obviously speculative at present, the suggestion is open to experiment. If PAF receptor binding and activation is important for proliferation, it follows that the growth advantage of at least some tumours may be determined by an increase in the number or the affinity of PAF receptors. This would render them particularly sensitive to cytotoxic PAF analogues; and might explain, in part, why these compounds are selectively cytotoxic to certain tumour cells. Whatever the explanation, our results encourage an investigation of PAF receptor levels in a variety of tumour cell types which are known to be sensitive to the PAF analogues used in this study.

In summary, *trans*-2,5-bis-(3,4,5-trimethoxyphenyl)-tetrahydrofuran (L-652,731), an antagonist which binds to platelet activating factor receptors, protects WEHI-3B myelomonocytic leukaemic cells against the toxic effect of two alkyl lysophospholipids, $C_{18}OCH_3$-PAF and SRI 62,834. Uptake of $^3HC_{18}OCH_3$-PAF was reduced in the presence of the antagonist. These findings suggest that the selective anti-tumour action of alkyl lysophospholipids may be related to differences of PAF receptor function in normal and tumour cells.

Acknowledgements—This work was supported by the Cancer Research Campaign and the Medical Research Council.

Paterson Institute for Cancer Research
Christie Hospital and Holt Radium Institute
Wilmslow Road
Manchester M20 9BX, U.K.

GEORGE W. BAZILL
T. M. DEXTER

REFERENCES

1. Andreesen R, Modollel M, Weltzien HU, Eibl H, Common HH, Lohr GW and Munder PG, Selective destruction of human leukaemic cells by alkyl lysophospholipids. *Cancer Res* **38**: 3894–3899, 1978.
2. Berdel WE, Andreesen R and Munder PG, In: *Phospholipids and Cellular Regulation* (Ed. Kuo JF), pp. 41–74. CRC Press, Boca Raton, FL, 1985.
3. Berdell WE, Greiner E, Fink U, Stavrou D, Reichert A, Rasletter J, Hoffman DR and Snyder F, Cytotoxicity of alkyl-lysophospholipid derivatives and low alkyl cleavage enzyme activities in rat brain tumour cells. *Cancer Res* **43**: 541–545, 1983.
4. Hoffman DR, Hadju J and Snyder F, Cytotoxicity of platelet activating factor and related alkyl-phospholipid analogues in human leukaemic cells, polymorphonuclear neutrophils and skin fibroblasts. *Blood* **63**: 545–552, 1984.
5. Berdel WE, Bausert WRE, Fink U, Rasletter J and Munder PG, Anti-tumour action of Alkyl-lysophospholipids. *Anti-cancer Res* **1**: 345–352, 1981.
6. Glasser L, Somberg LB and Vogler WR, Purging murine leukaemic bone marrow with alkyl lysophospholipids. *Blood* **64**: 1288–1291, 1984.
7. Yamamoto N and Ngwenya BZ, Activation of mouse peritoneal macrophages by lysophospholipids and ether derivations of neutral lipids and phospholipids. *Cancer Res* **47**: 2008–2013, 1987.
8. Soodsma JF, Piandosi C and Snyder F, The biocleavage of alkylglyceryl ethers in Morris hepatomas and other transplantable neoplasms. *Cancer Res* **30**: 309–311, 1970.
9. Modollel M, Andreeson R, Pahike W, Brugger U and Munder PG, Disturbance of phospholipid metabolism during selective destruction of tumour cells induced by alkyl lysophospholipids. *Cancer Res* **39**: 4681–4686, 1979.
10. Herrmann DBJ and Neumann HA, Cytotoxic ether phospholipids. *J Biol Chem* 7742–7747, 1986.
11. Biftu T, Gamble NF, Doebber T, San-Bao Hwang, Tsung-Ying Shen, Snyder J, Springer JP and Stevensen R, Conformation and activity of tetrahydrofuran lignans and analogues as specific platelet activating factor antagonists. *J Med Chem* **29**: 1917–1921, 1986.
12. Spooncer E, Heyworth CM and Dexter TM, Self-renewal and differentiation of Interleukin-3 dependent multi-potent stem cells are modulated by stromal cells and serum factors. *Differentiation* **31**: 111–118, 1986.
13. Bazill GW, Haynes M, Garland J and Dexter TM, Characterisation and partial purification of a growth factor in WEHI-3B cell conditioned medium. *Biochem J* **210**: 747–759, 1983.
14. San-bao Hwang, My-Hanh Lam, Biftu J, Beatie TR and Tsung-Ying Shen, *Trans*-2,5-*bis*-(3,4,5-trimethoxyphenyl)tetrahydrofuran. An orally active specific and competitive receptor antagonist of platelet activating factor. *J Biol Chem* **260**: 15639–15645, 1985.
15. Whetton AD, Bazill GW and Dexter TM, Stimulation of hexose uptake by haemopoietic growth factors occurs in WEHI-3B myelomonocytic leukaemia cells: a possible mechanism for loss of growth control. *J Cell Physiol* **123**: 73–78, 1985.

Biochemical Pharmacology, Vol. 38, No. 2, pp. 377–379, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

Cyclo-oxygenase inhibition does not unmask leukotriene release during ischaemia-reperfusion of the rat heart *in vitro*

(*Received* 14 *June* 1988; *accepted* 12 *September* 1988)

The calcium ionophore, A23187, stimulates leukotriene efflux from the rat isolated perfused heart [1, 2], probably reflecting calcium-induced activation of 5-lipoxygenase [3]. Reperfusion, following global ischaemia *in vitro*, promotes calcium accumulation by the myocardium (e.g. [4]), but does not induce leukotriene release [1, 2]. This stimulus does, however, promote prostaglandin synthesis, especially prostacyclin [2, 5]. Karmazyn [6] has recently reported that prostacyclin inhibits cardiac leukotriene release following calcium accumulation induced by the "calcium-paradox" in rat and guinea-pig, isolated perfused hearts. We have, therefore, determined the effect of cyclo-oxygenase inhibition on leukotriene release from the rat isolated perfused heart subjected to global ischaemia, followed by reperfusion.

Materials and methods

Male Wistar–Sprague–Dawley cross-bred rats (Glaxo Group Research Ltd., Ware, U.K.) were treated with aspirin (15 mg/kg i.v.) or vehicle (1% bicarbonate in 0.9% saline) at least 1 hr before sacrifice. Hearts were subsequently removed and perfused by the Langendorff tech-

nique, using modified Krebs solution containing 5 mM acetate [7] at a pressure of 80 mmHg (coronary flow = 20.7 ± 0.8 ml/min; mean ± SEM, N = 24). Following equilibrium (~20 min), global ischaemia was induced by reducing the flow rate to 0.7 ml/min for either 1 or 4 hr. Reperfusion was then initiated (at 50 mmHg) for either 15 min (1 hr ischaemia) or 60 min (4 hr ischaemia), following which, A23187 was infused at a final concentration of 10^{-6}M. Samples of coronary effluent were collected throughout both protocols and assayed for:

(i) Lactate dehydrogenase (LDH) activity [8].
(ii) 6-Keto-prostaglandin $F_{1\alpha}$ ($6KPGF_{1\alpha}$; the breakdown product of prostacyclin) by radioimmunoassay using a specific antiserum from Seragen (U.S.A.) $6KPGF_{1\alpha}$ was assayed in unextracted samples of coronary effluent.
(iii) Leukotriene C_4/D_4 and leukotriene B_4 by radioimmunoassay using antisera from New England Nuclear (U.S.A., LTC_4/D_4) and Amersham International (Amersham, U.K., LTB_4). Prior to assay, samples of coronary effluent were concentrated using Sep Pak C_{18} columns (Waters Associates, U.S.A.) as originally described by Eskra *et al.* [9]. Recovery of LTC_4/D_4 when applied at 5 ng/ml was 66.3 ± 6.9% (mean ± SEM, N = 15) and results were not corrected for extraction efficiency.

Results and discussion

The results are summarised in Tables 1 and 2. Induction of ischaemia did not significantly enhance the release of LTC_4/D_4, $6KPGF_{1\alpha}$ or LDH. Reperfusion, following either 1 or 4 hr of ischaemia, had no effect on leukotriene production, but significantly stimulated both $6KPGF_{1\alpha}$ synthesis and LDH release. Levels of LTB_4 were undetectable during both ischaemia and reperfusion (<0.03 ng/min). Assay of samples with an antibody recognising LTC_4/D_4 and LTE_4 (Amersham, U.K.) also failed to reveal any significant leukotriene synthesis during ischaemia-reperfusion (results not shown). The rat heart clearly had the capacity to synthesise leukotrienes under these conditions, since infusion of A23187 following reperfusion, significantly stimulated both LTC_4/D_4 and LTB_4 release, whilst that of $6KPGF_{1\alpha}$ was further enhanced. Pretreatment of animals with aspirin inhibited $6KPGF_{1\alpha}$ production in response to ischaemia-reperfusion and infusion of A23187. Inhibition of $6KPGF_{1\alpha}$ synthesis did not, however, result in any detectable release of leukotriene, nor did cyclo-oxygenase inhibition affect LDH release. Thus, in the rat isolated perfused heart, the inability of ischaemia-reperfusion to stimulate leukotriene production is probably not due to inhibition by prostacyclin or to "inactivation" or loss of 5-lipoxygenase during the experiment, since infusion of A23187 always uncovered leukotriene synthesis. The production of LTC_4/D_4 in response to A23187 was enhanced by pretreatment with aspirin, but this was not significantly different from non-aspirinised animals. The results of this study do not directly contradict those of Karmazyn [6], since the concentration of prostacyclin employed to inhibit leukotriene synthesis (~10 ng/min) was much higher than that measured in our studies employing ischaemia-reperfusion.

In summary, prostacyclin production is unlikely to be responsible for the inability of rat hearts to release leukotrienes in response to ischaemia-reperfusion *in vitro*. At present it is not clear why the stimulus of ischaemia-reperfusion does not cause the release of leukotrienes despite substantial cellular damage as judged by LDH efflux. The results suggest, however, that the cellular sources of LDH release and leukotriene synthesis are dissimilar.

Department of Respiratory Pharmacology & Biochemistry
Glaxo Group Research
Ware
Herts SG12 0DJ, U.K.

M. A. TREVETHICK*
A. K. BROWN
G. WRIGHT
P. STRONG

* To whom all correspondence should be addressed.

Table 1. Eicosanoid release during 1 hr ischaemia and 15 min reperfusion

		LTB_4 (ng/min)	LTC_4/D_4 (ng/min)	$6KPGF_{1\alpha}$ (ng/min)	LDH (mU/min)
Basal	—V	<0.03	0.1 ± 0.4	0.9 ± 0.2	49 ± 12
	—A	<0.03	0.2 ± 0.1	0.2 ± 0.1	44 ± 3
Ischaemia	—V	<0.03	<0.03	0.5 ± 0.1	19 ± 4
	—A	<0.03	0.06 ± 0.04	0.06 ± 0.04	13 ± 2
Reperfusion	—V	<0.03	0.26 ± 0.08	1.5 ± 0.4*	203 ± 53*
	—A	<0.03	0.29 ± 0.08	0.2 ± 0.1	199 ± 51*
A23187	—V	1.2 ± 0.02	14.4 ± 5.7	22.3 ± 2.2	121 ± 12
	—A	1.9 ± 0.01	26.4 ± 9.9	1.4 ± 0.9	188 ± 24

Table 2. Eicosanoid release during 4 hr ischaemia and 60 min reperfusion

		LTB_4 (ng/min)	LTC_4/D_4 (ng/min)	$6KPGF_{1\alpha}$ (ng/min)	LDH (mU/min)
Basal	—V	<0.03	0.5 ± 0.4	0.8 ± 0.3	67 ± 17
	—A	<0.03	0.2 ± 0.1	0.2 ± 0.1	60 ± 7
Ischaemia	—V	<0.03	<0.03	0.4 ± 0.2	28 ± 8
	—A	<0.03	<0.03	0.1 ± 0.1	22 ± 3
Reperfusion	—V	<0.03	0.05 ± 0.01	3.9 ± 1.3*	373 ± 65*
	—A	<0.03	0.10 ± 0.04	0.5 ± 0.2	293 ± 63*
A23187	—V	0.3 ± 0.05	2.2 ± 0.3	4.9 ± 1.3	244 ± 29
	—A	0.5 ± 0.2	5.3 ± 1.8	1.3 ± 0.3	375 ± 91

V = vehicle, A = aspirin. All values represent average release rates throughout the collection period. Results are expressed as mean ± SEM, N = 4 (Table 1), N = 3–7 (Table 2). * $P < 0.05$ when compared to basal values.

REFERENCES

1. Karmazyn M and Moffat MP, Calcium-ionophore stimulated release of leukotriene C_4-like immunoreactive material from cardiac tissue. *J Mol Cell Cardiol* **16**: 1071–1073, 1984.
2. Trevethick MA, Brown AK, Wright G and Strong P, Effect of A23187 and ischaemia-reperfusion on leukotriene release from rat isolated perfused heart. *Br J Pharmacol* **92**: 526p, 1987.
3. Lewis RA and Austen KF, The biologically active leukotrienes. *J Clin Invest* **73**: 889–897, 1984.
4. Grinwald PM, Calcium uptake during post-ischaemic reperfusion in the isolated rat heart: influence of extracellular sodium. *J Mol Cell Cardiol* **14**: 359–365, 1982.
5. Karmazyn M, Contribution of prostaglandins to reperfusion-induced ventricular failure in isolated rat hearts. *Am J Physiol* **251**: H133–H140, 1986.
6. Karmazyn M, Calcium paradox-evoked released of prostacyclin and immunoreactive leukotriene C_4 from rat and guinea-pig hearts. Evidence that endogenous prostaglandins inhibit leukotriene biosynthesis. *J Mol Cell Cardiol* **19**: 221–230, 1987.
7. Strong P, Mullings R and Illingworth JA, Aerobic lactate synthesis by cardiac muscle. *Eur J Biochem* **102**: 625–636, 1979.
8. Bergmeyer HU and Bernt E, UV-assay with pyruvate and NADH. In: *Methods of Enzymatic Analysis*, Vol. 2, pp. 574–576, Academic Press, London 1974.
9. Eskra JS, Pereira MJ and Ernest MJ, Solid-phase extraction and high performance liquid chromatography analysis of lipoxygenase pathway products. *Anal Biochem* **154**: 332–337, 1986.

Biochemical Pharmacology, Vol. 38, No. 2, pp. 379–381, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

Changes in polyamine content in primary cultures of adult rat hepatocytes

(*Received* 16 *May* 1988; *accepted* 3 *August* 1988)

Primary cultures of rat hepatocytes are a useful model system for the study of drug metabolism and toxicity [1, 2]. These are non-dividing cells which, when maintained under conventional culture conditions, exhibit rapid phenotypic changes resulting in a marked decrease in highly differentiated functions such as their ability to metabolise drugs via the cytochrome P-450 mono-oxygenase system [3, 4].

The polyamines, spermidine and spermine, and their diamine precursor, putrescine, are naturally occurring cellular polycations found in all living cells [5]. Their precise function within the cell is not known but increasing evidence indicates they play a positive role in the regulation of cell growth [6, 7].

Recently, it has been shown that polyamines have differential effects on the functions of freshly isolated hepatocytes. For example, polyamines can alter the cAMP-mediated stimulation of amino acid transport [8] and can inhibit the hormonal induction of tyrosine aminotransferase [9]. No study has, however, examined the changes in polyamine metabolism in primary cultures of hepatocytes. In this paper we have measured the alterations in the polyamine content of rat hepatocytes with time in primary culture and the effect inhibitors of polyamine metabolism have on these changes.

Materials and methods

Waymouth's medium was purchased from Flow Laboratories Ltd (Rickmansworth, U.K.). Donor horse serum, penicillin and streptomycin were from Gibco-BRL Ltd. Polyamines, hydrocortisone-21-acetate, insulin and inhibitors were from Sigma Chemical Co. (Poole, Dorset, U.K.). Collagenase was from Boehringer (Mannheim, F.R.G.).

Hepatocytes were isolated from male Sprague–Dawley rats (180–280 g) under aseptic conditions [10] and cultured on 10 cm dia. Petri dishes in Waymouth's medium supplemented with 1% (v/v) horse serum 10 μM-hydrocortisone and 1 μM insulin. Viability was assessed by Trypan blue exclusion and was initially 92% or greater. Polyamines were extracted from the cells in 0.2 M $HClO_4$ as described previously [11] and quantified by HPLC [12]. Protein content was determined by the method of Lowry *et al.* [13]. Each experiment is representative of a number carried out under the same conditions. In each case a range of the values obtained within one experiment is given. There was inter-rat variability in terms of the polyamine content but the results were consistent within each experiment. MGBG was extracted and quantified by HPLC [14].

Results and discussion

In rapidly growing cells the polyamine content on the cells increases with the cell growth rate [15]. In non-transformed cells, such as BHK-21/C13 cells, spermidine is the major polyamine within the cells and its intracellular concentration rises while the cells continue to grow. Primary cultures of hepatocytes do not grow in tissue culture but rather are maintained in a viable condition for several weeks [16]. As non-growing cells they therefore provide a novel system in which to study the role of polyamines. Initial experiments showed that the total polyamine content in freshly isolated hepatocytes was high and that this value decreased rapidly up to 24 hr. The total polyamine content of these cells remained relatively constant thereafter (Fig. 1). A more detailed analysis of the early times in culture showed that the total polyamine content remained approximately constant for 6–7 hr in culture; thereafter it decreased with time (Table 1). During this time the distribution of the individual polyamines was changing as seen by the continuous decline in the ratio of spermidine to spermine (Table 1). This ratio is a useful index of cell growth rate in non-transformed cells, high ratios of 1.5–2.0 being indicative of rapid rates of cell growth. This ratio falls markedly when cell growth slows down and, at confluence can be as low as 0.25 [15]. The low ratio in hepatocytes, even at early times, is indicative of their non-growing state.

Analysis of the individual polyamines showed spermine as the major polyamine within hepatocytes (Fig. 2). Putrescine, the precursor of spermidine and spermine was not detected at early times. Both spermidine and spermine decreased with time in culture, the former decreasing more quickly and hence accounting for the decline in the ratio of these two polyamines. Despite the fall in the content of the higher polyamines the total polyamine content was maintained, at least initially. This was due to the production of both putrescine and N^1-acetylspermidine from 3 hr onwards (Fig. 1). N^1-acetylspermidine is formed by the action of spermidine/spermine *N*-acetyltransferase which is the rate-limiting reaction in the breakdown or "so-called" retroconversion of the higher polyamines [17]. Putrescine