Anti-allergy properties of PRD-92-Ea [5,5-Dimethyl-11-oxo 5H, 11H-(2) benzopyrano (4,3-g) (1) benzopyran-9-carboxylic acid ethanolamine]

J.L. BEETS, H. MEADE & J. MORLEY

Department of Clinical Pharmacology, Cardiothoracic Institute & Brompton Hospital, Fulham Road, London SW3 6HP

PRD-92-Ea inhibits the PCA reaction in the rat when given by oral or intravenous routes (Stewart, Devlin & Freter, 1974). However, PRD-92-Ea has also been reported to antagonize *in vitro* 5-HT, bradykinin, SRS-A and histamine (Possanza, Bauen & Stewart, 1975). Therefore, the anti-allergy profile of this drug has been further investigated in the guinea-pig and rat using an isotopic method for measuring increased vascular permeability (Morley, Wolstencroft & Dumonde, 1973).

Guinea-pigs and rats received an intravenous injection of 1–2 μCi of [^{125}I]-HSA in 2% Evans Blue Dye. Inflammatory responses were elicited at 12 sites of flank skin by local injection of irritant (eg 48/80) or antigen (egg albumin in the rat and BGG in the guinea-pig) into passively sensitized sites. Drugs were given intravenously, orally or locally. The use of local administration of both antigen and drug permits dose response curves to anti-allergy drugs to be adequately defined with small numbers of animals.

In the guinea-pig PRD-92-Ea caused little inhibition of IgG mediated PCA reactions whereas in the rat reaginic PCA reactions were strongly inhibited by local ($ED_{50} = 0.35$ μg/site) or systemic ($ED_{50} = 0.5$ mg/kg) administration. However, responses to 48/80 (1 μg/site) and peptide 401 (1 μg/site) were partially antagonized (up to 50%) in both species by local injection of PRD-92-Ea. Inflammatory responses in the guinea-pig to local administration of histamine (1 μg/site), 5-HT (60 μg/site) or bradykinin (0.5 μg/site) were not inhibited by local PRD-92-Ea up to a concentration of 2 mg/site. Similarly in the rat, comparable responses to these mediators were not antagonized by local injection of PRD-92-Ea up to 0.5 mg/site. Systemic injection of PRD-92-Ea (75 mg/kg) into guinea-pigs was found to reduce PCA, Arthus, 48/80, histamine and bradykinin reactions but such concentrations greatly exceeded the systemic doses which effectively inhibit rat PCA responses; $ED_{50} = 0.5$ mg/kg (El-Azab & Stewart, 1977).

These properties of PRD-92-Ea are consistent with this drug acting *in vivo* specifically as an anti-allergy agent rather than as an antagonist of putative mediator responses.

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References

EL-AZAB, J. & STEWART, P.B. (1977). Pharmacological profile of a new anti-allergic compound PRD-92-Ea. *Int. Arch. Allergy Appl. Immunol.* (In press).

MORLEY, J., WOLSTENCROFT, R.A. & DUMONDE, D.C. (1973). The Measurement of Lymphokines. From: *Handbook of Experimental Immunology.*, ed. Weir, D.M. 2nd edition. pp. 281.1–281.6. Oxford: Blackwells.

POSSANZA, G.J., BAUEN, A. & STEWART, P.B. (1975). *In vitro* antagonism of the mediators of allergy by a benzopyrano-benzopyran carboxylic acid PRD-92-Ea. *Int. Arch. Allergy Appl. Immunol.*, **49**, 789.

STEWART, P.B., DEVLIN, J.P. & FRETER, K.R. (1974). Anti-allergic activity of a benzopyran PRD-92-Ea. *Fed. Proc. Fed. Amer. Soc. exp. Biol.*, **33**, No. 3, Part I.

Effects of D-penicillamine and levamisole on delayed hypersensitivity reactions in the rat and mouse

F.M. CUNNINGHAM, A.W. FORD-HUTCHINSON, M.J.H. SMITH & J.R. WALKER

Biochemical Pharmacology Research Unit, Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London, SE5 8RX

D-penicillamine and levamisole are antirheumatic drugs with a late onset of action and differ from conventional non-steroidal anti-inflammatory agents in showing no anti-inflammatory effects in animal models (Huskisson, Dieppe, Scott, Trapnell, Balme & Willoughby, 1976). Their mode of action in rheumatoid arthritis is unknown but levamisole has been reported to stimulate both humoral and cell mediated immunity (Renoux & Renoux, 1972; Tripodi, Parkes & Brugmans, 1973). It has been suggested that D-penicillamine may possess similar immunostimulatory properties (Dieppe, Willoughby, Huskisson & Arrigoni-Martelli, 1976).

The effects of daily oral administration of 25 mg/kg D-penicillamine and 5 mg/kg levamisole upon delayed hypersensitivity reactions to *Bacillus pertussis* in the rat, and sheep red blood cells in the mouse were examined. Rats were implanted with sponges soaked in *B. pertussis* (Ford-Hutchinson, Smith, Elliott, Bolam, Walker, Lobo, Badcock, Colledge & Billimoria, 1975) having been sensitized twelve days

previously with *B. pertussis* and incomplete Freund's adjuvant according to the directions of Dieppe *et al.*, (1976). Mice were sensitized by injection into the left hind paw and challenged four days later by injection into the right hind paw with sheep red blood cells (L'Age-Stehr & Diamastein, 1977). The drugs were administered 1 h prior to and 24 h, 48 h, 72 h and 96 h after sensitization and the reaction was monitored by measuring paw thickness after challenge.

Neither drug showed the effect on leucocyte migration observed with conventional anti-inflammatory drugs in non-sensitized rats (Walker, Smith & Ford-Hutchinson, 1976) confirming the absence of anti-inflammatory activity. When the delayed hypersensitivity reaction was superimposed on the sponge reaction significant enhancement of leucocyte migration was observed with both drugs. Similar effects occurred in the mouse, paw thicknesses being significantly increased by levamisole at 24 h and 48 h and by D-penicillamine at 48 h (% increase in paw thickness at 48 h: control 9.0 $\pm$ 3.9 $n = 8$, D-penicillamine 25.6 $\pm$ 7.2 $n = 6$ and levamisole 30.5 $\pm$ 4.5 $n = 5$). Levamisole and D-penicillamine thus enhance delayed hypersensitivity at dosages similar to those used in man. Whether this action in the animal models relates to their common action in rheumatoid arthritis remains to be determined.

References

DIEPPE, P.A., WILLOUGHBY, D.A., HUSKISSON, E.C. & ARRIGONI-MARTELLI, E. (1976). Pertussis vaccine pleurisy: a model of delayed hypersensitivity. *Agents and Actions*, **6**, 618–621.

FORD-HUTCHINSON, A.W., SMITH, M.J.H., ELLIOTT, P.N.C., BOLAM, J.P., WALKER, J.R., LOBO, A.A., BADCOCK, J.K., COLLEDGE, A.J. & BILLIMORIA, F.J. (1975). Effects of a human plasma fraction on leucocyte emigration into inflammatory exudates. *J. Pharm. Pharmacol.*, **27**, 106–112.

HUSKISSON, E.C., DIEPPE, P.A., SCOTT, J., TRAPNELL, J., BALME, M.W. & WILLOUGHBY, D.A. (1976). Immunostimulant therapy with levamisole for rheumatoid arthritis. *Lancet*, **i**, 393–395.

L'AGE-STEHR, J. & DIAMANSTEIN, T. (1977). Suppression and potentation of delayed type hypersensitivity by dextran sulphate. *Immunology*, **33**, 179–183.

RENOUX, G. & RENOUX, M. (1972). Levamisole inhibits and cures a solid malignant tumour and its pulmonary metastases in mice. *Nature New Biology*, **240**, 217–218.

TRIPODI, D., PARKES, L.C. & BRUGMANS, J. (1973). Drug-induced restoration of cutaneous delayed hypersensitivity in anergic patients with cancer. *New Eng. J. Med.*, **289**, 344—347.

WALKER, J.R., SMITH, M.J.H. & FORD-HUTCHINSON, A.W. (1976). Anti-inflammatory drugs, prostaglandins and leucocyte migration. *Agents and Actions*, **6**, 602–606.

Neuropharmacological studies on the central inhibition of oxytocin release

G. CLARKE, D.W. LINCOLN & P.v.d. SCHOOT

Department of Anatomy, The Medical School, University of Bristol, University Walk, Bristol BS8 1TD; and Department of Endocrinology, Growth & Reproduction, Erasmus University, Rotterdam, The Netherlands

The suckling-induced release of oxytocin may be blocked by emotional or stressful stimuli. Such a failure to release oxytocin, and thus eject the milk from the mammary alveoli, has been atrributed to a central inhibition of the reflex (Cross, 1955), and such inhibition possibly involves the cerebral cortex (Taleisnik & Deis, 1964). Moreover, we have recently observed a prominent correlation between the activity of the cerebral cortex and the release of oxytocin in anaesthetized lactating rats.

The electrocorticogram (ECoG) was recorded from bipolar platinum electrodes placed over the frontal lobes of lactating rats, anaesthetized with urethane (1.2 g/kg, i.p.). Typically, the ECoG alternated every few minutes between periods of large amplitude slow-waves and periods of low amplitude fast waves (arousal) (Lincoln, 1969). Within 1–2 min of the young being placed on the nipples, the ECoG changed and a continuous slow-wave pattern persisted for the next hour, or more. Thereafter, the 'cyclic-pattern' of ECoG activity returned. The continuous suckling activity of the young induced a series of reflex milk-ejections characterized by uniform increases in intramammary pressure at intervals of 5–15 min (Lincoln, Hill & Wakerley, 1973). Such milk ejections occurred only during periods of slow-wave ECoG activity and, when the ECoG was of the cyclical pattern, milk ejections were frequently observed 10–15 s after the transition from the 'aroused' to the 'sleep-like' state.

Suppression of cortical activity by the application of KCl to the exposed surface failed to modify the pattern of reflex milk ejection; the mean milk-ejection interval was 10.8 $\pm$ 3.6 (mean $\pm$ s.e. mean) min for controls ($n = 64$) and 10.0 $\pm$ 1.6 min during KCl depression ($N = 64$), and the amount of oxytocin released was similar in both situations. The injection of carbachol (0.1–0.2 μg in 1 μl) or bethanechol (1–2 μg) into the lateral ventricles caused an arousal of the ECoG and a parallel inhibition of the milk ejection reflex. Conversely, the systemic application of atropine (1 mg/kg, i.v.) prevented both the induced arousal and the associated inhibition of the reflex.