*Biochemical Pharmacology*, Vol. 33, No. 24, pp. 3919–3922, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00


# COMMENTARY

## PHARMACOLOGIC RECEPTORS FOR THE LEUKOTRIENES

JEROME H. FLEISCH,* LYNN E. RINKEMA and WINSTON S. MARSHALL
Lilly Research Laboratories, Indianapolis, IN 46285, U.S.A.

Leukotrienes are biologically active $C_{20}$ fatty acids derived from arachidonic acid [1–3]. The events leading to their synthesis and release can be initiated in a variety of cell types by either immunological or chemical stimuli [4–7]. Leukotrienes $B_4$, $C_4$, $D_4$ and $E_4$ ($LTB_4$, $LTC_4$, $LTD_4$ and $LTE_4$) are among the most pharmacologically significant members of this chemical family. $LTB_4$ has been shown to be chemotactic for polymorphonuclear leukocytes [8]. $LTC_4$, $LTD_4$ and $LTE_4$, the sulfidopeptide leukotriene components of slow-reacting substance, contract bronchial smooth muscle [9], increase mucus secretion [10], and depress myocardial contractility [11]. Although it is now appreciated that the leukotrienes exert profound pharmacologic effects, there is little information to suggest the physiologic functions that they might serve. Most of the available data gathered to date only provide information on the pathologic consequence of their generation. This has led to the hypothesis of leukotriene involvement in the clinical symptoms of asthma, inflammation, and other disease entities [12–14].

### *Leukotriene receptor antagonists*

If leukotrienes play a role in the pathogenesis of human disease, then it follows that pharmacologic antagonists, that is drugs capable of combining with leukotriene receptors but unable to activate these sites, should open a new avenue for novel medical therapy. In addition to their potential clinical utility, highly selective leukotriene antagonists are necessary tools for understanding the pharmacologic and biochemical nature of leukotriene receptors.

There have been two major approaches to the discovery of leukotriene antagonists. The first involved a search for molecules which possessed antagonist activity but seemed structurally unrelated to the natural agonists. This approach resulted in the development of FPL 55712 as an SRS-A antagonist [15]. The second line of research, synthesizing analogs of the natural leukotrienes, had to await elucidation of the leukotriene structures [16–18]. However, use of this approach to develop clinically acceptable drugs is fraught with uncertainties since agents resembling the naturally occurring agonist are more likely to be disposed of by the body in a rapid and efficient manner, in addition to having a greater chance of being encumbered with agonist activity.

The best characterized leukotriene antagonist is FPL 55712 which was developed from a series of compounds related to the antiasthma drug disodiumcromoglycate. Unfortunately, even though it lacks structural similarity to the natural agonist, FPL 55712 has a short biological half-life when given intravenously and is only poorly absorbed after oral administration [19]. Recently, however, O'Donnell and Welton [20] aerosolized FPL 55712 into guinea pigs, and this resulted in a longer biological half-life against $LTD_4$- and $LTE_4$-induced bronchoconstriction.

A variety of leukotriene antagonists has now been prepared. Chemically, they represent a diverse group of substances including hydrotropic acids [21], nitrocoumarins [22], imidodisulfamides [23, 24], pyrazolopyridines [25], and pyridoquinazolinecarboxylic acids [26]. However, most of them lack sufficient efficacy to make useful therapeutic agents. We recently reported on a selective $LTD_4$ antagonist, LY171883, a tetrazole-substituted acetophenone, which has potent *in vitro* and *in vivo* activity in addition to a relatively long biological half-life after oral administration.†–§. Although it is premature to speculate on its potential clinical usefulness, at the very least it should help to clarify the role of $LTD_4$ in some physiologic and pathologic processes.

### *Indirect action of leukotrienes*

Interaction of an agonist with a receptor is but the first in a series of steps that culminates in a pharmacologic effect. Generally, the reactions that follow this initiating event result in the synthesis or deployment of intracellular mediators that subsequently direct the cell to respond in an appropriate manner. For airway smooth muscle, the final step in the chain of events is a lengthening or shortening of the contractile apparatus. Pharmacologists have long appreciated that certain agonists cause the release of a second substance which modulates the initial response through its own receptor [27]. Such is the case with the leukotrienes in some organ systems.

Perhaps the earliest indication that responses to the leukotrienes might have an indirect component was the suggestion that guinea pig [28] or human SRS-A [29] released rabbit aorta contracting sub-

* Author to whom all correspondence should be sent.

† W. S. Marshall, T. Goodson, D. Swanson-Bean, L. E. Rinkema, K. D. Haisch, and J. H. Fleisch, *Prostaglandins and Leukotrienes '84: Their Biochemistry, Mechanism of Action and Clinical Applications*, Abstract 262 (1984).

‡ J. H. Fleisch, L. E. Rinkema, K. D. Haisch, T. Goodson, D. Swanson-Bean and W. S. Marshall, *Prostaglandins and Leukotrienes '84: Their Biochemistry, Mechanism of Action and Clinical Applications*, Abstract 261 (1984).

§ C. J. Parli and M. D'Onofrio, *Prostaglandins and Leukotrienes '84: Their Biochemistry, Mechanism of Action and Clinical Applications*, Abstract 260 (1984).

stance (mainly thromboxane $A_2$) and prostaglandins from perfused guinea pig lungs. Other studies from the same laboratory have extended these observations to chemically pure $LTC_4$ and $LTD_4$ [30]. The release of prostaglandins from airways is not unique to the leukotrienes. Orehek *et al.* [31], Grodzinska *et al.* [32], and Turker and Zengil [33] previously demonstrated release of the bronchodilator prostaglandin $E_2$ during tracheal contractions elicited by acetylcholine, histamine, or electrical stimulation. In these situations, the release of a second agonist was envisioned as a protective mechanism by the tissue to ward off an excessive contraction. In contrast, thromboxane $A_2$ released by the leukotrienes serves to enhance the direct bronchospastic actions of the leukotrienes. Numerous laboratories have now demonstrated both direct and indirect actions of the leukotrienes, with the relative proportions of each dependent on the tissue examined (*in vitro*) [34, 35], and the routes of administration (*in vivo*) [34, 36–38]. This complicates pharmacologic analysis since a response to the leukotrienes (e.g. bronchospasm) could conceivably be antagonized by a variety of different inhibitors. A case in point is the interesting agent SK & F 88046, an imidodisulfamide initially reported to be a receptor antagonist of $LTD_4$ in guinea pig parenchyma [39]. Weichman *et al.* [40] then demonstrated that, unlike FPL 55712, SK & F 88046 did not antagonize $LTD_4$-induced contractions of guinea pig trachea. Additional experiments showed that SK & F 88046 antagonized the thromboxane-sensitive component of $LTD_4$ on guinea pig parenchyma and eventually led these investigators to conclude that SK & F 88046 antagonized the action of thromboxane at the receptor level [40].

*Heterogeneity of leukotriene receptors: Pharmacologic studies*

Structural elucidation of the leukotrienes [16–18, 41], and their subsequent chemical synthesis [42, 43] have made chemically pure leukotrienes and leukotriene analogs available for characterizing leukotriene receptors. Based on a wealth of knowledge gathered from earlier drug receptor studies, it seemed reasonable to postulate *a priori* that there would be multiple receptors for the leukotrienes. Studies by Arunlakshana and Schild [44], Furchgott [45], Lands *et al.* [46], and numerous workers after them clearly demonstrated heterogeneity for a variety of receptors. For example, adrenergic receptors are divided into two major classes, $\alpha$ and $\beta$, and these can be further subdivided into $\alpha_1$, $\alpha_2$, $\beta_1$, and $\beta_2$. This knowledge has resulted in a wealth of new drugs that are relatively specific agonists and antagonists for the subpopulations of adrenergic receptors. Similar examples are also available for other pharmacologic receptor systems. Since the leukotrienes are a family of agonists, not only might each have its own specific class of receptors but each leukotriene might also have the capacity to interact with subsets of these receptors. The first indication of multiple leukotriene receptors came from Drazen *et al.* [47]. They found FPL 55712, which up to that point was considered a selective SRS-A antagonist, capable of antagonizing responses of guinea pig lung parenchymal strips to $LTD_4$ but unable to block contractile responses elicited by $LTC_4$. A similar observation was subsequently made by Krell *et al.* [48]. From these two studies, there was a clear indication that receptors for $LTC_4$ would ultimately be shown to differ from receptors for $LTD_4$. Using crude SRS-A as the agonist, we demonstrated that FPL 55712 was a more potent antagonist on guinea pig ileum than on lung parenchymal strips [7]. This led us to postulate the existence of at least two classes of SRS-A receptors represented by one group in the ileum and another in the lung. When chemically pure $LTD_4$ became available, we determined dissocation constants, $K_B$ and $pA_2$ values, for FPL 55712 on guinea pig ileum, trachea, and parenchyma [49]. The values for this competitive antagonist were similar in trachea and parenchyma but different from those in ileum, substantiating our previous results obtained with the crude material. More recent experiments from our laboratory using LY171883 support and extend our work with FPL 55712*. LY171883 is a competitive antagonist of $LTD_4$ on guinea pig ileum and lung parenchymal strips. The $K_B$ value was five times larger on parenchyma than on ileum. More interesting, however, was the noncompetitive nature of LY171883 as an $LTD_4$ antagonist on guinea pig trachea. $K_B$ values increased with increasing concentrations of LY171883. One possible explanation is the existence of a second receptor in trachea that recognizes $LTD_4$ but is not blocked by our antagonist. Krell *et al.* [50] had already suggested that two $LTD_4$ receptors might exist in trachea based on different affinities for FPL 55712. Further experiments with $LTE_4$ indicated to them that this leukotriene interacted preferentially with the high-affinity receptor. LY171883, like FPL 55712, does not measurably antagonize *in vitro* responses to $LTC_4$* and thus adds more credibility to the hypothesis of separate receptors for $LTC_4$ and $LTD_4$. Also, neither FPL 55712 [51] nor LY171883* antagonized the response of guinea pig lung parenchyma to $LTB_4$. This is not surprising considering the significant structural differences between $LTB_4$ and the sulfidopeptide leukotrienes. Further evidence showing that $LTB_4$ and $LTD_4$ do not recognize the same receptors can be found in the work of Sirois *et al* [51]. They found $LTB_4$ unable to contract guinea pig ileum, whereas $LTD_4$ and $LTC_4$ were powerful stimulants of this tissue. This implied a lack of $LTB_4$ receptors in guinea pig ileum. Sirois *et al.* [52] also characterized leukotriene receptors in guinea pig lung parenchyma using a desensitization technique. During infusion of $LTB_4$, the parenchyma became desensitized to $LTB_4$ but was still responsive to histamine and the other leukotrienes.

The above studies, taken together with others not cited, point to a heterogenity within and between the various classes of leukotriene receptors. However, these studies are not without their drawbacks. To properly characterize receptors with pharmacologic techniques, there are a number of criteria enumerated by Furchgott [53] that should be met. Unfor-

* J. H. Fleisch, L. E. Rinkema, K. D. Haisch, T. Goodson, D. Swanson-Bean and W. S. Marshall, *Prostaglandins and Leukotrienes '84: Their Biochemistry, Mechanism of Action and Clinical Applications*, Abstract 261 (1984).

tunately, there is not enough information presently available on the leukotrienes to satisfy all the criteria. For example, at the time a response is measured, the concentration of agonist and antagonist in the region of the receptors should be in equilibrium with the outside solution and a steady-state condition reached. The rate of drug loss in the tissue due to metabolism, uptake into the tissue, and binding should be negligible compared with the rate of diffusion into the outside solution. Little is known about leukotriene uptake mechanisms. Also, $LTC_4$ can be converted to $LTD_4$ which, in turn, can be metabolized to $LTE_4$. Inhibition of the conversion of $LTC_4$ to $LTD_4$ by isolated guinea pig trachea has been accomplished satisfactorily by an l-serine-borate complex [54]. However, the amounts required of this $\lambda$-glutamyl transpeptidase inhibitor are extremely high, up to 45 mM, and various nonspecific effects cannot be ruled out. Conversion of $LTD_4$ to $LTE_4$ seems to be satisfactorily prevented with a 15-fold lower concentration of cysteine, an inhibitor of the peptidase responsible for this reaction [54, 55]. In tissues with multiple receptor subtypes, dissociation constants are best obtained after treating the preparations with specific antagonists to pharmacologically eliminate any extraneous receptors. For example, analysis of histamine$_1$ receptors is optimally carried out in the presence of histamine$_2$ receptor antagonists and vice versa. A lack of highly specific leukotriene receptor antagonists has prevented this type of careful evaluation. Nevertheless, the studies to date have, for the most part, been state of the art. Improvements and re-evaluation will have to await future scientific advances.

*Heterogeneity of leukotriene receptors: Radioligand binding studies*

Despite any indecisiveness that may be inherent in the pharmacologic studies discussed above, the concept of multiple leukotriene receptors is on firm ground. Additional evidence in support of this hypothesis comes from radioligand-binding experiments using $^3$H-labeled leukotrienes as the agonists. Levinson [56], examined binding of [$^3$H]leukotriene $C_4$ to guinea pig uterine membranes. Specific saturable binding of $LTC_4$ at 4°, in the presence or absence of 10 mM serine borate [57], was found to be reversed on addition of unlabeled $LTC_4$. With $Ca^{2+}$ present, the affinity was in the 5 nM range, whereas in the absence of $Ca^{2+}$ the affinity decreased to approximately 70 nM. In contrast, [$^3$H]$LTD_4$ did not bind to the same membrane preparations, clearly suggesting a lack of affinity of $LTD_4$ for the $LTC_4$ binding site [57]. Similarly, specific receptors for $LTC_4$ were detected by Krilis *et al.* [58] on a cultured smooth muscle cell line derived from Syrian hamster vas deferens. To evaluate the specificity of these binding sites, FPL 55712, $LTD_4$, $LTE_4$ and various structural analogs of $LTC_4$ were assessed for their capacity to inhibit the binding of $LTC_4$. The results correlated with a highly specific receptor for $LTC_4$ which was not shared with the other components of SRS-A. Along the same lines, a binding site in rat lung membranes was found by Pong *et al.* [59] to be specific for $LTC_4$. Competition studies showed unlabeled $LTC_4$ with a lower $K_i$ than $LTD_4$, $LTE_4$, $LTB_4$, several prostaglandins, and a variety of other substances. Pong and DeHaven [60] also characterized $LTD_4$ receptors in guinea pig lung and found that $LTE_4$, but not $LTC_4$, has a high affinity for the $LTD_4$ binding site. These data are in concert with the pharmacologic observations of Krell *et al.* [50]. Distinct receptors for $LTC_4$ and $LTD_4$ have been found in guinea pig lung [61] and heart [62] by Hogaboom *et al.* using radioligand binding techniques. In their studies on leukotriene receptors in guinea pig lung, they made the interesting observation that guanine nucleotides could inhibit $LTD_4$ binding but had no effect on the binding by $LTC_4$.

Pharmacologic and biochemical studies have clearly separated $LTB_4$ receptors from those of the sulfidopeptide leukotrienes. A recent report by Goldman *et al.* [63] has taken this one step further by postulating subsets of $LTB_4$ receptors in human neutrophils. In their experiments, $LTB_4$ bound to a high-affinity site which mediated chemotactic responses and a low-affinity site which controlled release of lysosomal enzymes.

*Conclusions*

Pharmacologic and radioligand binding studies have provided presumptive evidence for the presence of heterogeneous leukotriene receptors. These reactive cellular elements appear to be selective for the individual leukotrienes. The possibility also exists for differences in leukotriene receptors from tissue to tissue, and for the presence of multiple receptor subtypes in the same tissue. The practical significance of these observations will be realized in the future with the development of novel leukotriene antagonists capable of specifically inhibiting leukotriene-mediated responses.

## REFERENCES


1. B. Samuelsson, *Science* **220**, 568 (1983).
2. H. R. Morris, G. W. Taylor, C. M. Jones, P. J. Piper, J. R. Tippins and M. N. Samhoun, in *SRS-A and Leukotrienes* (Ed. P. J. Piper), p. 19. John Wiley, New York (1981).
3. R. A. Lewis, J. M. Drazen, J. C. Figueiredo, E. J. Corey and K. F. Austen, *Int. J. Immunopharmac.* **4**, 85 (1982).
4. W. E. Brocklehurst, *J. Physiol. Lond.* **151**, 416 (1960).
5. M. K. Bach and J. R. Brashler, *J. Immun.* **113**, 2040 (1974).
6. P. J. Piper and J. P. Seale, *Br. J. Pharmac.* **67**, 67 (1979).
7. J. H. Fleisch, K. D. Haisch and S. M. Spaethe, *J. Pharmac. exp. Ther.* **221**, 146 (1982).
8. A. W. Ford-Hutchinson, M. A. Bray, M. V. Doig, M. E. Shipley and M. J. H. Smith, *Nature, Lond.* **286**, 264 (1980).
9. S. E. Dahlen, P. Hedqvist, S. Hammarstrom and B. Samuelsson, *Nature, Lond.* **288**, 484 (1980).
10. Z. Marom, J. H. Shelhamer, M. K. Bach, D. R. Morton and M. Kaliner, *Am. Rev. resp. Dis.* **126**, 449 (1982).
11. J. A. Burke, R. Levi, Z. Guo and E. J. Corey, *J. Pharmac. exp. Ther.* **221**, 235 (1982).
12. W. E. Brocklehurst, *Ann. Allergy* **40**, 1 (1978).
13. Z. Zukowska-Grojec, M. A. Bayorh, I. Yaar, I. J. Kopin and G. Feuerstein, in *Advances in Prostaglandin, Thromboxane and Leukotriene Research* (Eds. B. Samuelsson, R. Paoletti and P. Ramwell), Vol. 11, p. 407. Raven Press, New York (1983).

14. S. Samuelsson, in *Advances in Prostaglandin, Thromboxane, and Leukotriene Research* (Eds. B. Samuelsson, R. Paoletti and P. Ramwell), Vol. 11, p. 1. Raven Press, New York (1983).
15. J. Augstein, J. B. Farmer, T. B. Lee, P. Sheard and M. L. Tattersall, *Nature New Biol.* **245**, 215 (1973).
16. R. C. Murphy, S. Hammarstrom and S. Samuelsson. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4275 (1979).
17. H. R. Morris, G. W. Taylor, P. J. Piper and J. R. Tippins, *Nature, Lond.* **285**, 104 (1980).
18. R. A. Lewis, J. M. Drazen, K. F. Austen, D. A. Clark and E. J. Corey, *Biochem. biophys. Res. Commun.* **96**, 271 (1980).
19. P. Sheard, T. B. Lee and M. L. Tattersall, *Monogr. Allergy* **12**, 245 (1977).
20. M. O'Donnell and A. F. Welton, *Agents Actions* **14**, 43 (1984).
21. M. E. Greig and R. L. Griffin, *J. med. Chem.* **18**, 112 (1975).
22. D. R. Buckle, D. J. Outred, J. W. Ross, H. Smith, R. J. Smith, A. Spicer and B. C. Gasson, *J. med. Chem.* **22**, 158 (1979).
23. F. E. Ali, P. A. Dandridge, J. G. Gleason, R. D. Krell, C. H. Kruse, P. G. Lavanchy and K. M. Snader, *J. med. Chem.* **25**, 947 (1982).
24. F. E. Ali, J. G. Gleason, D. T. Hill, R. D. Krell, C. H. Kruse, P. G. Lavanchy and B. W. Volpe, *J. med. Chem.* **25**, 1235 (1982).
25. K. Nishino, H. Ohkubo, M. Ohashi, S. Hara, J. Kito and T. Irikura, *Jap. J. Pharmac.* **33**, 267 (1983).
26. J. W. Tilley, P. Levitan, A. F. Welton and H. J. Crowley, *J. med. Chem.* **26**, 1638 (1983).
27. J. H. Gaddum and Z. P. Picarelli, *Br. J. Pharmac. Chemother.* **12**, 323 (1957).
28. D. M. Engineer, H. R. Morris, P. J. Piper and P. Sirois, *Br. J. Pharmac.* **64**, 211 (1978).
29. J. P. Seale and P. J. Piper, *Eur. J. Pharmac.* **52**, 125 (1978).
30. P. J. Piper and M. N. Samhoun, *Prostaglandins* **21**, 793 (1981).
31. J. Orehek, J. S. Douglas, A. J. Lewis and A. Bouhuys, *Nature New Biol.* **245**, 84 (1973).
32. L. Grodzinska, B. Panczenko and R. J. Gryglewski, *J. Pharm. Pharmac.* **27**, 88 (1975).
33. R. K. Turker and H. Zengil, *Archs int. Physiol. Biochim.* **84**, 833 (1976).
34. B. M. Weichman, R. M. Muccitelli, R. R. Osborn, D. A. Holden, J. G. Gleason and M. A. Wasserman, *J. Pharmac. exp. Ther.* **222**, 202 (1982).
35. N. Feuerstein, M. Foegh and P. W. Ramwell, *Br. J. Pharmac.* **72**, 389 (1981).
36. B. B. Vargaftig, J. Lefort and R. C. Murphy, *Agents Actions* **11**, 574 (1981).
37. R. Hamel, P. Masson, A. W. Ford-Hutchinson, T. R. Jones, G. Brunet and H. Piechuta, *Prostaglandins* **24**, 419 (1982).
38. A. G. Leitch, E. J. Corey, K. F. Austen and J. M. Drazen, *Am. Rev. resp. Dis.* **128**, 639 (1983).
39. J. G. Gleason, R. D. Krell, B. M. Weichman, F. E. Ali and B. Berkowitz, in *Advances in Prostaglandin, Thromboxane and Leukotriene Research* (Eds. B. Samuelsson and R. Paoletti), Vol. 9, p. 243. Raven Press, New York (1982).
40. B. M. Weichman, M. A. Wasserman and J. G. Gleason, *J. Pharmac. exp. Ther.* **228**, 128 (1984).
41. S. Hammarstrom, R. C. Murphy, B. Samuelsson, D. A. Clark, C. Mioskowski and E. J. Corey, *Biochem. biophys. Res. Commun.* **91**, 1266 (1979).
42. E. J. Corey, D. A. Clark, G. Goto, A. Marfat, C. Mioskowski, B. Samuelsson and S. Hammarstrom, *J. Am. chem. Soc.* **108**, 1436 (1980).
43. S. R. Baker, W. B. Jamieson, D. J. Osborne and W. J. Ross, *Tetrahedron Letts.* **22**, 2505 (1981).
44. O. Arunlakshana and H. O. Schild, *Br. J. Pharmac. Chemother.* **14**, 48 (1959).
45. R. F. Furchgott, *Ann. N.Y. Acad. Sci.* **139**, 553 (1967).
46. A. M. Lands, A. Arnold, J. P. McAuliff, F. P. Luduena and T. G. Brown, *Nature, Lond.* **214**, 597 (1967).
47. J. M. Drazen, K. F. Austen, R. A. Lewis, D. A. Clark, G. Goto, A. Marfat and E. J. Corey, *Proc. natn. Acad. Sci. U.S.A.* **77**, 4354 (1980).
48. R. D. Krell, R. Osborn, L. Vickery, K. Falcone, M. O'Donnell, J. Gleason, C. Kinzig and D. Bryan, *Prostaglandins* **22**, 387 (1981).
49. J. H. Fleisch, L. E. Rinkema and S. R. Baker, *Life Sci.* **31**, 577 (1982).
50. R. D. Krell, B. S. Tsai, A. Berdoulay, M. Barone and R. Giles, *Prostaglandins* **25**, 171 (1983).
51. P. Sirois, S. Roy, J. P. Tetrault, P. Borgeat, S. Picard and E. J. Corey, *Prost. Med.* **7**, 327 (1981).
52. P. Sirois, S. Roy and P. Borgeat, *Prostaglandins* **26**, 91 (1983).
53. R. F. Furchgott, in *Handbook of Experimental Pharmacology* (Eds. O. Eichler, A. Farah, H. Herken and A. D. Welch), Vol. 33, p. 283. Springer, New York (1972).
54. D. W. Snyder, M. Barone, M. P. Morrissette, P. R. Bernstein and R. D. Krell, *Pharmacologist* **25**, 205 (1983).
55. D. E. Sok, J. K. Pai, V. Atrache and C. J. Sih, *Proc. natn. Acad. Sci. U.S.A.* **77**, 6481 (1980).
56. S. L. Levinson, *Pharmacologist* **25**, 201 (1983).
57. S. L. Levinson, *Prostaglandins*, in press.
58. S. Krilis, R. A. Lewis, E. J. Corey and K. F. Austen, *J. clin. Invest.* **72**, 1516 (1983).
59. S. S. Pong, R. N. DeHaven, F. A. Kuehl and R. W. Egan, *J. biol. Chem.* **258**, 9616 (1983).
60. S. S. Pong and R. N. DeHaven, *Proc. natn. Acad. Sci. U.S.A.* **80**, 7415 (1983).
61. G. K. Hogaboom, S. Mong, H. L. Wu and S. T. Crooke, *Biochem. biophys. Res. Commun.* **116**, 1136 (1983).
62. G. K. Hogaboom, S. Mong and S. T. Crooke, *Fedn. Proc.* **43**, 1037 (1984).
63. D. W. Goldman, L. A. Gifford and E. J. Goetzl, *Fedn. Proc.* **43**, 845 (1984).