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REFERENCES

1. P. J. Magistretti, J. H. Morrison, W. J. Shoemaker, V. Sapin and F. E. Bloom, *Proc. natn. Acad. Sci. U.S.A.* **78**, 6535 (1981).
2. C. Kerins and S. I. Said, *Proc. Soc. exp. Biol. Med.* **142**, 1014 (1973).
3. C. Balabaud, M. Noel and J. Dangomau, *J. Pharmac. (Paris)*, **8**, 191 (1977).
4. G. L. Ricci, R. Michiels, J. Fevery and J. De Groote, *Hepatology* **4**, 651 (1984).
5. C. Balabaud, D. Peron-Marc, M. Noel, and J. Dangomau, *J. Pharmac. (Paris)* **7**, 265 (1976).
6. G. L. Ricci, R. Michiels, J. Fevery, J. De Groote and K. P. M. Heirwegh, *Gastroenterology* **84**, 1393(A) (1983).
7. L. Thulin and M. Hellgreen, *Acta chir. scand.* **142**, 235 (1976).
8. S. D. Turley and J. M. Dietschy, *J. Lipid Res.* **19**, 924 (1978).
9. F. P. Van Roy and K. P. M. Heirwegh, *Biochem J.* **107**, 507 (1968).
10. N. Blanckaert, *Biochem J.* **185**, 115 (1980).
11. C. Balabaud, K. A. Kron and J. J. Gumucio, *J. Lab. Clin. Med.* **89**, 393 (1977).
12. A. L. Baker, R. A. B. Wood, A. R. Moossa and J. L. Boyer, *J. clin. Invest.* **64**, 312 (1979).
13. I. M. Coupar, *Clin. Exp. Pharmac. Physiol.* **3**, 615 (1976).
14. N. B. Javitt, *N. Engl. J. Med.* **295**, 1464 (1976).
15. G. L. Ricci and J. Fevery, *Gastroenterology* **81**, 552 (1981).
16. I. Magnusson and L. Thulin, *Acta physiol. scand.* **160**, 293 (1979).
17. G. L. Ricci, R. Michiels and K. P. M. Heirwegh, *Clin. Sci.* **64**, 53P (1983).
18. G. J. Wishart and M. T. Campbell, *Biochem. J.* **178**, 443 (1979).

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Effect of plasma on human erythrocyte *beta*-adrenergic receptors

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Beta-adrenergic receptors were until recently considered absent on human erythrocytes. Catecholamine sensitive adenylate cyclase activity was found to be extremely low in the human red cell membrane [1–5] and *beta*-adrenergic receptor binding sites were not detectable [6, 7] until recent observations [8–10] showed a small, but reproducible number of binding sites for *beta*-adrenergic ligands.

The binding of the *beta*-adrenergic blockers (±)-propranolol and (−)-alprenolol to intact human erythrocytes involved saturable and non saturable binding [11]. Using a model with one class of saturable binding sites, it was found that each cell contained approximately 6000 high affinity binding sites in whole blood. By washing the number of binding sites was reduced to approximately 600 per cell, but after resuspension of washed cells in autologous plasma, the binding capacity became similar to that of erythrocytes in whole blood. These results indicated that the saturable, but not the non saturable binding to human erythrocytes was influenced by the presence of plasma and by washing.

The present study was undertaken for further characterization of the influence of autologous plasma and washing on binding capacity for propranolol and to determine whether plasma influences the catecholamine sensitive adenylate cyclase through these binding sites.

Materials and methods

The following chemicals were employed in the study: [^{3}H]-(−)-propranolol (spec. act. 19.6 Ci/mmol), [^{3}H]-adenosine 3′,5′-cyclic monophosphate (spec. act. 37 Ci/mmol) New England Nuclear, Dreieich, F.R.G.; unlabelled (−)-propranolol from Radiochemical and Pharmaceutical Division of Imperial Chemical Industries Ltd., London, U.K.; (−)-isoproterenol hydrochloride, theophylline and adenosine 3′,5′-cyclic monophosphate from Sigma Chemical Corp., St. Louis, MI.; sodium heparin from NOVO industries, Copenhagen, Denmark. Other chemicals were of analytical grade.

Washing and incubation buffer (modified Krebs Ringer phosphate buffer): NaCl 122 mM, KCl 4.9 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 1.3 mM, Na_2HPO_4 15.9 mM, d-glucose 10 mM, heparin 10 IU/ml. pH was adjusted to 7.40.

Blood was obtained from young, healthy individuals without any medication after an overnight fast and collected in plain glass tubes containing heparin to achieve a final concentration of 10 IU/ml. Whole blood was gassed with 5% CO_2 in air to achieve pH 7.4. The erythrocytes were separated from plasma and buffer by centrifugation at 1000 *g* for 15 min and plasma including buffy coat was aspirated. After one, four and nine washes with buffer at 22°, the cells were resuspended in the buffer at pH 7.4. The original hematocrit was maintained during the washing and in the final resuspension.

Platelet poor plasma was obtained by centrifugation of plasma for 20 min at 105,000 *g*. Carbon dioxide was partially removed from the plasma by gentle stirring at 22° for 3 hr and pH was adjusted to 7.4 with 0.1 M HCl.

The binding of (−)-propranolol to cells was determined by simultaneous addition of labelled and unlabelled ligand to achieve unbound concentrations from 0.1 nM to 100 μM in 2 ml of whole blood or in cell suspensions with original hematocrit. After 1 hr at 22°, the cells were separated from the incubation medium (plasma or buffer) by a centrifugation assay described before [11]. (−)-Propranolol binding to the erythrocytes was calculated from the equation

$$R = T \times (1/H) - P \times (1-H)/H,$$

T representing total radioligand concentration in the incubation mixture, R and P being the radioligand concentration in erythrocytes and supernatant (plasma or buffer) and H being the hematocrit. From the distribution of radioligand and the ratio between labelled and unlabelled ligand, the total (−)-propranolol binding to erythrocytes was calculated. Non saturable binding (B_{NS}) to the

cells was determined for 100 μM (−)-propranolol because the ratio bound/unbound (B/F) did not decrease above that concentration.

Plasma protein binding was determined for (−)-propranolol concentration ranging from 0.1 nM to 1 mM by equilibrium dialysis [12]. 500 μl of the incubation medium (plasma or washing buffer which contained proteins) with labelled and unlabelled ligand was dialyzed against 500 μl buffer for 16–18 hr at 22° using a dialysis membrane 20/32 (Union Carbide Corp., Chicago, IL) clamped between two Perspex cells. From the distribution of radioactivity and ratio between labelled and unlabelled ligand, the plasma protein binding was calculated. Non saturable plasma protein binding (B_{NS}) was determined for 1 mM (−)-propranolol because the ratio bound/unbound (B/F) did not decrease above that concentration.

Saturable binding (B_S) to cells and to plasma proteins was calculated according to Chamness and McGuire [13] by the equation

$$B_S = B_T - (B/F)_{NS} \times F,$$

where B_T and F represents total and unbound ligand concentration, respectively. Saturable (−)-propranolol binding became curvilinear in a Scatchard plot [14] for both cells and plasma proteins. Assuming the simple model where the ligand binds with different affinities to two classes of binding sites, the Scatchard plot was analyzed by the computer based iterative method described by Minneman *et al.* [15].

cAMP concentrations were determined by a radioimmunoassay [16]. $1–2 \times 10^8$ cells were incubated in absence or presence of 25% (v/v) platelet poor autologous plasma in a total volume of 1 ml. After 30 min incubation with 8 mM theophylline in plasma at 32°, the cells were exposed to different concentrations of (−)-isoproterenol. The reaction was terminated after 2.5 min when the increase in cAMP accumulation was linear both in absence and presence of plasma. In separate experiments 25% (v/v) platelet containing and platelet poor plasma was incubated as described above, but without cells. The cAMP standard curves in absence and presence of plasma became identical after blank substraction.

Protein concentrations were determined according to Lowry *et al.* [16]. The number of blood cells and platelets was determined in a Coulter Counter, Model S5, Coulter Electronics Ltd., England. Hematocrit was determined in an International Micro Capillary Centrifuge, Model MB.

Results

Previously [11] we found that the capacity of saturable (±)-propranolol binding to human erythrocytes was markedly reduced after cell washing. To decide whether this effect was a graded response dependent on the extent of washing, binding capacity and affinity of (−)-propranolol was determined at different stages of the washing procedure. The total binding of (−)-propranolol to intact human erythrocytes reflected one component of saturable and another of non saturable binding. Non saturable binding was not affected by removal of plasma and the washing (Table 1). The saturable binding gave a curvilinear Scatchard plot which was decomposed into one class of high affinity and another class of low affinity binding sites. The binding capacity of both classes of binding sites decreased in parallel with the extent of washing as reflected by the reduction of protein concentration in the incubation media (Table 1). After more than nine washes we were not able to detect any saturable binding by the applied binding assay. The binding affinity of both classes of binding sites was unaltered during the washes (Table 1). A marked reduction in number leucocytes and platelets was observed after one wash, but further washing caused a minimal change in the number of these blood elements (Table 1).

In the earlier study [11], we found that the erythrocyte capacity of saturable (±)-propranolol binding increased when washed cells were resuspended in autologous plasma. The present results show that plasma increased the binding capacity for both classes of binding sites, leaving the affinity unchanged (Fig. 1 and Table 2). Non saturable binding was unaffected (results not shown). (−)-Propranolol binding to plasma proteins, represented by saturable and non saturable binding (results not shown), was determined to establish free concentration in equilibrium with the cells and to characterize the binding parameters of plasma. The

Table 1. (−)-Propranolol binding to human erythrocytes at 22°, number of erythrocytes (RBC), leucocytes (WBC), blood platelets (TRC) and protein concentration in the incubation medium was determined in whole blood and after one, four and nine washes as described in the methods. Results are presented as mean value ± S.D., N = 5

Number of washes	ZERO	ONE	FOUR	NINE
Saturable high affinity binding				
N (Sites/cell)	1.800 ± 200	520 ± 60	160 ± 40	70 ± 30
K_d (nM)	1.0 ± 0.2	1.1 ± 0.4	0.9 ± 0.3	1.0 ± 0.3
Saturable low affinity binding				
N (Sites/cell)	43.000 ± 5.000	23.000 ± 4.000	11.000 ± 2.000	9.000 ± 3.000
K_d (nM)	70 ± 10	70 ± 20	70 ± 10	70 ± 30
Non saturable binding				
B/F (nM^{-1})	190 ± 50	170 ± 60	170 ± 30	150 ± 60
Number RBC (10^{12}/l)	4.5 ± 0.3	4.2 ± 0.2	4.2 ± 0.3	4.4 ± 0.4
Number WBC (10^{-9}/l)	7.5 ± 0.5	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.2
Number TRC (10^{-9}/l)	250 ± 50	40 ± 20	30 ± 10	20 ± 10
Protein concentration (g/l)	70 ± 10	0.88 ± 0.29	0.26 ± 0.05	0.16 ± 0.05

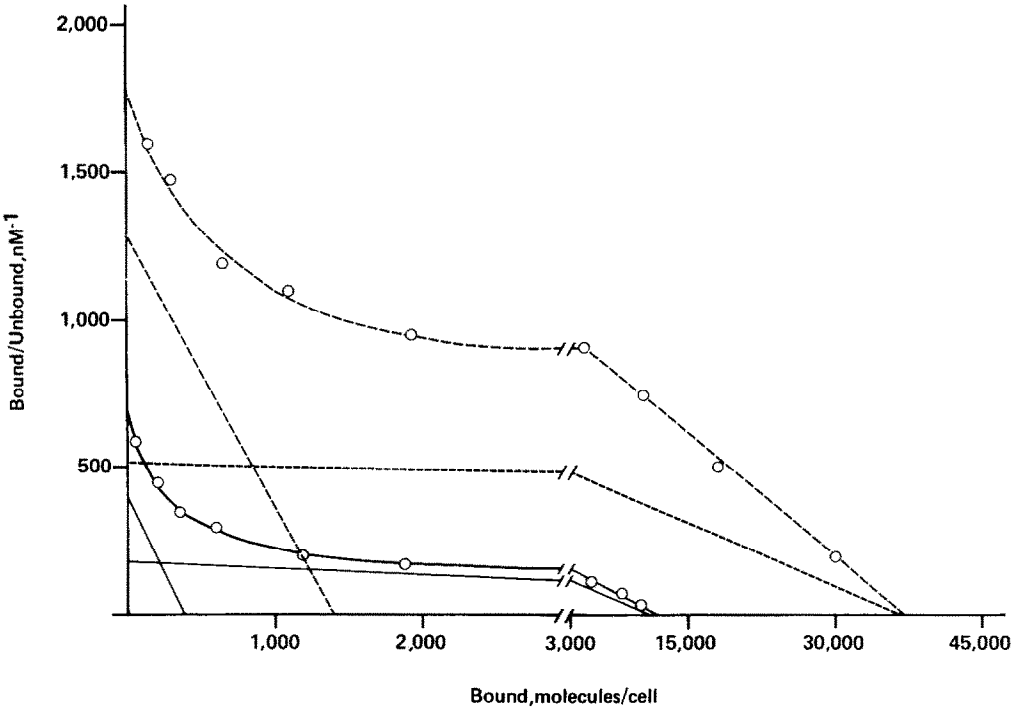

Fig. 1. Saturable binding of (−)-propranolol to intact human erythrocytes, after one wash, in absence (solid curve/lines) and presence (broken curve/lines) of 25% (v/v) autologous platelet poor plasma, dissected into two binding components, represented by the binding lines for the high and low affinity binding sites. The results from one representative experiment are presented.

Table 2. Saturable binding of (−)-propranolol to human erythrocytes (RBC) and to plasma. After one wash, the binding to cells (N = 3) in absence and presence of platelet poor plasma (PPP) was determined by centrifugation [11]. Protein binding (N = 3) in platelet containing (PCP) and platelet poor plasma (PPP) was determined by equilibrium dialysis [12]. Basal and (−)-isoproterenol (10 μM) stimulated cAMP levels of intact erythrocytes, after one wash, and in plasma were determined as described in the methods. The cellular levels were determined in absence or presence of 25% (v/v) PPP (N = 5). The cAMP levels were determined in plasma containing platelets (PCP) and in plasma poor of platelets (PPP), (N = 5)

	RBC/buffer*	RBC/25% (v/v) PPP*	PPP†	PCP†
Saturable high affinity binding				
B_{max}	400 ± 100	1.400 ± 400	160 ± 30	150 ± 20
K_d (nM)	1.0 ± 0.4	1.2 ± 0.4	400 ± 40	300 ± 60
Saturable low affinity binding				
B_{max}	18.000 ± 3.000	41.000 ± 7.000	800 ± 200	900 ± 100
K_d (μM)	0.07 ± 0.02	0.08 ± 0.01	12 ± 0.8	10 ± 1
cAMP levels				
Basal	2.1 ± 0.8	1.8 ± 0.6	0.13 ± 0.03	0.13 ± 0.03
10 μM (−)-IPR	8.2 ± 1.8	14.7 ± 3.7	0.13 ± 0.03	0.18 ± 0.02

* Binding capacity and cAMP levels are given as sites/cell and pmol/10^8 cells, respectively.
† Binding capactiy and cAMP levels are both given as pmol/mg protein.

saturable binding was decomposed into two classes of binding sites. Significant higher affinities were observed for the binding to the erythrocytes than to plasma, the dissociation constants were approximately 1 and 300 nM for the high affinity binding sites and approximately 70 nM and 10 μM for the low affinity binding sites (Table 2). The binding of (−)-propranolol in plasma was unaffected by the presence of platelets (Table 2).

To decide whether the observed change in number of high affinity binding sites represented *beta*-adrenergic receptors [8–10] functionally coupled to adenylate cyclase [5], the effect of plasma on isoproterenol induced cAMP accumulation was determined. The cells were resuspended in platelet poor plasma since the presence of platelets gave a small, but concentration dependent cAMP elevation when exposed to isoproterenol (Table 2). The presence of plasma

reduced basal ($P < 0.01$), but increased maximal cAMP levels ($P < 0.01$), observed for 10 μM isoproterenol (Table 2 and Fig 2). The sensitivity was unchanged, EC_{50} being 44 ± 16 nM and 36 ± 28 nM, in absence and presence of plasma, respectively. At higher isoproterenol concentrations the cAMP levels decreased, but were still higher in the presence of plasma (Fig. 2).

Discussion

In a previous study [11] we interpreted the total binding of propranolol to human erythrocytes according to a model with only one class of saturable binding sites. However, when using a higher ligand concentration to define non saturable binding and then apply a wider range of ligand concentrations, the saturable binding curve became distinct curvilinear, when plotted according to Scatchard [14]. The presence of a low affinity binding component explains the lower binding capacity of the erythrocyte high affinity binding in whole blood and after similar washing in the present study. By the same reason, we presently also found lower non saturable binding than previously [11]. The dissociation constant for the high affinity binding site in the present study is similar to that reported for (−)-propranolol inhibition of [^{3}H]-(−)-dihydroalprenolol binding to *beta*-adrenergic receptors on intact human leucocytes [18] and erythrocytes [19]. The present results are supported by the earlier reports of *beta*-adrenergic receptors present on human erythrocytes [8–10].

The number of receptors decreased after removal of plasma and washing, confirming our earlier observation [11]. The mechanism of this effect is unclear. Solubilization of the binding sites is one possibility, but the high affinity binding of propranolol in plasma had a dissociation constant approximately 300 times greater than that of the erythrocyte *beta*-adrenergic receptor. Another possibility is that washing removes plasma constituents in equilibrium with the cell membrane or membrane constituents necessary for the structural integrity of the binding sites. Denaturation of the receptors is less likely since the affinity is unaffected by washing and the binding capacity is restored by resuspension of washed cells in plasma.

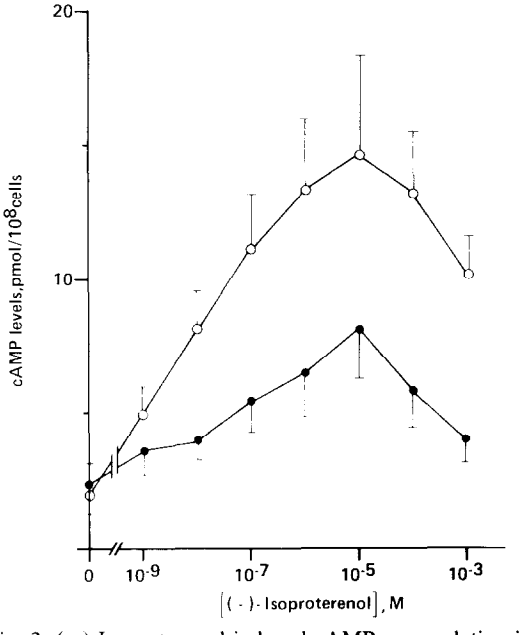

Fig. 2. (−)-Isoproterenol induced cAMP accumulation in intact human erythrocytes, after one wash, in absence (●—●) and in presence (○—○) of 25% (v/v) autologous platelet poor plasma. The results represent mean value ± S.D. from five separate experiments.

Also other constituents of the *beta*-adrenergic effector system in human erythrocytes seem to be vulnerable to preparation procedures. In a study of catecholamine sensitive protein kinase, intact cells were preferred since leaky ghosts caused little or no stimulation [20]. In addition, it was shown that the activity was markedly reduced after four washes of intact cells. Catecholamine sensitive adenylate cyclase activity has been found to be extremely low [1–4], but a recent study showed that enzyme lability during isolation of red cell ghosts could partly account for the low activity [5]. In the present study with intact cells, isoproterenol caused a concentration dependent cAMP accumulation with levels in close agreement to earlier observations [8, 19].

By the present experimental procedures resuspension of washed erythrocytes in plasma increased the response to isoproterenol with higher cAMP levels, but without affecting sensitivity. This strongly indicates that the increase of high affinity binding capacity represents an elevation of *beta*-adrenergic receptors functionally linked to adenylate cyclase.

Propranolol was also bound to a class of low affinity sites on the erythrocytes. Low affinity binding for [^{3}H]-(−)-dihydroalprenolol has previously been reported to human erythrocytes [7, 19] and to intact human leucocytes [18]. The low affinity binding capacity did also increase in the presence of plasma. It is possible that this binding represents adsorbed plasma proteins since the dissociation constant (approximately 70 nM) was comparable to the dissociation constant of high affinity binding in plasma (approximately 300 nM). Non saturable propranolol binding was not affected by the presence of plasma or by washing, probably reflecting a partitioning of propranolol to the lipid phase of the cell membranes.

The present study adds further evidence to the existence of *beta*-adrenergic receptors on human erythrocytes and demonstrates that the presence of plasma and the degree of washing influence these receptors. Studies are in progress to determine whether similar effects can be demonstrated for other human blood cells.

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REFERENCES

1. G. Kaiser, K. Quiring, D. Gauger, D. Palm, H. Becker and W. Schoeppe, *Blut* **29**, 115 (1974).
2. S. R. Pfeffer and N. I. Swislocki, *Archs Biochem. Biophys.* **177**, 117 (1976).
3. S. B. Rodan, G. A. Rodan and R. I. Sha'afi, *Biochim. biophys. Acta* **428**, 509 (1976).
4. J. P. Piau, J. Delaunay, S. Fischer, M. Tortolero and G. Schapira, *Blood* **56**, 963 (1980).
5. M. Nakagawa, J. Willner, C. Cerri and P. Reydel, *Biochim. biophys. Acta* **770**, 122 (1984).
6. L. T. Williams, R. Snyderman and R. J. Lefkowitz, *J. clin. Invest.* **57**, 149 (1976).
7. C. M. Mendel and R. R. Almon, *Gen. Pharmac.* **10**, 31 (1979).

8. G. Sager, *Biochem. Pharmac.* **31**, 99 (1982).
9. J. A. Heinsimer, A. O. Davies, R. W. Downs, M. A. Levine, A. M. Spiegel, M. K. Drezner, A. DeLean, K. A. Wreggett, M. G. Caron and R. J. Lefkowitz, *J. Clin. Invest.* **73**, 1335 (1984).
10. F. Bree, I. Gault, P. d'Athis and J.-P. Tillement, *Biochem. Pharmac.* **33**, 4045 (1984).
11. G. Sager and S. Jacobsen, *Biochem. Pharmac.* **28**, 2167 (1979).
12. G. Sager, O. G. Nilsen and S. Jacobsen, *Biochem. Pharmac.* **28**, 905 (1979).
13. G. C. Chamness and W. L. McGuire, *Steroids* **26**, 538 (1975).
14. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
15. K. P. Minneman, L. R. Hegstrand and P. B. Molinoff, *Molec. Pharmac.* **16**, 34 (1979).
16. T. Skomedal, B. Grynne, J. B. Osnes, A. E. Sjetnan and I. Øye, *Acta Pharmac. Tox.* **46**, 200 (1980).
17. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
18. H. Meurs, W. van den Bogaard, H. F. Kauffman and P. L. B. Bruynzeel, *Eur. J. Pharmac.* **85**, 185 (1982).
19. G. Sager, *Biochem. Pharmac.* **32**, 1946 (1983).
20. T. Tsukamoto and M. Sonenberg, *J. clin. Invest.* **64**, 534 (1979).

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Sodium *n*-butyrate enhancement of prostaglandin D_2 antitumor efficacy

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While the cell growth inhibitory effects of prostaglandins of the E and A series have been well described [1, 2], the potent antiproliferative activity of prostaglandin D_2 (PGD_2) has been reported only recently. Fukushima *et al.* [3], for example, examined the *in vitro* growth inhibitory effects of PGD_2 against the murine leukemia L1210 as well as several human leukemia cell lines. They have suggested that PGD_2 may represent a new and promising antineoplastic agent due to its potency and reduced potential to produce adverse effects on the systemic circulation. Other recent preclinical studies have shown that PGD_2 reduces the metastatic potential of B16 melanoma cells [4] and significantly inhibits the growth of a mastocytoma cell line [5].

The potential for short chain fatty acids such as *n*-butyrate to result in apparent differentiation of certain leukemia cell lines *in vitro* and *in vivo* has been documented by several groups of investigators [6–9]. The ability of *n*-butyrate to induce fatty acid cyclooxygenase activity in specific cell culture lines *in vitro*, however, is a relatively new observation [10, 11]. Since activation of cyclooxygenase activity results in the endogenous production of prostaglandins including PGD_2, we reasoned that *n*-butyrate may augment the cytotoxicity of exogenously administered PGD_2. We, therefore, examined the ability of noncytotoxic and clinically achievable concentrations of *n*-butyrate to increase the antitumor activity of PGD_2 and PGE_2 against murine B16 melanoma as well as established human breast, uterine, and lung cancer cell lines.

Materials and methods

Prostaglandins D_2 and E_2 were purchased from the Cayman Chemical Co. (Denver, CO). [*Methyl*-^{3}H]Thymidine (5 ci/mmole) was purchased from the New England Nuclear Corp. (Boston, MA). Sodium *n*-butyrate, acetylsalicylic acid and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] were purchased from the Sigma Chemical Co. (St. Louis, MO).

Cell culture. Maximally tolerated yet noncytotoxic concentrations of sodium *n*-butyrate were determined for each cell line utilizing a 72-hr drug exposure period. Cytotoxicity of prostaglandins D_2 or E_2 in the presence or absence of sodium *n*-butyrate was determined by a recently developed dimethylthiazol tetrazolium (MTT) dye method [12] or by uptake and incorporation of [^{3}H]thymidine into acid insoluble material. Since cytotoxicity determinations utilizing the MTT method produced results which were similar to that produced by measurement of [^{3}H]thymidine incorporation, the former method was routinely used throughout these studies.

Three human (NCI H-69, BT-475, and MES-SA) and one murine cell line (B16) were utilized for this study. The human small cell lung carcinoma cell line (NCI H-69) was obtained from Dr. Desmond N. Carney, NCI Navy Medical Oncology Branch, Bethesda, MD. The human breast carcinoma cell line (BT-475) was obtained from Dr. Helene S. Smith, Peralta Cancer Research Institute, Oakland, CA. The murine melanoma cell line (B16) was obtained from EG & G Mason Research Institute, Worcester, MA. The human sarcoma cell line (MES-SA) was developed in the laboratory of Dr. B. Sikic at Stanford University. The origin, characteristics and cytogenetics of this cell line have been described previously [4]. H-69 and BT-475 cells were grown in RPMI medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% fetal calf serum (FCS), insulin (5 μg/ml), penicillin (100 μg/ml), and streptomycin (100 μg/ml). MES-SA and B16 cells were maintained in a 1:1 mixture of McCoy's 5A and Waymouth's media supplemented with 15% newborn calf serum, insulin, penicillin, and streptomycin. H-69 cells, growing in suspension culture, were disaggregated by vigorous pipetting. The three other cell lines were grown in monolayer culture and harvested using 0.06 M EDTA. Cell number and viability were routinely determined by hemocytometer and trypan blue exclusion methods.

Results and discussion

B16 murine melanoma was chosen since the effects of PGD_2 on cell growth and macromolecular synthesis have been well characterized in this cell line [5]. Three established human cell culture lines were also examined to provide an estimate of the relative antitumor sensitivity to prostaglandins D_2 and E_2. In agreement with studies by Simmet and Jaffe [5], B16 cells were found to be very sensitive to PGD_2 (Fig. 1A) with an estimated IC_{50} value of 2.8 μg/ml (Table 1). In contrast, melanoma cells were far less sensitive to PGE_2; the IC_{50} for PGE_2 was greater than 50 μg/ml. Adding a noncytotoxic concentration (0.01 mM) of sodium *n*-butyrate to B16 cultures increased the inhibition of cell growth produced by PGD_2 by an average of 150–200%, resulting in an IC_{50} of 1.4 μg/ml. Sodium *n*-butyrate did not, however, significantly potentiate the cytotoxicity of PGE_2 in these melanoma cells.

Human uterine sarcoma and lung adenocarcinoma were both 6-fold less sensitive *in vitro* to the cytotoxic effects of PGD_2 than was melanoma (Table 1). The cytotoxic activity of PGD_2, however, could be significantly potentiated by exposure of the cells to a noncytotoxic concentration of sodium *n*-butyrate (Figs. 2 and 3). Neither human cell line was particularly sensitive to incubation with PGE_2. While