The Journal of Experimental Biology 200, 155–160 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
JEB0603



# CHARACTERISATION OF THE BINDING OF LEUKOTRIENE $B_4$ TO MACROPHAGES OF THE RAINBOW TROUT *ONCORHYNCHUS MYKISS*

LINDA A. BOWDEN[1], STEFANO FIORE[2], COLIN J. RESTALL[1], CHARLES N. SERHAN[2] AND ANDREW F. ROWLEY[1,*]

[1]*School of Biological Sciences, University of Wales Swansea, Singleton Park, Swansea SA2 8PP, UK* and [2]*Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesia, Brigham and Women's Hospital and Harvard Medical School, Brookline, MA 02115, USA*

*Accepted 10 October 1996*

## Summary

**The binding of leukotriene $B_4$ ($LTB_4$) to macrophages from the head kidney of the rainbow trout *Oncorhynchus mykiss* was measured. Binding of $[^3H]LTB_4$ achieved a steady state after approximately 30 min of incubation and was 30 % reversible in the presence of a minimum of 1000-fold excess of $LTB_4$. Scatchard analysis of the kinetics of $LTB_4$ binding over a range of $[^3H]LTB_4$ concentrations indicated the existence of only a single class of receptor with a dissociation constant, $K_D$, of 0.14 nmol $l^{-1}$ and a maximum receptor density, $B_{max}$, of approximately 17 800 sites per macrophage. The $LTB_4$ receptor antagonist LY223982 was ineffective in inhibiting the binding of $[^3H]LTB_4$ to trout macrophages, although another receptor antagonist, $LTB_4$-dimethylamide, displaced a maximum of 25 % of the total binding. $LTB_5$ was equally effective as $LTB_4$ at displacing $[^3H]LTB_4$, while other eicosanoids tested were without significant effect. It is suggested that the putative receptors for $LTB_4$ on trout macrophages are similar to the high-affinity receptors for this compound reported to occur on mammalian granulocytes, although any structural similarities of the binding sites await further investigation.**

Key words: leukotriene receptor, rainbow trout, macrophage, eicosanoids, LY223982, *Oncorhynchus mykiss*.

## Introduction

Leukotriene $B_4$ [5(*S*),12(*R*)-8,10-*trans*-6,14-*cis*-eicosatetraenoic acid, $LTB_4$] is a key pro-inflammatory compound in mammals promoting leucocyte chemotaxis, aggregation, superoxide anion generation and degranulation (Samuelsson *et al.* 1987). Production of $LTB_4$ has been found in several cell types, including granulocytes, lymphocytes, mononuclear phagocytes and keratinocytes in which the required 5-lipoxygenase and $LTA_4$ hydrolase activities are found (Samuelsson *et al.* 1987; Ford-Hutchinson *et al.* 1994). $LTB_4$ interacts with a number of target cells *via* a single type of receptor, termed B-LT (Metters, 1995). This can exist in two states, at least in some cell types. First, high-affinity receptors which are coupled to guanine-nucleotide-binding G-proteins and are involved in chemotactic and chemokinetic responses in granulocytes (Sherman *et al.* 1988; Mong, 1991). The second state is represented by the low-affinity forms which are uncoupled from G-proteins and are associated with degranulation and secretion events in human granulocytes (e.g. Kreisle *et al.* 1985; Mong, 1991). Both forms of the receptor are interconvertible; for example, the receptor can be converted from high to low affinity by treatment with GTP analogues (Sherman *et al.* 1988; Slipertz *et al.* 1993). The B-LT protein has been identified as having an $M_r$ of either 60 kDa (Goldman *et al.* 1991) or 70–80 kDa (Harvey *et al.* 1992), although further structural elucidation is still awaited.

Significantly less is known about $LTB_4$ generation and its biological activities in non-mammalian vertebrates. In amphibians, $LTB_4$ and $LTB_5$ (the latter derived from eicosapentaenoic acid) cause contraction of bullfrog (*Rana catesbeiana*) lung (Andazola *et al.* 1992) but their potential pro-inflammatory ability is apparently untested. The generation and biological activities of leukotrienes in fish, in particular the rainbow trout *Oncorhynchus mykiss*, are well studied. $LTB_4$ biosynthesis occurs both in macrophages and in platelet equivalent cells, termed thrombocytes, in rainbow trout (Pettitt *et al.* 1991; Lloyd-Evans *et al.* 1994). As eicosapentaenoate (20:5, *n*-3) is a common fatty acid in trout phospholipids, $LTB_5$ is also generated in similar amounts to $LTB_4$ by these cells. Unspecified cell types in the brain, skin, ovary, heart and alimentary canal also have $LTB_{4/5}$ biosynthetic capacity (Knight *et al.* 1995). Functional studies have demonstrated that $LTB_4$ is a chemotactic agent for trout leucocytes (Sharp *et al.* 1992), causes transient increases in intracellular $[Ca^{2+}]$ in macrophages (Knight *et al.* 1993) but

*Author for correspondence (e-mail: a.f.rowley@swansea.ac.uk).

does not affect enzyme secretion from trout macrophages (Knight, 1995; Rowley, 1996) or the uptake of foreign test particles by these cells (Knight *et al.* 1993). Experimentally induced inflammatory exudates in the peritoneal cavity of rainbow trout exhibit enhanced levels of $LTB_4$ and other eicosanoids, including prostaglandin $E_2$ and lipoxin $A_4$ (Rowley, 1996). Despite this knowledge of the biosynthesis and functioning of $LTB_4$ in trout, nothing is known about its binding to target cells and the specificity of this reaction. Therefore, the present study characterises the binding of $LTB_4$ to rainbow trout macrophages. This work is the first report of eicosanoid binding sites on non-mammalian leucocytes.

## Materials and methods

### *Chemicals*

$[^3H]LTB_4$ (specific activity 193 Ci mmol$^{-1}$; 7.14 TBq mmol$^{-1}$) was obtained from Amersham Life Sciences (Little Chalfont, UK), while $LTB_4$, $LTB_4$-dimethylamide, $LTB_5$, $LTC_4$, lipoxin $A_4$ ($LXA_4$), 12(*S*)-hydroxyeicosatetraenoic acid [12(*S*)-HETE] and 12(*S*)-hydroxyeicosapentaenoic acid [12(*S*)-HEPE] were obtained from Cascade Biochem Ltd (Reading, UK). LY223982, an $LTB_4$ receptor antagonist, was kindly provided by Lilly Research Laboratories (Indianapolis, USA). All other reagents were obtained from the Sigma Chemical Co. Ltd (Poole, UK), unless otherwise stated, and were the highest grade available.

### *Fish*

Adult triploid rainbow trout, *Oncorhynchus mykiss* (Walbaum), approximate mass 200–250 g, were obtained from Pontlliw Trout Farm, (South Wales, UK), maintained in outdoor freshwater concrete tanks at 10–15 °C and fed *ad libitum* on Mainstream expanded diet (B.P. Nutrition Ltd, Cheshire, UK) prior to use.

### *Macrophage isolation and maintenance*

Trout were killed by immersion in a lethal dose of ethyl *p*-aminobenzoate (0.1 g l$^{-1}$), exsanguinated and the head kidneys removed into $Ca^{2+}/Mg^{2+}$-free Hanks balanced salt solution (HBSS). Each kidney was disrupted through a fine plastic mesh and the resulting cell suspension centrifuged (1500 ***g*** for 5 min at 4 °C) to remove soluble material and cell debris. The cell pellet was resuspended, layered onto a preformed 55 % Percoll gradient prepared as described previously (Pettitt *et al.* 1991) and centrifuged at 3000 ***g*** for 30 min at 4 °C. The layer containing macrophages and other contaminating leucocytes (mainly lymphocytes) was removed and the cells washed by centrifugation in $Ca^{2+}/Mg^{2+}$-free HBSS. The cells obtained from approximately six fish were then pooled and approximately $2\times10^7$ adherent cells were placed in 9 cm tissue-culture-grade Petri dishes (Nunc, Paisley, UK) containing 5 ml of HBSS and left to attach for 30–60 min. The dishes were then gently agitated to resuspend any non-adherent cells, which were discarded. The remaining adherent cells were incubated in 5 ml of RPMI (Gibco, Paisley, UK) containing 5 % heterologous fish serum overnight at 18 °C. After incubating the cells for approximately 15 min at 18 °C with $Ca^{2+}/Mg^{2+}$-free HBSS containing 5 mmol l$^{-1}$ EDTA, the remaining cells were resuspended by gentle aspiration, washed once with $Ca^{2+}/Mg^{2+}$-free HBSS and counted using a haemocytometer. Typically in excess of 90 % of the recovered cells re-adhered to glass and were judged to be macrophages on the basis of their morphology.

### *Binding time-course experiments*

Macrophages ($2\times10^7$ ml$^{-1}$) in $Ca^{2+}/Mg^{2+}$-containing HBSS ([$Ca^{2+}$] 1.3 mmol l$^{-1}$; [$Mg^{2+}$] 0.4 mmol l$^{-1}$) were incubated at less than 4 °C in a salt ice-water bath in parallel with either 0.03 nmol l$^{-1}$ $[^3H]LTB_4$ alone or 0.03 nmol l$^{-1}$ $[^3H]LTB_4$ together with a $10^4$-fold excess of $LTB_4$ to establish the levels of total and non-specific binding respectively. At designated time intervals (1, 5, 10, 30 and 60 min), samples (0.5 ml) were removed from both incubations and layered onto 350 µl of silicone oil (relative density 1.03) in Eppendorf tubes which were centrifuged (10 000 ***g*** for 1 min) to pellet the cells, isolating them from the unbound label. The upper aqueous phase was removed with a small amount of the silicone oil and the tube and oil interface were washed with methanol (750 µl) which was drawn off with the remainder of the oil. The pellet was then disrupted with 30 % ethanol, 20 % trichloroacetic acid in $Ca^{2+}/Mg^{2+}$-free HBSS (200 µl) and the tube and contents mixed with 5 ml of Pico Fluor 40 (Packard Instruments B.V., Groningen, The Netherlands). The radioactivity associated with the pellet was determined over a 5 min counting period using a 1217 Rackbeta liquid scintillation counter (LKB, Pharmacia, Milton Keynes, UK).

### *Determination of the quantity of cold homoligand required to displace specific binding*

Macrophages ($2\times10^7$ ml$^{-1}$) in $Ca^{2+}/Mg^{2+}$-containing HBSS were incubated with 0.03 nmol l$^{-1}$ $[^3H]LTB_4$ and a range of concentrations between 0.3 nmol l$^{-1}$ and 0.3 µmol l$^{-1}$ of cold homoligand ($LTB_4$) for 30 min at less than 4 °C. Samples (0.5 ml) of the incubation mixture were then layered onto cushions of silicone oil and treated as described above.

### *Scatchard analysis of $LTB_4$ binding to macrophages*

Macrophages ($2\times10^7$ ml$^{-1}$) in $Ca^{2+}/Mg^{2+}$-containing HBSS were incubated with a range of concentrations of $[^3H]LTB_4$ (0.05, 0.1, 0.3, 0.6, 0.9 and 1.2 nmol l$^{-1}$) both in the presence and in the absence of a 1000-fold excess of cold $LTB_4$ for 30 min at less than 4 °C. Samples (0.5 ml) of the cell suspension were then removed and layered onto a silicone oil cushion and centrifuged to separate the cells from the unbound label. The upper aqueous phase was carefully removed, mixed with scintillation fluid and the quantity of label present was determined. The tube was then washed, the cell pellet disrupted and the quantity of label present was determined.

### *Determination of the specificity of the $LTB_4$ binding sites*

Macrophages ($2\times10^7$ ml$^{-1}$) in $Ca^{2+}/Mg^{2+}$-containing HBSS were incubated with a variety of related eicosanoids [$LTB_4$,

$LTB_5$, $LXA_4$, $LTC_4$, 12(*S*)-HETE, 12(*S*)-HEPE] or the $LTB_4$ receptor antagonists LY223982 and $LTB_4$-dimethylamide, at three different concentrations ($3\times10^{-8}$, $3\times10^{-9}$ or $3\times10^{-10}$ mol l$^{-1}$) together with 0.03 nmol l$^{-1}$ [$^3$H]$LTB_4$ for 30 min at less than 4 °C. The results were expressed as a percentage of the label bound to cells incubated with radioactive ligand in the absence of a competing compound.

## Results

The non-specific association of the radioactive label with trout macrophages with time was established by incubating the cells with both [$^3$H]$LTB_4$ and a $10^4$-fold excess of cold $LTB_4$. Such conditions have been calculated to prevent 99.9 % of receptor occupancy by the radiolabel (Hulme and Birdsall, 1992), thereby identifying the quantity of radioactivity that had partitioned into the membrane or other hydrophobic areas, had become entrapped in the cell pellet or had become associated with the microcentrifuge tube. Throughout the incubation, the levels of non-specific binding did not exceed 8.2±1.0 % (mean ± S.D., *N*=6) of the total binding. The level of specific binding was calculated by subtracting the non-specific binding from the total amount of label associated with the cell pellet.

Both the total and specific binding of [$^3$H]$LTB_4$ achieved a steady state at approximately 30 min (Fig. 1). A 30 min incubation time was therefore used subsequently to ensure that a suitable concentration of unlabelled (cold) homoligand had been used to determine non-specific binding. At least a 1000-fold excess of cold $LTB_4$ was required to compete with the specifically bound radioactive ligand to display a constant level of non-specific binding. Furthermore, once at equilibrium, this binding was partially reversible with approximately 30 % reduction in binding following the addition of a 1000-fold excess of cold $LTB_4$ after 20 min of incubation. As shown in Fig. 2A, as the concentration of [$^3$H]$LTB_4$ increased, the specific binding approached saturation. When replotted as a Scatchard plot (Fig. 2B), linear regression analysis indicated that there is a single class of receptor sites with a $K_D$ of 0.14±0.003 nmol l$^{-1}$ and a $B_{max}$ of 0.295±0.006 pmol per $10^7$ macrophages or 17 800±3600 binding sites per cell (mean values ± S.E.M., *N*=3). The linear nature of the Scatchard plots

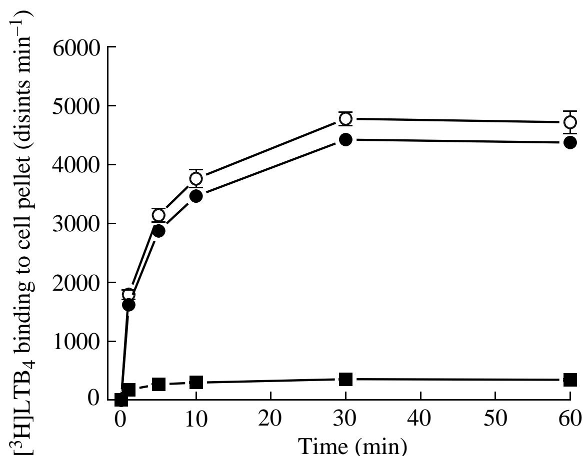


Fig. 1. Time course of [$^3$H]$LTB_4$ binding to trout macrophages determined at less than 4 °C. Macrophages ($2\times10^7$) were incubated with 0.03 nmol l$^{-1}$ [$^3$H]$LTB_4$ in the absence (○) or presence (■) of a $10^4$-fold excess of cold homoligand. Specific binding (●) was calculated by subtracting the non-specific bound label from the total radiolabel. Results shown are means ± S.E.M of three separate experiments using different batches of cells performed with duplicate determinations at each time interval.

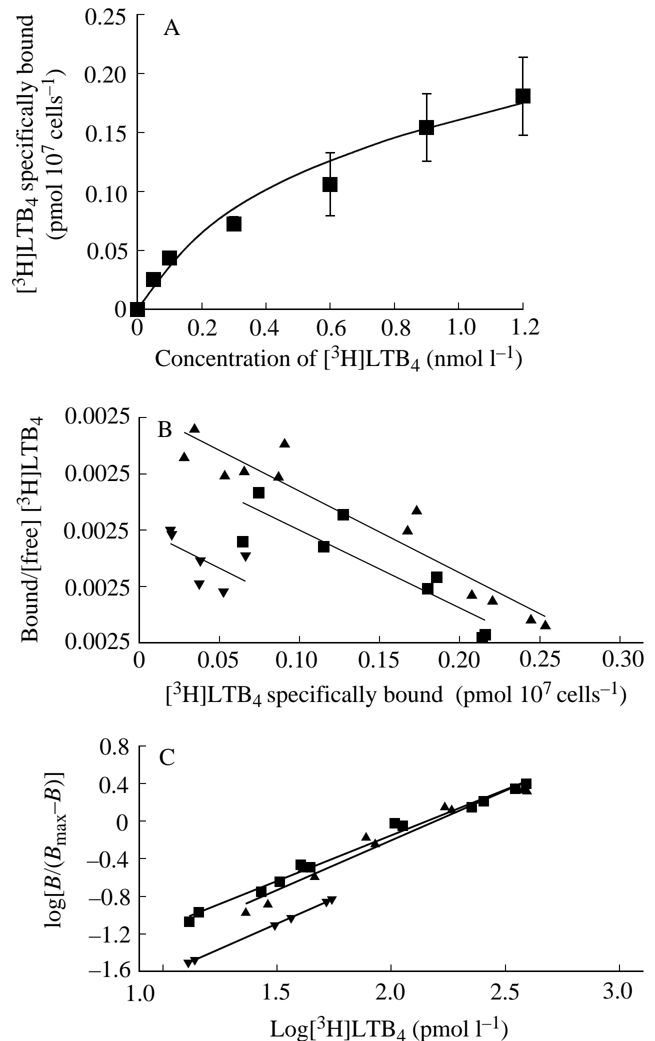


Fig. 2. (A) Saturation analysis of [$^3$H]$LTB_4$ binding to rainbow trout macrophages. Values are means ± S.E.M of three separate experiments each performed in duplicate. (B) Scatchard analysis of [$^3$H]$LTB_4$ binding to rainbow trout macrophages. The lines represent linear regression analyses of data from three separate experiments where $r$=−0.887 (■), −0.953 (▲) and −0.585 (▼). The linear nature of these plots suggests a single binding site with a mean $K_D$ of 0.14 nmol l$^{-1}$ and $B_{max}$ of approximately 17 800 binding sites per cell. (C) Hill analysis of [$^3$H]$LTB_4$ binding to rainbow trout macrophages. The lines represent linear regression analyses of three experiments where $r$=0.994 (■), 0.979 (▲) and 1.00 (▼). The mean Hill coefficient was determined to be 1.03.

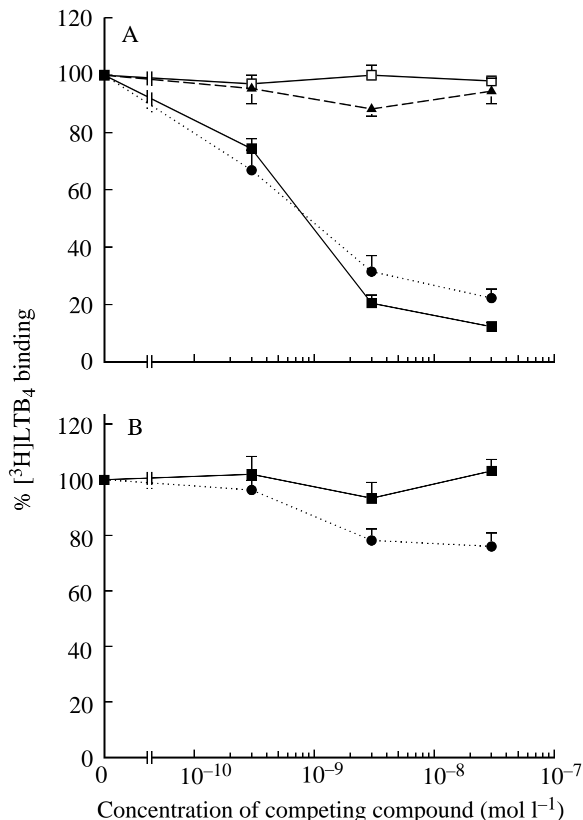


Fig. 3. (A) Competition of [$^3$H]LTB$_4$ binding with structurally related compounds: LTB$_4$ (■), LTB$_5$ (●), 12-(*S*)HETE (□) and LXA$_4$ (▲), and (B) with the known LTB$_4$ mammalian receptor antagonists LY223982 (■) and LTB$_4$-dimethylamide (●). The results are expressed as a percentage of total binding in the absence of any competing compound and represent the mean ± S.E.M. of 3–4 separate experiments. For the sake of clarity, the results for two other compounds, 12-(*S*)HEPE and LTC$_4$, are not shown, but they were not significantly different from those shown for LXA$_4$ and 12-(*S*)HETE.

did not suggest the existence of cooperativity, and the Hill plot, which yielded a coefficient of 1.03, confirmed this (Fig. 2C).

Fig. 3 demonstrates the ability of several other lipoxygenase products previously shown to be generated by trout macrophages [LXA$_4$, 12-(*S*)HETE, 12-(*S*)HEPE, LTB$_5$, LTC$_4$; Pettitt *et al.* 1991; Knight *et al.* 1995] to antagonise the binding of [$^3$H]LTB$_4$ to macrophages. It was found that LTB$_5$ interacted with the binding site in a similar manner to LTB$_4$, with an estimated IC$_{50}$ value of $8\times10^{-10}$ mol l$^{-1}$. The other predominant trout macrophage-derived lipoxygenase products had no significant effect upon the ability of the putative receptor to bind radiolabelled LTB$_4$ at the concentrations tested. The LTB$_4$ receptor antagonist LY223982 had no significant displacement effect on [$^3$H]LTB$_4$ binding to macrophages over the concentration range used, although LTB$_4$-dimethylamide, a partial LTB$_4$ receptor antagonist in mammals (Falcone and Aharony, 1990), did displace over 25 % of the total binding (IC$_{50}$>$1\times10^{-8}$ mol l$^{-1}$).

## Discussion

The results of the present work demonstrate that LTB$_4$ binding to rainbow trout macrophages is structurally specific, apparently saturable, but only approximately 30 % reversible after 20 min in the presence of a 1000-fold excess of unlabelled homoligand. Whilst under ideal conditions binding would be fully reversible to non-specific binding levels, other authors have also reported similar difficulties with human granulocytes with levels of displacement of approximately 70 % (Goldman and Goetzl, 1982). This non-displacement may be due to internalisation of the label similar to that described by Lin *et al.* (1984) and is usually prevented in studies with mammalian cells by lowering the temperature sufficiently to prevent possible internalisation and metabolism of the label. The fish used in these experiments were maintained at temperatures between 10 and 15 °C and the experiments were performed at approximately 2 °C. Consequently, the temperature difference between the two conditions may not have been sufficient to prevent internalisation of the label especially as fish leucocytes show a high degree of homeoviscous adaptation, allowing them to maintain cellular processes, such as endocytosis, at low environmental temperatures (Bowden *et al.* 1996). The label may also have been internalised by a transport system similar to that described for LXA$_4$ (Simchowitz *et al.* 1994), although the only similar system identified for LTB$_4$ in humans results in the release of this compound from cells (Lam *et al.* 1990) and no import systems have been described.

Analysis of the kinetics of LTB$_4$ binding over the [$^3$H]LTB$_4$ concentration range used indicated that only a single class of receptor was present with a $K_D$ of 0.14 nmol l$^{-1}$ and a $B_{max}$ of 17 800 binding sites per macrophage. This does not appear to resemble the high- and low-affinity B-LT states found in some mammalian neutrophils and eosinophils (e.g. Goldman and Goetzl, 1984; Goldman *et al.* 1986; Maghni *et al.* 1991; Sehmi *et al.* 1992), although Kreisle *et al.* (1985) were able to identify only one high-affinity LTB$_4$ receptor type on rat neutrophils.

The putative rainbow trout LTB$_4$ receptors most closely resemble the mammalian high-affinity receptors, which transduce the chemotactic response to LTB$_4$ (Sehmi *et al.* 1992). Trout head kidney leucocytes display both a chemotactic response to LTB$_4$ (Sharp *et al.* 1992) and an increase in the intracellular concentration of $Ca^{2+}$ (EC$_{50}$=1.2 nmol l$^{-1}$) in the presence of this eicosanoid (Knight *et al.* 1993). It is also interesting that Knight (1995) was unable to demonstrate an increase in degranulation of these cells in response to LTB$_4$ at concentrations above $10^{-5}$ mol l$^{-1}$, which is a cellular response attributed to the mammalian low-affinity receptors (Goldman and Goetzl, 1984). Whilst it is tempting to speculate that only one receptor state exists, and that receptor occupancy may result in a rise in intracellular [$Ca^{2+}$] and induce a chemotactic response, further work demonstrating the antagonistic effects of certain compounds on both these responses and on receptor binding is required.

In the present study, it was not possible to demonstrate the inhibition of [$^3$H]LTB$_4$ binding by LY223982, although LTB$_4$-

dimethylamide was a partial antagonist and may demonstrate further inhibition at higher concentrations. The ineffectiveness of a further mammalian $LTB_4$ receptor antagonist, LY255283, was also reported by Andazola *et al.* (1992) whilst characterising the bullfrog lung $LTB_4$ receptor and together these results suggest that the piscine and anuran leukotriene receptors are both different from their mammalian counterparts in terms of their structure. Further insight into the potential dissimilarity between eicosanoid receptors in fish and mammals is reflected in the finding that the human cDNA probe for the $LXA_4$ receptor on human granulocytes (Fiore *et al.* 1994) did not hybridize with trout macrophage mRNA (S. Fiore, C. N. Serhan, L. A. Bowden and A. F. Rowley, unpublished observations), suggesting that sequence homology between these two 'receptors' in fish and humans may be limited.

Finally, the structural specificity of this binding site elucidated by the competition studies is also similar to that for bullfrog lung (Andazola *et al.* 1992). Both display a higher affinity for the eicosapentaenoic-acid-derived lipoxygenase product $LTB_5$ than for the equivalent mammalian receptor (Falcone and Aharony, 1990) and neither interacts with peptido-leukotrienes. This former observation is not unexpected since membranes of trout macrophages contain a high proportion of eicosapentaenoic acid and consequently produce similar amounts of $LTB_5$ and $LTB_4$ (Pettitt *et al.* 1989, 1991). Furthermore, both 4- and 5-series leukotrienes have similar biological potency in fish (Secombes *et al.* 1994), unlike the situation in mammals where $LTB_5$ is a weak chemotactic factor in comparison with $LTB_4$ (e.g. Lee *et al.* 1984). The similarity in receptor occupancy by $LTB_4$ and $LTB_5$ reported in the present study with trout is therefore not surprising and may reflect an evolutionary adaptation to the situation where both 4- and 5-series lipoxygenase products naturally exist in these animals.

We wish to thank Dr R. Newton, University of Wales Swansea, for helpful discussion during these studies. This work was supported by the Agricultural and Food Research Council (grant AG58/510 to A.F.R.), NIH (grants GM38765 and PO1-DK50305 to C.N.S.) and a studentship to L.A.B from the Natural Environment Research Council. We also thank Lilly Research Laboratories for the generous provision of LY223982.

## References

ANDAZOLA, J. J., UNDERWOOD, J. A., CHIONO, M., TORRES, O. A. AND HERMAN, C. A. (1992). Leukotriene $B_4$ and leukotriene $B_5$ have binding sites on lung membranes and cause contraction on bullfrog lung. *J. Pharmac. exp. Ther.* **263**, 1111–1117.

BOWDEN, L. A., RESTALL, C. J. AND ROWLEY, A. F. (1996). The influence of environmental temperature on membrane fluidity, fatty acid composition and lipoxygenase product generation in head kidney leucocytes of the rainbow trout, *Oncorhynchus mykiss*. *Comp. Biochem. Physiol.* (in press).

FALCONE, R. C. AND AHARONY, D. (1990). Modulation of ligand binding to leukotriene $B_4$ receptors on guinea pig lung membranes by sulfhydryl modifying reagents. *J. Pharmac. exp. Ther.* **255**, 565–571.

FIORE, S., MADDOX, J. F., PEREZ, H. D. AND SERHAN, C. N. (1994). Identification of human cDNA encoding a functional high affinity lipoxin $A_4$ receptor. *J. exp. Med.* **180**, 253–260.

FORD-HUTCHINSON, A. W., GRESSER, M. AND YOUNG, R. N. (1994). 5-Lipoxygenase. *A. Rev. Biochem.* **63**, 383–417.

GOLDMAN, D. W., ENKEL, H., GIFFORD, L. A., CHENOWETH, D. E. AND ROSENBAUM, J. T. (1986). Lipopolysaccharide modulates receptors for leukotriene $B_4$, C5a and formyl-methionyl-leucyl-phenylalanine on rabbit polymorphonuclear leukocytes. *J. Immunol.* **137**, 1971–1976.

GOLDMAN, D. W., GIFFORD, L. A., YOUNG, R. N., MAROTTI, T., CHEUNG, K. L. AND GOETZL, E. J. (1991). Affinity labelling of the membrane protein-binding component of human polymorphonuclear leukocyte receptors for leukotriene $B_4$. *J. Immunol*. **146**, 2671–2677.

GOLDMAN, D. W. AND GOETZL, E. J. (1982). Specific binding of leukotriene $B_4$ to receptors on human polymorphonuclear leukocytes. *J. Immunol.* **129**, 1600–1604.

GOLDMAN, D. W. AND GOETZL, E. J. (1984). Heterogeneity of human polymorphonuclear leukocyte receptors for leukotriene B. *J. exp. Med.* **153**, 482–487.

HARVEY, J. P., KOO, C. H., BOGGS, J. M., YOUNG, R. N. AND GOETZL, E. J. (1992). Mouse monoclonal antibody to a latent epitope of leucocyte receptors for leukotriene $B_4$. *Immunology* **76**, 122–128.

HULME, E. C. AND BIRDSALL, N. J. M. (1992). Strategy and tactics in receptor-binding studies. In *Receptor Ligand Interactions* (ed. E. C. Hulme), pp. 63–176. Oxford: Oxford University Press.

KNIGHT, J. (1995). Distribution and function of lipoxins and other eicosanoids in the rainbow trout, *Oncorhynchus mykiss*. PhD thesis, University of Wales.

KNIGHT, J., HOLLAND, J. W., BOWDEN, L. A., HALLIDAY, K. AND ROWLEY, A. F. (1995). Eicosanoid generating capacities of different tissues from the rainbow trout, *Oncorhynchus mykiss*. *Lipids* **30**, 451–458.

KNIGHT, J., LLOYD-EVANS, P., ROWLEY, A. F. AND BARROW, S. E. (1993). Effects of lipoxins and other eicosanoids on phagocytosis and intracellular calcium mobilisation in rainbow trout (*Oncorhynchus mykiss*) leukocytes. *J. Leukocyte Biol*. **54**, 518–522.

KREISLE, R. A., PARKER, C. W., GRIFFIN, G. L., SENIOR, R. M. AND STENSON, W. F. (1985). Studies of leukotriene $B_4$-specific binding and function in rat polymorphonuclear leukocytes: absence of a chemotactic response. *J. Immunol*. **134**, 3356–3363.

LAM, B. K., GAGNON, K., AUSTEN, F. AND SOBERMAN, R. J. (1990). The mechanisms of leukotriene $B_4$ export from human polymorphonuclear leukocytes. *J. biol. Chem.* **265**, 13438–13441.

LEE, T. H., MENCIA-HEURTA, J.-M., SHIH, C., COREY, E. J., LEWIS, R. A. AND AUSTEN, K. F. (1984). Characterisation of biologic properties of 5,12-dihydroxy derivatives of eicosapentaenoic acid, including leukotriene $B_5$ and the double lipoxygenase product. *J. biol. Chem.* **259**, 2383–2389.

LIN, A. H., RUGGSED, P. L. AND GORMAN, R. R. (1984). Leukotriene $B_4$ binding to human neutrophils. *Prostaglandins* **28**, 837–842.

LLOYD-EVANS, P., BARROW, S. E., HILL, D. J., BOWDEN, L. A., RAINGER, G. E. AND ROWLEY, A. F. (1994). Eicosanoid generation and effects on the aggregation of thrombocytes from the rainbow trout, *Oncorhynchus mykiss*. *Biochim. biophys. Acta* **1215**, 291–299.

MAGHNI, K., DE BRUM-FERNANDES, A. J., FOLDES-FILEP, E., GAUDRY,

M., BORGEAT, P. AND SIROIS, P. (1991). Leukotriene $B_4$ receptors on guinea pig alveolar eosinophils. *J. Pharmac. exp. Ther.* **258**, 784–789.

METTERS, K. M. (1995). Leukotriene receptors. *J. Lipid Mediators Cell Signalling* **12**, 413–427.

MONG, S. (1991). Receptors and receptor antagonists for mammalian 5-lipoxygenase products. In *Lipoxygenases and Their Products* (ed. S. T. Crooke and A. Wong), pp. 185–206. London: Academic Press.

PETTITT, T. R., ROWLEY, A. F. AND BARROW, S. E. (1989). Synthesis of leukotriene B and other conjugated triene lipoxygenase products by blood cells of the rainbow trout, *Salmo gairdneri. Biochim. biophys. Acta* **1003**, 1–8.

PETTITT, T. R., ROWLEY, A. F., BARROW, S. E., MALLET, A. I. AND SECOMBES, C. J. (1991). Synthesis of lipoxins and other lipoxygenase products by macrophages from the rainbow trout, *Oncorhynchus mykiss*. *J. biol. Chem.* **266**, 8720–8726.

ROWLEY, A. F. (1996). The evolution of inflammatory mediators. *Mediators Inflammation* **5**, 3–13.

SAMUELSSON, B., DAHLEN, S.-E., LINDGREN, J. Å., ROUZER, C. A. AND SERHAN, C. N. (1987). Leukotrienes and lipoxins: Structures, biosynthesis and biological effects. *Science* **237**, 1171–1176.

SECOMBES, C. J., CLEMENTS, K., ASHTON, I. AND ROWLEY, A. F. (1994). The effect of eicosanoids on rainbow trout, *Oncorhynchus mykiss*, leucocyte proliferation. *Vet. Immunol. Immunopathol.* **42**, 367–378.

SEHMI, R., ROSSI, A. G., KAY, A. B. AND CROMWELL, O. (1992). Identification of receptors for leukotriene $B_4$ expressed in guinea-pig peritoneal eosinophils. *Immunology* **77**, 129–135.

SHARP, J. E., PETTITT, T. R., ROWLEY, A. F. AND SECOMBES, C. J. (1992). Lipoxin- induced migration of fish leukocytes. *J. Leukocyte Biol*. **51**, 140–145.

SHERMAN, J. W., GOETZL, E. J. AND KOO, C. H. (1988). Selective modulation by guanine nucleotides of the high affinity subset of plasma membrane receptors for leukotriene $B_4$ on human polymorphonuclear leukocytes. *J. Immunol.* **140**, 3900–3904.

SIMCHOWITZ, L., FIORE, S. AND SERHAN, C. N. (1994). Carrier-mediated transport of lipoxin $A_4$ in human neutrophils. *Am. J. Physiol*. **267**, C1525–C1534.

SLIPERTZ, D. M., SCOGGAN, K. A., NICHOLSON, D. W. AND METTERS, K. M. (1993). Photoaffinity labelling and radiation inactivation of the leukotriene $B_4$ receptor in human myeloid cells. *Eur. J. Pharmac.* **244**, 161–173.