Biochemical Pharmacology, Vol. 30, No. 7, pp. 697–702, 1981.
Printed in Great Britain.

0006-2952/81/070697-06 $02.00/0

INHIBITION OF LUNG CYCLIC AMP- AND CYCLIC GMP-PHOSPHODIESTERASES BY FLAVONOIDS AND OTHER CHROMONE-LIKE COMPOUNDS*

M. RUCKSTUHL and Y. LANDRY†

Laboratoire de Pharmacologie and Laboratoire de Pharmacodynamie (CNRS ERA 787), Faculté de Pharmacie-Université Louis Pasteur, B.P. 10, 67048 Strasbourg-Cedex, France

(*Received* 28 *July* 1980; *accepted* 16 *October* 1980)

Abstract—A series of 17 flavonoids and related compounds were tested as inhibitors of bovine lung cyclic AMP- and cyclic GMP-phosphodiesterase, comparatively to 12 reference substances, including antianaphylactic drugs. Most of the chromone-like compounds, including flavonoids disodium cromoglycate, gentiacaulein (a xanthone) and gentiacaulein (an anthraquinone) exhibited a higher potency for the inhibition of cyclic GMP hydrolysis with respect to cyclic AMP hydrolysis. The highest selectivities were observed using pelargonidin chloride and gentiacaulein with potencies similar to that of 1-methyl 3-isobutyl xanthine. Catechins were also highly selective of the cyclic GMP hydrolysis, but showed smaller potencies. These selectivities, however, remained smaller than observed when using 2′deoxy cyclic GMP, cyclic IMP, ICI 74917 and M&B 22948. An opposite selectivity was observed using khellin, a xanthone, papaverine, ZK62711 and Ro-20 1724, which show higher potencies of inhibition of cyclic AMP hydrolysis with respect to cyclic AMP hydrolysis. Altogether, these data suggest that the antianaphylactic properties of chromone-like compounds might be related to the increase of both cyclic AMP and cyclic GMP through the inhibition of phosphodiesterase.

Various pharmacological effects have been attributed to flavonoids and structurally-related compounds since Szent-Györgi asserted that some possess vitamin-like properties [1]. These effects include antiasthmatic properties [2–7] which might be related to both inhibition of mast cell degranulation and relaxation of lung smooth muscle—two cyclic nucleotide-dependent phenomena [8, 9]. The observations that flavonoids could induce an increase of cyclic AMP in cells [10] and that they inhibited cyclic AMP hydrolysis by phosphodiesterase were consistent with this hypothesis. However, it has also been suggested that the pharmacological increase of cyclic GMP is involved in antianaphyllactic properties of drugs. Indeed, cyclic nucleotide phosphodiesterase exists in different molecular forms with various affinities for cyclic AMP and cyclic GMP. The selective inhibition of one form might induce a selective increase of one cyclic nucleotide [14]. In a preliminary communication, we reported that some flavonoids were selective inhibitors of cyclic GMP hydrolysis [15]. This study was extended to 12 flavonoids and five related compounds, comparatively to 12 reference inhibitors, using a bovine lung preparation containing two phosphodiesterase molecular forms, selective of the hydrolysis of cyclic AMP and cyclic GMP, respectively.

* This work was supported by grants from INSERM (CRL 79-1-171-3) and DRET (78-1240).

† To whom all correspondence should be sent.

MATERIALS AND METHODS

Chemicals. [8-^{3}H]cyclic AMP (34.4 Ci/mole), [8-^{3}H]cyclic GMP (8.28 Ci/mole), [U-^{14}C]guanosine (427 mCi/mmole) were purchased from New England Nuclear Chemicals; [U-^{14}C]adenosine (567 mCi/mmole) from Radiochemical Center Amersham; 5′-nucleotidase (snake venom from *Ophiophagus hannah*) and bovine serum albumin fraction V from Sigma Chemical Corp. (USA). DEAE (diethylaminoethyl)–Cellulose DE 11 'Whatman' from Reeve Angel; 2′deoxy-cyclic AMP, 2′deoxy-cyclic GMP and cyclic IMP from Boehringer-Mannheim (FRG). ZK 62711 [16] (Rolipram) was a gift from Schering-Berlin (FRG), Ro 20–1724 [17] from Hoffman–LaRoch (CH), papaverine from Synthelabo (F), fluphenazine from Olmaco (F), ICI 74917 [19] from ICI-pharma (F), M&B 22948 (20) from May and Baker (GB), disodium cromoglycate from Fisons (GB), and amentoflavone from Dr. H. Wagner, Institut für pharmazeutische Arzneimittellehre, München (FRG). Theophylline and MIX (1-methyl-3-isobutyl xanthine) [18] were obtained from Aldrich Chemical Corp. (USA), catechin, quercetin, dihydroquercetol and apigenin from Carl Roth (FRG). Pelargonidin chloride was purchased from Fluka (CH). All other flavonoids were gifts from Dr. R. Anton, Faculté de Pharmacie, Strasbourg (F).

EGTA (ethylenebis (oxyethylenenitrilo)-tetraacetic acid), EDTA ((ethylenedinitrilo)-tetraacetic acid) and all other chemicals were of analytical grade.

Phosphodiesterase preparation. Bovine lung was obtained from a local slaughterhouse and kept at 4°

until dissection (within 2 hr). Samples of 50 g of parenchymal tissues were cut into small pieces (about 30 mm^3) and washed for a period of 24 hr with 700 ml of a medium containing 2mM Mg Cl_2, 3mM 2-mercaptoethanol and 20mM Tris–HCl pH 7.5. The washing fluids were discarded and the tissues homogenized with one volume of the same medium using a Vir Tis 60K homogenizer at 40,000 rpm for 2 min. The homogenate was centrifuged at 3000 *g* for 20 min. and at 105,000 *g* for 60 min. The last supernatant was frozen at −20° in small volumes and used to test phosphodiesterase inhibitors without further purification.

Phosphodiesterase assay. A modified [21] batch assay originally described by Thompson and Appleman [22] was used, with 1 μM cyclic AMP or cyclic GMP as substrate including 30,000 cpm of tritiated cyclic nucleotide. The reaction, performed at 37° for 3 min, was initiated by adding enzyme to the reaction medium preincubated for 2 min at 37°. The reaction was stopped by immersing the test tubes in boiling water for 1 min. Then 120 μg of snake venom and 10,000 cpm of [^{14}C]adenosine or guanosine were added to each tube. After 10 min of incubation of 37°, 1 ml of QAE-Sephadex A25 slurry prepared according to Schultz [23] was added. After 30 min at room temperature, the mixture was centrifuged, and 0.5 ml of the supernatant was counted in the scintillation fluid. The results were corrected for the yield of adenosine or guanosine recovered in each sample.

All assays were performed with both substrates and in duplicate. Blanks were run under identical conditions, with buffer replacing the enzyme, since in preliminary experiments we verified that blanks using boiled enzyme were identical. Phosphodiesterase concentrations used in the assay were ajusted to give linear reaction rates under the conditions described above. The separation of the final products by thin layer chromatography [24] gives the same percentage of hydrolysis. This shows that deaminases and other enzymes metabolizing the products of the reaction [25] did not interfere in the experimental conditions used (low substrates concentration, low incubation time, and low enzyme sample). Inhibitors were solubilized in ethanol or dimethylsulfoxyde. No analytical interference was observed under the conditions used.

Expression of the data. I_{50} were calculated by interpolating four values of inhibition, ranging from 35 to 70 per cent against the logarithm of inhibitor concentrations. The direct comparison of I_{50} among them suffice to determine the relative efficiency of inhibitors with respect to a single enzyme activity [26], as the assays were performed under identical conditions and assuming identical mechanisms of action. In order to compare the selectivity of inhibitors from the first enzyme to the second (cyclic AMP- and cyclic GMP-phosphodiesterase), K_i were calculated according to Cheng and Prussof [26] assuming competitive inhibitions ($K_i = I_{50}/1 + S/K_m$; S was the substrate concentration 1 μM, and K_M the Michaelis constant determined on Fig. 1 for low substrate concentrations, i.e. corresponding to physiological concentrations occurring in lung tissue). Competitive inhibitions of phosphodiesterase were previously reported by others, namely for flavonoids [13], disodium cromoglycate [3], 2'deoxycyclic nucleotide and cyclic IMP [27], and methylxanthines [28, 29]. For papaverine, both competitive [27, 30] and non-competitive [28, 30] inhibitions have been described.

RESULTS

Properties of the lung phosphodiesterase preparation

A soluble fraction of bovine lung was prepared by centrifugation at 105,000 *g* (see Materials and Methods). The phosphodiesterase activities of this fraction were representative of 90 per cent (cyclic AMP) and 96 per cent (cyclic GMP) of the tissues as previously

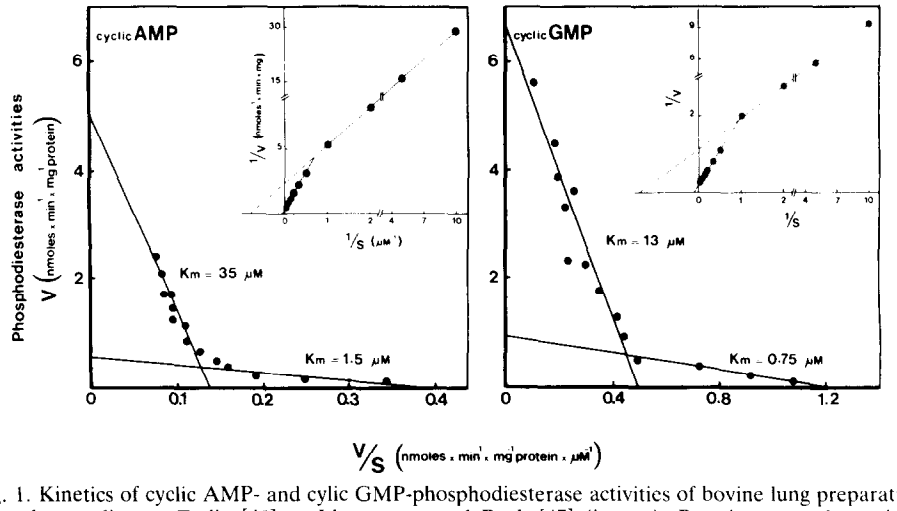

Fig. 1. Kinetics of cyclic AMP- and cylic GMP-phosphodiesterase activities of bovine lung preparation plotted according to Eadie [46] or Lineweaver and Burk [47] (inserts). Proteins were determined according to Lowry *et al.* [48] with bovine serum albumin as standard.

determined by differential centrifugation [31]. The ratio of cyclic AMP to cyclic GMP-phosphodiesterase activity was around 0.5. As currently reported, a limited linearity of hydrolyzing activities for both substrates according to incubation time and enzyme concentration was observed with various phosphodiesterase preparations (for a review, see [32]). Consequently, a 3 min incubation time was used for the following experiments with a maximum of 20 per cent hydrolyzed substrate. As often observed with phosphodiesterases [32], two apparent K_m's were determinable for each substrate (Fig. 1) according to the range of substrate concentrations. In the following experiments, 1 μM substrate concentrations were used, within the range of naturally occurring cyclic nucleotide levels in lung [9].

Table 1 indicates that high cyclic AMP and cyclic GMP concentrations were necessary to inhibit, respectively, the hydrolysis of cyclic GMP and cyclic AMP. The selectivity of inhibition was calculated as the ratio of K_i (see Materials and Methods) for each enzyme activity (Table 1). 2'Deoxy-substrates were selective inhibitors of the hydrolysis of their non-reduced analogs. Cyclic IMP was a selective inhibitor of the hydrolysis of cyclic GMP. In comparison with results obtained by Davis and Kuo [27] who used purified cyclic AMP- and cyclic GMP-phosphodiesterases from guinea pig, with similar inhibition features by cyclic nucleotides, these results suggested the presence in the bovine lung preparation of two enzymes selective of the hydrolysis of cyclic AMP and cyclic GMP, respectively. Indeed these activities could be separated from bovine lung using a DEAE cellulose column chromatography eluted with a NaCl gradient [31]. However, in order to facilitate the comparative study of inhibition of both enzymes, the bovine lung preparation was used, in the following experiments, without further purification.

Selective inhibition of cyclic AMP- and cyclic GMP-phosphodiesterase from bovine lung

Besides cyclic nucleotides, other reference inhibitors were used (Table 1) to further characterize the enzymes before studying chromone-like compounds. The cylic AMP-phosphodiesterase was inhibited by micromolar concentrations of ZK 62711 and papaverine, other compounds being 10- to 100-fold less potent. The cyclic GMP phosphodiesterase was inhibited in the micromolar range by MIX, ICI 74917, M&B 22948, and papaverine. Theophylline and fluphenazine slightly inhibited both enzymes.

ZK 62711 and Ro 20–1724 appeared as highly selective of the cyclic AMP phosphodiesterase whereas M&B 22948, ICI 74917, and MIX were selective inhibitors of the cyclic GMP-enzyme. The selectivity of papaverine for the cyclic AMP-enzyme or of theophylline and fluphenazine for the cyclic GMP-enzyme was lower than 2.

Table 2 shows results obtained with chromone-like compounds and emodin, an anthraquinone. Most of the listed compounds were potent inhibitors of the cyclic AMP- and/or the cyclic GMP-phosphodiesterase. With the exception of khellin, these compounds were selective inhibitors of the cyclic GMP enzyme. The rank order of selectivity for cyclic GMP hydrolyzing activity was disodium cromoglycate > (+)-catechin > gentiacaulein > epicatechine > pelargonidan. Among these compounds, gentiacaulein (a xanthone) and pelargonidin (a flavylium) showed the highest potencies for both enzymes. Some flavonoids (amentoflavone, kaempferol, luteolin,

Table 1. Inhibition of cyclic AMP and cyclic GMP phosphodiesterase activities from bovine lung 105,000 *g* supernatant by cyclic nucleotides and reference compounds

Compounds	I_{50} (μM)		Calculated K_i (μM)		$\frac{a}{b}$
	Cyclic AMP	Cyclic GMP	Cyclic AMP (a)	Cyclic GMP (b)	
Cyclic nucleotides					
Cyclic AMP	—	520	—	223	—
Cyclic GMP	800	—	482	—	—
2'Deoxy-cyclic AMP	12	380	7.2	163	0.04
2-Deoxy-cyclic GMP	1100	7	662	3	220
Cyclic IMP	325	14	196	6	33
Reference compounds					
ZK 62711	4	500	2.4	214	0.01
Ro 20-1724	45	380	27.1	163	0.17
Papaverine	5	11	3	4.7	0.64
Theophylline	300	310	181	133	1.36
Fluphenazine	300	225	181	97	1.86
MIX	35	7	21	3	7
ICI 74917	325	14	196	6	33
M&B 22948	120	3	72	1.3	56

Assays were performed using 1 μM substrates. I_{50} and K_i were calculated as described in Materials and Methods. (Means of two determinations in duplicate.)

Table 2. Inhibition of cyclic AMP and cyclic GMP phospodiesterases from bovine lung 105,000 *g* supernatant by flavonoids and related compounds

Trivial and systematic name	I_{50}		Calculated K_i (μM)		$\frac{a}{b}$
	Cyclic AMP	Cyclic GMP	Cyclic AMP (a)	Cyclic GMP (b)	
Flavonoids					
Apigenin *4',5,7-Trihydroxyflavone*	53	35	31.9	15	2.13
Amentoflavone *(I-3', II-8) biapigenin*	0.66	0.54	0.40	0.23	1.74
Kaempferol *3,4',5,7-Tetrahydroxyflavone*	18	23	10.8	9.9	1.09
Luteolin *3',4',5,7,-Tetrahydroxyflavone*	19	19	11.4	8.1	1.4
Eriodictyol *3',4',5,7-Tetrahydroxyflavanone*	48	33	28.9	14.2	2.03
Pelargonidin chloride *3,4',5,7-Tetrahydroxy-flavylium chloride*	70	23	42.2	9.9	4.37
Morin *2',3',4',5,7-Pentahydroxyflavone*	44	30	26.5	12.9	2.05
Quercetin *3,3',5,7-Pentahydroxyflavone*	23	15	13.8	6.5	2.14
Dihydroquercetin *3,3',4',5,7-Pentahydroxyflavanone*	320	170	193	73	2.64
(+)-Catechin	640	170	385	73	5.27
(−)-Catechin	630	300	379	129	2.93
(−)-Epicatechin	1900	600	1145	257	4.45
Chromones					
Isoluteolin *3',4',5,7-Tetrahydroxy-phenyl-3-chromone*	42	54	25.3	23.2	1.09
Khellin	125	320	75	142	0.53
Disodium cromoglycate	>2000	250	>1200	107	>11.2
Xanthone					
Gentiacaulein	70	20	42	8.6	4.9
Anthraquinone					
Emodin	16	16	9.6	6.9	1.40

Assays were performed with 1 μM substrate, I_{50} and K_i were calculated as described in Materials and Methods (Means of two determinations in duplicate).

eriodictyol, isoluteolin), and emodin were potent inhibitors of the two enzymes but their selectivity for the cyclic GMP-enzyme was slight.

DISCUSSION

Multiple forms of cyclic nucleotide phosphodiesterase have been shown to exist in various tissues with different substrate selectivities, and with or without sensitivity to calmodulin (for a review, see [32]). The data shown in Table 1 suggested the presence in the preparation used of two enzymes hydrolyzing selectivity cyclic AMP and cyclic GMP respectively, and insensitive to calmodulin. This hypothesis was confirmed by the separation of the two enzymes, using DEAE cellulose chromatography [31]. Their properties were similar to those of enzymes purified from guinea pig [27] and human [29] lung. We also observed a calmodulin-sensitive phosphodiesterase in bovine lung [31] with properties similar to those previously reported for human lung [33] and bovine aorta [34, 35]. This calmodulin-sensitive enzyme hydrolyzed cyclic AMP and cyclic GMP with similar kinetics, and chemical inhibitors were equally active with respect to both substrates. Moreover, ZK 62711 [35] and Ro 20 1724 [33, 35] were inefficient in inhibiting its activity. The present lung preparation, previously referred to as "washed lung supernatant" [31], did not contain calmodulin-sensitive phosphodiesterase and allowed an easy analysis of the selective inhibition of cyclic AMP-phosphodiesterase and cyclic GMP-phosphodiesterase, respectively. This preparation was representative of 90 to 96 per cent of the phosphodiesterase activity of lung [31].

Structure–activity relationships of flavonoids as inhibitors of cyclic AMP-phosphodiesterase from bovine heart [11] and liver fluke [13] were recently reported. The present data allow the activity of flavonoids and other chromone-like compounds as inhibitors of cyclic AMP-phosphodiesterase, to be compared with their activity as inhibitors of cyclic

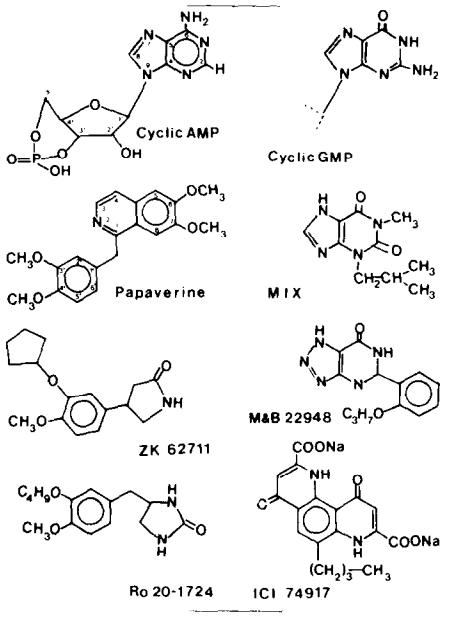

Fig. 2. Structures of substrates and selective inhibitors of cyclic AMP-phosphodiesterase and cyclic GMP-phosphodiesterase.

GMP-phosphodiesterase, from a preparation containing the two enzymes. Considering the inhibition of both enzymes, it appears that the order of inhibitory potencies among flavonoids was biflavone > flavone > flavone > catechin. The main feature of the chemical structure influencing activities were the following: the addition of one hydroxyl group to trihydroxyflavone at C-3 or C-3′ increased the activities (compare apigenin to kaempferol and luteolin); the addition of one hydroxyl group at C-3 to tetrahydroxyflavone did not modify the inhibitor activities (compare quercetin to luteolin), whereas a fifth hydroxy-group added at C-2′ lowered the activities (compare kaemferol to morin); the double bond between C-2 and C-3 seemed to be especially important for the activities (compare flavones to flavonones). Moreover, a better potency for the cyclic GMP-enzyme was constantly observed, with different selectivities from one molecule to another. The order of selectivity for the inhibition of cyclic GMP hydrolysis, with respect to cyclic AMP was catechin > flavylium > pentahydroxyflavones, pentahydroxyflavonones, trihydroxyflavones > tetrahydroxyflavones. This selectivity was favoured by some structures: whereas the C ring of flavylium, as opposed to flavone, was less active considering the inhibition of cyclic AMP hydrolysis, a similar activity was observed for both molecules considering the inhibition of cyclic AMP hydrolysis (compare kaempferol and pelargonidin); whereas the stereochemistry of catechins did not influence their activity as inhibitors of cyclic AMP hydrolysis [13], it modified their activity as inhibitors of cyclic GMP hydrolysis (Table 2).

These data also show that besides flavonoids other chromone-like compounds are also selective inhibitors of cyclic GMP hydrolysis with respect to cyclic AMP, with the exception of khellin, selective of cyclic AMP. The highest selectivity for cyclic GMP hydrolysis was observed with disodium cromoglycate, an antiallergic compound currently used in therapeutics [2–4]. Gentiacaulein, a xanthone, was also selective of cyclic GMP hydrolysis. Its structure is closely related to other xanthones with antiasthmatic properties [5]. In the area, Coulson *et al.* [36] suggested that histamine release was reduced more effectively when cyclic GMP levels were increased with respect to cyclic AMP levels. Indeed, as cromoglycate [2], quercetin [37, 38] was shown to inhibit histamine release. Moreover, the increase of both cyclic AMP and cyclic GMP levels was suggested during pulmonary smooth muscle relaxation [9, 39, 40].

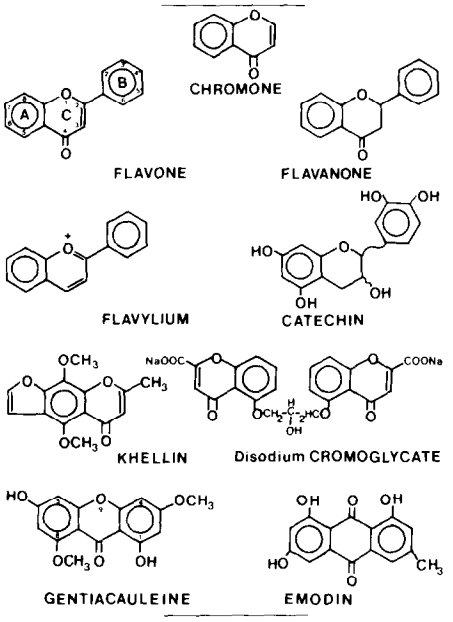

Fig. 3. Structures of chromone, flavonoids and other chromone-like compounds tested as inhibitors of cyclic nucleotide phosphodiesterases.

These results suggest that the pharmacological effects of chromone-like compounds, and especially their antianaphyllatic properties, might be related to the increase of both cyclic AMP and cylic GMP levels through the inhibition of phosphodiesterases in various tissues. However, other enzymes, including various ATPases [38, 41–44], are also sensitive to these drugs, and their pharmacological effects might result from the respective balance of these various targets in a selected tissue. It could be pointed out that, though in another field, the potency of emodin for inhibiting cyclic nucleotide phosphodiesterase suggests that cyclic nucleotides might be related to the mechanism of action of cathartic anthraquinones. As observed with cholera toxin [45] the increase of cyclic AMP in the intestine wall by anthraquinones might induce an influx of water to produce the cathartic phenomena.

Acknowledgements—The authors with to thank Dr R. Anton, Dr A. Beretz and Dr J. C. Stoclet for helpful suggestions throughout this work.

REFERENCES

1. St. Rusznyak and A. Szent-Györgi, *Nature* **138**, 27 (1936).
2. J. S. C. Cox, *Nature* **216**, 1328 (1967).
3. A. S. Roy and B. T. Warren, *Biochem. Pharmac.* **23**, 917 (1974).
4. N. Lavin, G. S. Rachelefsky and S. A. Kaplan, *J. Allergy Clin. Immun.* **57**, 80 (1976).
5. E. S. K. Assem, J. A. Evans and M. McAllen, *Br. Med. J.* **2**, 93 (1974).
6. J. R. Pfister, R. W. Ferraresi, I. T. Harrison, W. H. Rooks, A. P. Roszkowski, A. Van Horn and J. H. Fried, *J. Med. Chem.* **15**, 1032 (1972).
7. H. Bergstrand, J. Kristofferson, B. Lundquist and A. Schurmann, *Molec. Pharmac.* **13**, 38 (1977).
8. M. Kaliner and K. F. Austen, *Biochem. Pharmac.* **23**, 763 (1974).
9. W. N. Gold, in *Physiology and Pharmacology of the Airways* (Ed. J. A. Nadel) p. 123. Marcel Dekker, New York (1980).
10. Y. Graziani and R. Chayoth, *Biochem. Pharmac.* **26**, 1259 (1977).
11. A. Beretz, R. Anton and J. C. Stoclet, *Experientia* **34**, 1054 (1978).
12. A. Beretz, M. Joly, J. C. Stoclet and R. Anton, *Planta Medica, J. Med. Plant Res.* **36**, 193 (1979).
13. J. E. Ferrel, P. D. G. Chang Sing, G. Loew, R. King, J. M. Mansour and T. E. Mansour, *Molec. Pharmac.* **16**, 556 (1979).
14. B. Weiss and W. H. Hait, *A. Rev. Pharmac. Toxic.* **17**, 441 (1977).
15. M. Ruckstuhl, A. Beretz, R. Anton and Y. Landry, *Biochem. Pharmac.* **28**, 535 (1979).
16. U. Schwabe, M. Miyake, Y. Ohga and J. W. Daly, *Molec. Pharmac.* **12**, 900 (1976).
17. H. Sheppard and G. Wiggan, *Molec. Pharmac.* **7**, 111 (1971).
18. J. A. Beavo, N. C. Rogers, O. B. Crofford, J. G. Hardman, E. W. Sutherland and E. U. Neuman, *Molec. Pharmac.* **6**, 597 (1970).
19. D. P. Evans and Thomson D. S., *Br. J. Pharmac.* **53**, 409 (1975).
20. B. J. Broughton, P. Chaplen, P. Knowless, E. Lunt, D. L. Pain, K. R. H. Wolldridge, R. Ford, S. Marshall, J. L. Walker and D. R. Maxwell, *Nature* **251**, 650 (1974).
21. C. Lugnier and J. C. Stoclet, *Biochem. Pharmac.* **28**, 3581 (1979).
22. W. J. Thompson and M. M. Appleman, *Biochemistry* **10**, 331 (1971).
23. G. Shultz, E. Böhme and J. G. Hardman, in *Methods in Enzymology* (Eds J. G. Hardman and B. W. O'Malleg), Vol. 38, p. 13. Academic Press, New York (1974).
24. E. Böhme and G. Schultz, in *Methods in Enzymology* (Eds J. G. Hardman and B. W. O'Malley), Vol. 38, p. 27. Academic Press, New York (1974).
25. W. J. Rutten, B. M. Schoot and J. J. H. H. M. De Pont, *Biochim. biophys. Acta* **315**, 378 (1973).
26. Y. C. Cheng and W. H. Prusoff, *Biochem. Pharmac.* **22**, 3099 (1973).
27. C. W. Davis and J. F. Kuo, *Biochem. Pharmac.* **27**, 89 (1978).
28. U. Schwabe, E. W. Bernt and R. Ebert, *Arch. Pharmak.* **273**, 62 (1972).
29. W. J. Rutten, B. M. Schoot, J. J. H. H. M. De Pont and S. L. Bonting, *Biochim. biophys. Acta* **315**, 384 (1973).
30. C. Lugnier and J. C. Stoclet, *Biochem. Pharmac.* **23**, 3071 (1974).
31. M. Ruckstuhl, A. Morel, B. Ilien and Y. Landry, submitted for publication.
32. J. N. Wells and J. G. Hardman, *Adv. Cyclic. Nucleotide Res.* **8**, 119 (1977).
33. H. Bergstrand, B. Lundquist and A. Schurmann, *J. biol. Chem.* **253**, 1881 (1978).
34. B. Ilien, A. Stierle, C. Lugnier, J. C. Stoclet and Y. Landry, *Biochem. biophys. Res. Commun.* **83**, 486 (1978).
35. B. Ilien, M. Ruckstuhl and Y. Landry, submitted for publication.
36. C. J. Coulson, R. E. Ford, S. Marshall, J. L. Walker, K. R. H. Wooldridge, K. Bowden and T. J. Coombs, *Nature* **265**, 545 (1977).
37. C. M. S. Fewtrell and B. D. Gomperts *Biochim. biophys. Acta* **469**, 52 (1977).
38. C. M. S. Fewtrell and B. D. Gomperts, *Nature* **265**, 635 (1977).
39. J. B. Dalson, J. J. Krzanowski, D. F. Fitzpatrick and A. Sentivanyi, *Biochem. Pharmac.* **27**, 254 (1978).
40. J. B. Polson, J. J. Krzanowski, W. H. Anderson, D. F. Fitzpatrick, D. P. G. Huang and A. Szentivanyi, *Biochem. Pharmac.* **28**, 1391 (1979).
41. E. M. Suolinna, D. E. Lang and E. Racker, *J. natn. Cancer Inst.* **53**, 1515 (1974).
42. Y. Kuriki and E. Racker, *Biochemistry* **15**, 4951 (1976).
43. A. Di Pietro, C. Godinot, M. L. Bouillant and D. C. Gautheron, *Biochimie* **57**, 959 (1975).
44. A. Di Pietro, C. Godinot, D. C. Guatheron and P. Foulhoux, *Biochem. Pharmac.* **26**, 1021 (1977).
45. D. M. Gill, *Adv. Cyclic. Nucleotide Res.* **8**, 85 (1977).
46. G. S. Eadie, *J. biol. Chem.* **146**, 85 (1942).
47. H. Lineweaver and D. Burk, *J. Am. Chem. Soc.* **56**, 658 (1944).
48. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall. *J. biol. Chem.* **193**, 265 (1951).