	66 (14 – 312)
Chlordiazepoxide	133 (49 – 363)
Diazepam	59 (28 – 122)
Chlorpromazine	64 (24 – 176)
Chlorprothixene	37 (12 – 177)
Flupenthixol	10 (2 – 48)
Clozapine	27 (8 – 89)
Haloperidol	103 (7 –1557)
Tranylcypromine	>500
Nialamide	38 (6 – 624)
Iproniazid	376 (61 –2300)
Phenelzine	2·4 (0·5– 12)
Tetrabenazine	~500
Reserpine	17 (0·2–1646)

* 95% confidence limits in brackets. n = 3–6.

Table 4. *IC50 values (μM) of some standard non-steroidal anti-inflammatory agents against PG-synthetase compared to the effect obtained in the chopped lung tissue model.*

Synthetase used	Indo-methacin	Flufenamic acid	Ibuprofen	Phenyl-butazone	Acetyl-salicylic (acid)	Naproxen	References
Dog spleen	0·17	0·64		7·25	37·0		Flower & others, 1972
BSV	2	48	1200	420	820	220	Takeguchi & Sih, 1972
BSV	40		2000	1400	9000	370	Flower & others, 1973
SSV	0·45	2·5	1·5	12·3	83·4	6·1	Ham & others, 1972
Chopped lung tissue	0·10	0·17	0·62	2·8	11	0·27	

BSV: Bovine seminal vesicle. SSV: Sheep seminal vesicle.
Kendall coefficient of concordance: $P < 0.05$.

correlation inhibition of PG-synthesis and inhibition of biogenic amine uptake. This is not so for the six antidepressants examined in this study (Carlsson, Corrodi & others, 1969; Maxwell, Eckhardt & others, 1971). The marked effect of monoamine oxidase inhibitors in comparison with indomethacin observed by Lee (1974), was not obtained in this study. However phenelzine was as potent as phenylbutazone. Perhaps this is because of the free hydrazine group (phenelzine analogues, containing free hydrazine groups, were also potent, unpublished).

The absence of effect of hydrocortisone in the present study is in accordance with data of Flower & others (1972), although Greaves & McDonald-Gibson (1972) found the drug active in rat skin. Recently Lewis & Piper (1975) suggested, that the effect of hydrocortisone on fat cells was due to inhibition of PG release but not synthesis.

Stimulation of PG biosynthesis has been described for benzydamine, apomorphine, morphine and other substances (Flower, 1974; Collier, McDonald-Gibson & Saeed, 1974; 1975). With chopped lung release of PG-like activity was noticed with benzydamine 100–200 μM but not with apomorphine or morphine. None of the substances mentioned potentiated the release of PG-like substances when the period of mechanical agitation was reduced from 45 to 15–30 s. However, it should be mentioned that the present model is not suitable for estimating PG-stimulating properties, as a maximal substrate stimulation is necessary to obtain reproducible results (unpublished).

From the literature and the results obtained in the present study, it seems that guinea-pig chopped lung is an appropriate pharmacological model for studying PG-biosynthesis. It seems relatively selective and sensitive for the NAIAs, but it must be borne in mind that drugs which affect PG degradation could also influence the results.

REFERENCES

Carlsson, A., Corrodi, H., Fuxe, K. & Hökfelt, T. (1969). *Eur. J. Pharmac.*, **5**, 357–366.

Collier, H. O. J., McDonald-Gibson, W. J. & Saeed, S. A. (1974). *Nature*, **252**, 56–58.

Collier, H. O. J., McDonald-Gibson, W. J. & Saeed, S. A. (1975). *Lancet*, 702.

Ferreira, S. H. & Vane, J. R. (1974). *Ann. Rev. Pharmac.*, **14**, 57–73.

Finney, D. J. (1952). *Probit analysis*. 2nd edn, pp. 20–64. Cambridge: Cambridge University Press.

Fjalland, B. (1974). *J. Pharm. Pharmac.*, **26**, 448–451.

Flower, R. J. (1974). *Pharmac. Rev.*, **26**, 33–67.

Flower, R. J., Cheung, H. S. & Cushman, D. W. (1973). *Prostaglandins*, **4**, 325–341.

Flower, R. J., Gryglewski, R., Herbacsynska-Cedro, K. & Vane, J. R. (1972). *Nature New. Biol.*, **238**, 104–106.

Flower, R. J. & Vane, J. R. (1974). *Biochem. Pharmac.*, **23**, 1439–1450.

Gilmore, N., Vane, J. R. & Willie, J. H. (1968). *Nature*, **218**, 1135–1140.

Greaves, M. N. & McDonald-Gibson, W. (1972). *Br. med. J.*, **2**, 83–84.

HAM, E. A., CIRILLO, W. J., ZANETTI, M., SHEN, T. Y. & KUEHL, F. A. JR. (1972). In: *Prostaglandins in Cellular Biology*, pp. 345–352. Editors: Ramwell, P. W. & Pharris, B. B.

KRUPP, P. & WESP, M. (1975). *Experientia*, **31**, 330–331.

KUNZE, H., BOHN, E. & BAHRKE, G. (1975). *J. Pharm. Pharmac.*, **27**, 880–881.

KUNZE, H., BOHN, E. & VOGT, W. (1974). *Naunyn-Schmiedebergs Arch. Pharmac.*, **285**, Suppl. R 49.

LEE, R. E. (1974). *Prostaglandins*, **5**, 63–72.

LEWIS, G. P. & PIPER, P. J. (1975). *Nature New. Biol.*, **254**, 308–311.

MAXWELL, R. A., ECKHARDT, S. B., CHAPLIN, E. & BURCSU, J. (1971). In: *Physiology and Pharmacology of Vascular Neuroeffector Systems*, pp. 98–110. Editors: Bevan, J. A., Furchgott, R. F., Maxwell, R. A. & Somlego, A. P. Basel: Karger.

PALMER, M. A., PIPER, P. J. & VANE, J. R. (1973). *Br. J. Pharmac.*, **49**, 226–242.

PIPER, P. & VANE, J. (1971). *Ann. N.Y. Acad. Sci.*, **180**, 363–385.

TAKEGUCHI, C. & SIH, C. J. (1972). *Prostaglandins*, **2**, 169–184.

VANE, J. R. (1957). *Br. J. Pharmac.*, **12**, 344–349.

VANE, J. R. (1971). *Nature New Biol.*, **231**, 232–235.