detected at the time of weaning. Both chemical estimation[6] and isotopic experiments revealed the same trend. It may be mentioned that the concentration of CPIB in the serum and liver of the offspring was only about a tenth of that found in the adult animal[6].

The above results show that the compound crosses the placental barrier and enters foetal circulation. Also, the drug may be transferred to the pup by way of mother's milk. In agreement with this, placenta collected before birth from clofibrate-fed mothers was found to contain about 80 nmoles of CPIB per g fresh weight. Milk collected from mothers fed with radioactive CPIB showed substantial amounts of radioactivity (data not given).

The passage of CPIB into the young ones resulted in increases in liver size and glycerolphosphate dehydrogenase activity at birth and during lactation, but did not lead to proliferation of hepatic mitochondria. We have also observed that hepatic catalase activity was higher in the newborn experimental pups, but the increase was not maintained during the suckling phase (data not given).

The reasons for the differential effects are not at once obvious. It is possible that adequate quantities of CPIB are not reaching the offsprings. It has been shown[20] that body weight and age of the animal influence the effect of the drug. Weanling rats do not readily respond to the action of the drug[21]. Even in the adult animal, all the lobes of the liver are not affected equally by the drug[22]. Another possibility is that the animal may not be in a state of 'competance' to respond to the stimulus. The secretion of throxine which has been implanted to mediate in the action of clofibrate[23] begins only at the late fetal stage[5] and this could also limit the response. Administration of the hormone to the mature foetus directly or to the pregnant mother increases foetal liver glycerolphosphate dehydrogenase activity[5]. The rapid decrease in the enzyme activity on weaning is consistent with our earlier observation[8] that where administration of the drug is discontinued the effects are reversed and that the compound practically disappears from the serum in 24 hr[6]. It is also consistent with the view that CPIB passes to the offspring *via* the mother's milk.

Acknowledgements—We wish to thank Dr. J. M. Thorp, Imperial Chemical Industries Ltd., U.K., for generously providing gift samples of Clofibrate and [^{14}C] CPIB used in these experiments.

SUVIRA CHHABRA
C. K. RAMAKRISHNA KURUP
Department of Biochemistry
Indian Institute of Science
Bangalore-5600 12, India

REFERENCES

1. M. M. Best and C. H. Duncan, *J. Lab clin. Med.* **64**, 634 (1964).
2. C. K. R. Kurup, H. N. Aithal and T. Ramasarma, *Biochem. J.* **116**, 773 (1970).
3. T. P. Krishnakantha and C. K. R. Kurup, *Biochem. J.* **130**, 167 (1972).
4. R. Hess, W. Staubli and W. Riess, *Nature, Lond.* **208**, 856 (1965).
5. O. Greengard, in *Essays in Biochemistry*, (Eds. P. N. Campbell and F. Dickens), Vol. 7, pp. 159–205, Academic Press, London, (1971).
6. C. K. R. Kurup and S. Chhabra, *Indian J. Biochem. Biophys.* **14**, 267 (1977).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
8. T. P. Krishnakantha and C. K. R. Kurup, *Environ. Physiol. Biochem.* **4**, 80(1974).
9. M. McKellar, *Am. J. Anat.* **85**, 263 (1949).
10. T. Gustafson, *Int. Rev. Cytol.* **3**, 277 (1954).
11. J. A. Ontko, *Life Sci.* **5**, 817 (1966).
12. A. Herzfeld, M. Federman and O. Greengard, *J. Cell. Biol.* **57**, 475 (1973).
13. A. R. L. Gear, A. D. Albert and J. M. Bednarek, *J. biol. Chem.* **249**, 6495 (1974).
14. M. Subramanian, S. S. Katyare, P. Fatterkaper and A. Sreenivasan, *Indian J. Biochem. Biophys.* **12**, 307 (1975).
15. R. G. Vernon and D. G. Walker, *Biochem. J.* **118**, 531 (1970).
16. M. A. De Vos, C. W. Wilmink and F. A. Hommes, *Biol. Neonatorum* **13**, 83 (1968).
17. P. Hemon. *Biochim. Biophys. Acta.* **132**, 175 (1967).
18. M. T. Kahonen, H. Ylikahari and I. Hassinen. *Life Sci.* **10**, 661 (1971).
19. J. M. Thorp, *Lancet*, **1** 1323 (1962).
20. D. S. Platt and J. M. Thorp, *Biochem. Pharamac.* **15**, 915 (1966).
21. W. W. Westerfeld, D. A. Richert and W. R. Ruegamer, *Biochem. Pharmac.* **17**, 1003 (1968).
22. T. P. Krishnakantha and C. K. R. Kurup. *Environ. Physiol. Biochem.* **4**, 243 (1974).
23. J. M. Thorp, *J. Atheroscler. Res.* **3**, 737 (1963).

Biochemical Pharmacology, Vol. 27, pp. 2065–2067.

0006-2952/78/0815-2065$02.00/0

Inhibition of cyclic nucleotide phosphodiesterase by FPL 55712, an SRS-A antagonist

(*Received* 24 *October* 1977; *accepted* 27 *January* 1978)

It has been suggested that the antiallergic drug disodium cromoglycate prevents allergic asthmatic attacks by inhibiting the release of chemical mediators of immediate allergic reactions through the inhibition of cyclic AMP phosphodiesterase[1] and the subsequent increase in intracellular cyclic AMP levels. In addition, the potential antiallergic compounds doxantrazole [3-(5-tetrazolyl)-thioxanthone 10,10-dioxide] and CTD (3-carboxythioxanthone 10,10-dioxide) inhibit the phosphodiesterase of human and guinea pig lung and beef heart, suggesting that their antiallergic activity may be related to their ability to elevate intracellular cyclic AMP levels by inhibiting phosphodiesterase[2, 3].

The present study explores the inhibition of cyclic

nucleotide phosphodiesterase by FPL 55712, 7 - (3 - hydroxy - 2 - propylphenoxy [2 - hydroxypropoxyl] - 4 - oxo - 8 - propyl - 4H - 1 - benzopyran - 2 - carboxylate), a member of the class of chromone-2 carboxylic acids. FPL 55712 somewhat antagonizes the activity of ECF-A (eosinophil chemotactic factor anaphylaxis) on the induced chemotaxis of eosinophils and has been reported to be a potent and specific inhibitor of SRS-A (slow reacting substance of anaphylaxis) activity on the guinea pig ileum[4, 5], suggesting that FPL 55712 may be a useful agent for studying the effects of the SRS-A released by antigen in the lung during an asthmatic attack, as well as potentially providing therapeutic value in the treatment of asthma.

The FPL 55712 used in these studies was generously provided by P. Sheard of Fisons, Ltd., Bakewell Rd., Loughborough, Leicestershire, LE 11 0QY, England, and SQ 20,009 by S. M. Hess of Squibb, Princeton, NJ. Cyclic AMP and cyclic GMP phosphodiesterase assays were conducted following a modification of the radio-displacement assay of Brooker *et al.*[6], as described by Chasin and Harris[7], utilizing enzyme isolated from rat brain and a substrate concentration of 0.16 μM. The results of both the cyclic AMP and cyclic GMP phosphodiesterase assays for FPL 55712 and the known phosphodiesterase inhibitors, SQ 20,009[8–10], papaverine[11] and theophylline[12], are shown in Table 1. As can be seen, FPL 55712 was as potent as papaverine and SQ 20,009, and forty times more potent than theophylline in inhibiting cyclic AMP phosphodiesterase activity. It was also three, four and twenty-five times more potent than SQ 20,009, papaverine and theophylline, respectively, in inhibiting cyclic GMP phosphodiesterase. In fact, this is the most potent cyclic GMP phosphodiesterase inhibitor yet reported.

To determine the mechanism and kinetic constants of the inhibition of phosphodiesterase by FPL 55712, the experiments depicted by the double reciprocal plots in Figs. 1 and 2 were conducted for cyclic AMP and cyclic GMP respectively. Experimental points and computer-calculated linear regression "best-fit" lines are shown. FPL 55712 was a noncompetitive inhibitor of the hydrolysis of both cyclic nucleotides, with a K_i of 2.8 ± 0.1 μM (mean ± S.E. of five determinations) for cyclic AMP hydrolysis, and a K_i of 11.7 ± 0.8 μM for cyclic GMP hydrolysis. The noncompetitive nature of the inhibition was confirmed by the method of Eisenthal and Cornish-Bowden[13].

Reversibility of the inhibition by FPL 55712 was demonstrated by incubation of a concentrated enzyme solution with 0.05 M Tris buffer containing 100 μM FPL 55712. After incubation for 30 min at room temperature and dialysis overnight to remove residual drug, the enzyme solutions were diluted and assayed; total enzyme activities for both untreated enzyme and enzyme incubated with FPL 55712 were identical.

A second demonstration of the reversibility of this inhibition was accomplished by incubation of a concentrated enzyme solution with high concentrations of FPL 55712. Dilution of this mixture prior to assay resulted in no greater inhibition than was produced by the final concentration of FPL 55712. Finally, preincubation of phosphodiesterase with FPL 55712 for periods up to 1 hr resulted in no increase in the observed inhibition.

Table 1. Inhibition of cyclic AMP and cyclic GMP phosphodiesterase activities by SQ 20,009, papaperine, theophylline and FPL 55712

Compound	Phosphodiesterase inhibition I_{50} (μM) Cyclic AMP	Cyclic GMP
SQ 20,009	2.5	38
Papaverine	1.3	56
Theophylline	130	310
FPL 55712	3.0	13

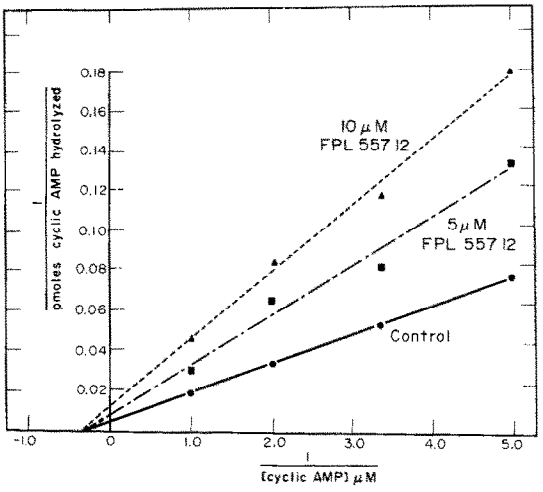

Fig. 1. Double reciprocal plot of cyclic AMP hydrolysis and the effect of 5 and 10 μM FPL 55712. The points are experimentally determined enzymatic rates, and the lines are computer generated linear regression "best-fit" lines ($r = 0.99$ for all lines).

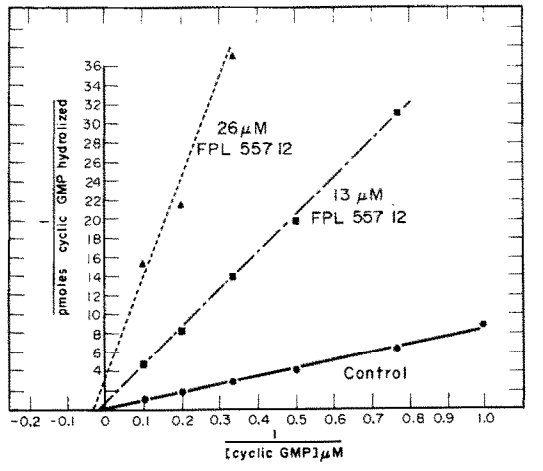

Fig. 2. Double reciprocal plot of cyclic GMP hydrolysis and the effect of 13 and 26 μM FPL 55712. The points are experimentally determined enzymatic rates, and the lines are computer generated linear regression "best-fit" lines ($r = 0.99$ for all lines).

FPL 55712 has been reported to be a specific antagonist of SRS-A[4, 5]. SRS-A exists preformed in human lung[14], and probably can induce the typical bronchospasms of human asthmatic attacks[15]. Therefore, a specific inhibitor of SRS-A would be extremely valuable both as a tool to investigate the role of SRS-A in human allergic asthma and as a potential therapy for the disease, much as specific pharmacological antagonists of other mediators have been used to elucidate the role of specific mediators in a variety of disease states.

In the present study, FPL 55712 has been shown to be a potent, reversible cyclic nucleotide phosphodiesterase inhibitor. In fact, to our knowledge it is the most potent inhibitor of the hydrolysis of cyclic GMP yet reported. Allergen-induced release of both histamine[16–18] and

SRS-A[19] is inhibited by agents capable of increasing cellular cyclic AMP levels, such as those stimulating adenylate cyclase activity or those inhibiting phosphodiesterase activity. Kaliner *et al.*[18] have further demonstrated potentiation of antigen-induced histamine release by increased intracellular cyclic GMP. It is clear, therefore, that a potent inhibitor of the hydrolysis of both cyclic AMP and cyclic GMP has the potential of dramatically affecting the release of several mediators of the allergic response. Therefore, use of an agent such as FPL 55712 as a tool to elucidate the role of one specific mediator may be premature at this time, until a careful investigation into the role of mediator release in model systems has been conducted.

This does not preclude the carefully controlled use of FPL 55712 as an antagonist of SRS-A. Perhaps its activity as a phosphodiesterase inhibitor, particularly of the hydrolysis of cyclic GMP, may be of benefit for the therapy of asthma, as suggested by Coulson *et al.*[20] and Bergstrand *et al.*[21].

In summary, the potent SRS-A inhibitor FPL 55712, a chromone-2-carboxylic acid, demonstrated marked activity as an inhibitor of both cyclic AMP and cyclic GMP phosphodiesterase activity. Kinetic experiments indicated that the interaction of this drug with rat brain phosphodiesterase is both noncompetitive and reversible. These experiments strongly suggest that investigators using this compound as an inhibitor of SRS-A should be aware of other potential activities of this compound which may complicate interpretation of such experiments.

Division of Biochemical Research, Ortho Pharmaceutical Corporation, Raritan, NJ 08869, *U.S.A.*

MARK CHASIN*
CYNTHIA SCOTT

REFERENCES

1. N. Lavin, G. S. Rachelefsky and S. A. Kaplan, *J. Allergy clin. Immun.* **57**, 80 (1976).
2. J. E. Tateson and D. G. Trist, *Life Sci.* **18**, 153 (1976).
3. D. G. Jones and A. B. Kay, *J. Pharm. Pharmac.* **26**, 918 (1974).
4. J. Augstein, J. B. Farmer, T. B. Lee, P. Sheard and M. L. Tattersall, *Nature New Biol.* **245**, 215 (1973).
5. R. A. Appleton, J. R. Bantick, T. R. Chamberlain, D. N. Hardern, T. B. Lee and A. D. Pratt, *J. med. Chem.* **20**, 371 (1977).
6. G. Brooker, L. J. Thomas and M. M. Appleman, *Biochemistry* **7**, 4177 (1971).
7. M. Chasin and D. N. Harris, in *Methods in Cyclic Nucleotide Research* (Ed. M. Chasin), p. 169. Marcel Dekker, New York (1972).
8. M. Chasin, *Fedn Proc.* **30**, 1268 (1971).
9. M. Chasin, D. N. Harris, M. B. Phillips and S. M. Hess, *Biochem. Pharmac.* **21**, 2443 (1972).
10. I. Weinryb, B. Beer, M. Chasin, E. B. Proctor and S. M. Hess, *Excerpta med. Int. Cong* **359**, 857 (1975).
11. W. R. Kukovetz and G. Poch, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **267**, 189 (1970).
12. R. W. Butcher and E. W. Sutherland, *J. biol. Chem.* **237**, 1244 (1962).
13. R. Eisenthal and A. Cornish-Bowden, *Biochem. J.* **139**, 715 (1974).
14. L. S. Turnbull, D. G. Jones and A. B. Kay, *Immunology* **31**, 813 (1976).
15. P. Sheard and A. M. J. N. Blair, *Int. Archs Allergy appl. Immun.* **38**, 217 (1970).
16. L. M. Lichtenstein and S. Margolis, *Science, N.Y.* **161**, 902 (1968).
17. R. P. Orange, M. A. Kaliner, P. J. Laraia and K. F. Austen, *Fedn Proc.* **30**, 1725 (1971).
18. M. Kaliner, R. P. Orange and K. F. Austen, *J. exp. Med.* **136**, 556 (1972).
19. W. J. Koopman, R. P. Orange and K. F. Austen, *J. Immun.* **105**, 1096 (1970).
20. C. J. Coulson, R. E. Ford, S. Marshall, J. L. Walker, K. R. H. Wooldridge, K. Bowden and T. J. Coombs, *Nature, Lond.* **265**, 545 (1977).
21. H. Bergstrand, J. Kristoffersson, B. Lundquist and A. Schurmann, *Molec. Pharmac.* **13**, 38 (1977).

*To whom reprint requests should be addressed.