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Differential effect of acetylsalicylic acid and dipyrone on prostaglandin production in human fibroblast cultures

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1 Human skin fibroblasts incubated with arachidonic acid in culture show basal release of prostaglandins. They produce the same prostaglandins after stimulation with bradykinin.

2 Basal release of prostaglandins I_2 (6-oxo-$PGF_{1\alpha}$), $F_{2\alpha}$ and E_2 is inhibited dose-dependently by both acetylsalicylic acid (ASA) and dipyrone ($P<0.05$). The examined dose-range was 10^{-7} to 10^{-4} M for both drugs. During the first 5 min after removal of the drugs from the incubation medium, bradykinin-stimulated release remains dose-dependently inhibited ($P<0.001$) in ASA-, but not in dipyrone-treated cultures. The difference between the effects of ASA and of dipyrone is highly significant ($P<0.0001$), whereas the dipyrone-treated cultures are not different from controls.

3 The findings are consistent with cyclo-oxygenase inhibition by ASA as well as by dipyrone. However, the data demonstrate rapid reversibility of the effect of dipyrone. This suggests that in contrast to ASA, dipyrone does not inhibit cyclo-oxygenase by binding covalently to the enzyme.

Introduction

Cultured human skin fibroblasts produce prostaglandins of the same type as other human cell lines (Baenziger, Dillender & Majerus, 1977; Weksler, Ley & Jaffe, 1978; Baenziger, Becherer & Majerus, 1979). The ready availability of the human fibroblast model facilitates the study of drug actions on arachidonic acid metabolism (Oetliker & Mestel, 1981).

Vane (1971) showed that aspirin-like drugs exert their action on prostaglandin metabolism by inhibiting cyclo-oxygenase. Roth & Siok (1978) and Domschke & Domschke (1980) postulated that acetylsalicylic acid acetylates cyclo-oxygenase and thus binds covalently to the enzyme.

Dipyrone belongs to the group of non-steroidal analgesic and anti-inflammatory drugs (Flower, 1974; Flower, Moncada & Vane, 1980). It inhibits prostaglandin synthesis from arachidonic acid in microsomal preparations from bovine or ram seminal vesicles, and it produces 'aspirin-induced asthma' in patients with hypersensitivity to inhibitors of prostaglandin biosynthesis (Szczeklik, Gryglewski & Czerniawska-Mysik, 1977). Although inhibition of cyclo-oxygenase by dipyrone might therefore be expected, its chemical structure differs considerably from that of aspirin (Gryglewski, 1974) and the question arises whether dipyrone, like aspirin, forms a covalent linkage with the active sites of the enzyme.

Our study was undertaken in order to compare the action of ASA with that of dipyrone on prostaglandin production by cultured human fibroblasts, (i) under conditions of basal release of prostaglandins from the arachidonic acid supplied in the growth medium, and (ii) from the arachidonic acid derived from the cell membrane lipids of the same culture after stimulation with bradykinin and with the drug removed from the surrounding medium. A drug which binds covalently to cyclo-oxygenase should exert its action under both conditions.

Methods

Human fibroblasts were obtained from the superfluous skin of a 4-year-old healthy boy who underwent surgical correction of hypospadias. The skin was sliced and placed in Medium 199 (Boehringer) supplemented with 10% foetal calf serum. The cells were cultured at 37°C in a 5% CO_2 environment. The medium was renewed twice weekly. After confluent monolayers had been formed the cultures were exposed to trypsin (0.025%) for 5 min and multiplied in

subcultures thereafter. The experiment was performed on the 12th subculture.

For the study, the cell lines were cultivated in culture dishes until confluent. Twenty-four hours before the experiment, the individual cultures were given new medium in which the arachidonic acid (AA) was labelled with 1 μCi of [^{14}C]AA (New England Nuclear, sp. act.: 58.4 mCi mmol^{-1}). After 24 h the medium was removed, and the prostaglandins were extracted twice with 50 ml of diethylether at pH 3.5. This extract was evaporated under nitrogen and the dry products were redissolved in 3 ml of methanol/chloroform (1:1). This extraction represented the material for analysis of basal prostaglandin production.

The culture dishes were rinsed three times with Hank's solution and stimulated for 5 min in 10 ml HEPES buffer (0.02 M, pH 7.4) containing 1 μg bradykinin. The released products were again extracted according to the procedure described above. The redissolved products were used to assay stimulated prostaglandin production.

The cell layers were removed in Hank's solution using a rubber spatula. The sediment was resuspended in 1 ml 0.05 Tris-buffer (pH 8) and the cells were lysed by sonication. The protein content of the lysates was determined by the photometric method of Lowry, Rosebrough, Farr & Randall (1951).

The extract representing basal or stimulated prostaglandin production was dried again under nitrogen and redissolved in 50 μl methanol/chloroform (1:1). Half of this was spotted on Merck thin layer chromatography plates (Silica gel 60/Kieselgur F_{254}, layer thickness 0.25 mm). In addition, non-radioactive standards of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2 and AA were spotted in the same place. The plates were developed in a mixture of isooctane, ethyl acetate, acetic acid (50:110:20) which had been saturated with water. After development, the unlabelled standards were visualized with phosphomolybdic acid (Merck 3,5%). According to the localization of the standards, 3 cm^2 areas of Silica gel were scraped off and suspended in Aquassure (New England Nuclear). The radioactivity was counted in a liquid scintillation counter and the results are given as disintegrations per minute per mg protein (d min^{-1} mg^{-1} protein).

The recovery of prostaglandins and of the precursor from medium and buffer has been established separately by utilizing tritiated 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, and AA. The results are shown in Table 1.

The entire study was performed on one cell line and in parallel cultures. During the 24 h before the experiment the cultures were exposed either to ASA or to dipyrone at 4 different concentrations (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} M). Parallel controls were used without exposure to drugs.

In parallel experiments with the same cell line AA was not labelled so that newly synthesized PGE_2 could be measured by radioimmunoassay (RIA) according to a modified method of Jaffe, Behrman & Parker (1973). Aliquots (1 ml) of medium or buffer were taken and [^{3}H]-PGE_2 (New England Nuclear, Boston, Mass., sp. act.: 165.0 Ci mM^{-1}) 5000 d min^{-1} in 0.05 ml added. Each aliquot was extracted with 3.0 ml of petroleum ether to remove neutral lipids. The aqueous layer was then mixed with 3 ml of 3:3:1 ethyl acetate : isopropanol : 0.1 M HCl, approximate pH 5.8, vortexed, and a mixture of 2.0 ml of ethyl acetate and 3.0 ml of water was added. After further mixing, the two phases were separated by centrifugation (2000 rev min^{-1} for 5 min at ambient temperatures). A volume of 3.0 ml of the 3.5 ml organic phases supernatant was removed by aspiration, dried in a vacuum dessicator at ambient temperatures overnight and dissolved in 0.1 ml of phosphate buffer 0.05 M, pH 7.4. For the RIA, the unknown samples in buffer as well as unlabelled prostaglandin E_2 standard (Sigma) in a range of 2.5 to 200 pg 100 μl^{-1} were incubated with 30 μl of appropriately diluted PGE_2 antibody (Sigma) and 130 μl phosphate buffer for 15 min at ambient temperatures. After adding 4000–6000 d min^{-1} of tritiated PGE_2 the assay was again incubated at 37°C for 1 h and then at 4°C for at least 12 h. Separation of bound antibody from unbound [^{3}H]-PGE_2 was accomplished by adding 1.0 ml of BSA-coated charcoal (250 mg charcoal, 25 mg bovine serum albumin in 100 ml phosphate buffer) on ice; within 30 min the tubes were centrifuged at 4°C for 10 min at 3000 rev min^{-1}. One ml of supernatant was transferred into 10 ml Aquasure (New England Nuclear) and radioactivity was counted in a liquid scintillation counter. The calibration curves plotted as percentage inhibition (logit)

Table 1 Extraction-recovery of the examined prostaglandins and arachidonic acid (AA)

	% recovery from medium (basal release)	*% recovery from buffer (stimulated release)*
6-oxo-$PGF_{1\alpha}$	78.3 ± 5.7 (n = 7)	77.3 ± 10.7 (n = 7)
$PGF_{2\alpha}$	89.0 ± 6.1 (n = 7)	90.4 ± 7.4 (n = 7)
PGE_2	84.7 ± 7.1 (n = 6)	96.3 ± 3.9 (n = 4)
AA	43.4 ± 8.9 (n = 7)	118.4 ± 7.3 (n = 7)

Table 2 Effects of acetylsalicylic acid (ASA) on basal and stimulated release

On basal release (d min^{-1} mg^{-1} protein)						
ASA (M)	n	*6-oxo-PGF$_{1\alpha}$*	*% control*	n	*PGF$_{2\alpha}$*	*% control*
0	6	9689 ± 4290	100 ± 44	6	8520 ± 2595	100 ± 30
10^{-7}	3	6917 ± 2729	71 ± 28	3	8992 ± 2696	106 ± 32
10^{-6}	3	7194 ± 3533	74 ± 36	3	5426 ± 1389	64 ± 16
10^{-5}	3	4715 ± 1659	49 ± 17	3	3124 ± 443	37 ± 5
10^{-4}	3	3466 ± 670	36 ± 7	3	4050 ± 429	48 ± 5
ASA (M)	n	*PGE$_2$*	*% control*	n	*AA*	*% control*
0	6	23071 ± 9434	100 ± 41	6	222748 ± 107817	100 ± 48
10^{-7}	3	13966 ± 6743	61 ± 29	3	213860 ± 9274	96 ± 4
10^{-6}	3	13077 ± 6512	57 ± 28	3	166423 ± 10495	75 ± 5
10^{-5}	3	5486 ± 1453	24 ± 6	3	169301 ± 10048	76 ± 5
10^{-4}	3	6172 ± 1314	27 ± 6	3	149407 ± 24205	67 ± 11
RIA-PGE$_2$ (pg mg^{-1} protein)						
0	2	2293 ± 429	100 ± 19	d min^{-1} ng^{-1}: 10061		
10^{-4}	2	900 ± 335	39 ± 15	d min^{-1} ng^{-1}: 6857		
On stimulated release (d min^{-1} mg^{-1} protein)						
ASA (M)	n	*6-oxo-PGF$_{1\alpha}$*	*% control*	n	*PGF$_{2\alpha}$*	*% control*
0	6	6804 ± 1824	100 ± 27	6	2763 ± 849	100 ± 31
10^{-7}	3	3734 ± 1738	55 ± 26	3	1644 ± 625	60 ± 23
10^{-6}	3	1367 ± 307	20 ± 5	3	787 ± 288	28 ± 10
10^{-5}	3	773 ± 378	12 ± 6	3	613 ± 221	22 ± 8
10^{-4}	3	789 ± 138	12 ± 6	2	540 ± 143	20 ± 5
ASA (M)	n	*PGE$_2$*	*% control*	n	*AA*	*% control*
0	6	15541 ± 3443	100 ± 22	6	6197 ± 1625	100 ± 26
10^{-7}	3	8460 ± 3612	54 ± 23	3	8214 ± 2232	133 ± 36
10^{-6}	3	2109 ± 343	14 ± 2	3	8104 ± 3317	131 ± 54
10^{-5}	3	941 ± 473	6 ± 3	3	6664 ± 1591	108 ± 26
10^{-4}	2	338 ± 124	2 ± 1	3	8807 ± 2713	143 ± 44
RIA-PGE$_2$ (pg mg^{-1} protein)						
0	2	5126 ± 2660	100 ± 52	d min^{-1} ng^{-1}: 3031		
10^{-4}	2	390 ± 132	8 ± 3	d min^{-1} ng^{-1}: 866		

versus log prostaglandin were linear over the range of 2.5 to 2000 pg. Prostaglandin concentrations in medium or buffer were calculated by a computer programme, using the best fit of data to a straight line.

All data are given as average values with one standard deviation, and for statistical analysis of dose-dependent inhibition, variance analysis was used (Engelman, Frane & Jennrich, 1979).

Results

The basal and bradykinin-stimulated release of prostaglandins under the influence of various concentrations of ASA and dipyrone as well as under control conditions are presented in Tables 2 and 3. The PGE_2 production estimated by RIA under the influence of either drug and under control conditions is added to the Tables.

ASA inhibits both the basal and stimulated release of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 (Table 2). The dose-dependent inhibition is significantly different from control for basal release of 6-oxo-$PGF_{1\alpha}$ ($P < 0.05$) and for basal $PGF_{2\alpha}$ and PGE_2 release, as well as for stimulated release of all prostaglandins ($P < 0.001$). Changes of AA are not significantly different from control. The production of PGE_2 determined by RIA is clearly inhibited by ASA concentrations of 10^{-4} M. The basal and stimulated re-

Table 3 Effects of duotribe on basal and stimulated release

On basal release (d min^{-1} mg^{-1} protein)						
Dipyrone (M)	n	*6-oxo-PGF$_{1\alpha}$*	*% control*	n	*PGF$_{2\alpha}$*	*% control*
0	6	12678 ± 4033	100 ± 32	6	6967 ± 1546	100 ± 22
10^{-7}	3	7218 ± 1456	57 ± 11	3	4866 ± 1426	70 ± 20
10^{-6}	3	7931 ± 1024	63 ± 38	3	4642 ± 608	67 ± 9
10^{-5}	3	6239 ± 834	49 ± 7	3	4751 ± 729	68 ± 10
10^{-4}	3	4149 ± 298	33 ± 2	3	2975 ± 161	43 ± 2
Dipyrone (M)	n	*PGE$_2$*	*% control*	n	*AA*	*% control*
0	6	18553 ± 4805	100 ± 26	6	185183 ± 31825	100 ± 17
10^{-7}	3	12361 ± 4541	67 ± 24	3	164321 ± 20748	89 ± 11
10^{-6}	3	11932 ± 1417	64 ± 8	3	162522 ± 27211	88 ± 15
10^{-5}	3	6028 ± 1120	32 ± 6	3	160851 ± 17207	87 ± 9
10^{-4}	3	2771 ± 68	15 ± 0.4	3	157451 ± 18730	85 ± 10
RIA-PGE$_2$ (pg mg^{-1} protein)						
0	2	3124 ± 687	100 ± 22	d min^{-1} ng^{-1}: 5938		
10^{-4}	2	1049 ± 128	34 ± 4	d min^{-1} ng^{-1}: 2641		
On stimulated release (d min^{-1} mg^{-1} protein)						
Dipyrone (M)	n	*6-oxo-PGF$_{1\alpha}$*	*% control*	n	*PGF$_{2\alpha}$*	*% control*
0	5	4586 ± 1053	100 ± 23	5	2060 ± 353	100 ± 17
10^{-7}	3	3448 ± 517	75 ± 11	3	1544 ± 140	75 ± 7
10^{-6}	3	3963 ± 1186	86 ± 26	3	1653 ± 261	80 ± 13
10^{-5}	3	3758 ± 1244	82 ± 27	3	1550 ± 429	75 ± 21
10^{-4}	3	3806 ± 169	83 ± 4	3	1641 ± 125	80 ± 6
Dipyrone (M)	n	*PGE$_2$*	*% control*	n	*AA*	*% control*
0	5	11475 ± 2189	100 ± 19	5	5230 ± 989	100 ± 19
10^{-7}	3	8893 ± 402	77 ± 4	3	4798 ± 958	90 ± 18
10^{-6}	3	9636 ± 2372	84 ± 21	3	5728 ± 2911	110 ± 56
10^{-5}	3	9945 ± 3085	87 ± 27	3	3360 ± 687	64 ± 13
10^{-4}	3	9731 ± 397	85 ± 3	3	4013 ± 258	77 ± 5
RIA-PGE$_2$ (pg mg^{-1} protein)						
0	2	4144 ± 627	100 ± 15	d min^{-1} ng^{-1}: 2769		
10^{-4}	2	4032 ± 503	97 ± 12	d min^{-1} ng^{-1}: 2413		

lease of 6-oxo-PGF$_{1\alpha}$, PGF$_{2\alpha}$ and PGE$_2$ is shown in Table 3. The basal release of all examined prostaglandins is dose-dependently and significantly inhibited by dipyrone ($P < 0.0001$). AA remains uninfluenced. However, in stimulated release dipyrone does not significantly inhibit any of the prostaglandins, nor AA. The data for PGE$_2$ are confirmed by RIA showing an inhibition of the basal release and no inhibition of the stimulated release after incubation with 10^{-4} M dipyrone.

The variance analysis of difference between the dose-dependent inhibition by ASA and dipyrone are shown in Table 4. It can be seen that both drugs show a similar inhibition pattern on basal prostaglandin release. Except for PGF$_{2\alpha}$, no statistically significant difference was found. Nevertheless, both ASA and dipyrone significantly inhibit PGF$_{2\alpha}$-synthesis as compared with control. In stimulated release, there was no inhibition of prostaglandin synthesis by di-

Table 4 Variance analysis of differences between inhibitory effects of acetylsalicylic acid and dipyrone

	Basal release	*Stimulated release*
6-oxo-PGF$_{1\alpha}$	$P = 0.0683$	$P < 0.0001$
PGF$_{2\alpha}$	$P = 0.0433$	$P < 0.0001$
PGE$_2$	$P = 0.0988$	$P < 0.0001$
Arachidonic acid	$P = 0.4195$	$P < 0.0001$

pyrone and comparison with that of ASA revealed a highly significant difference ($P<0.0001$). Comparison of the influences of dipyrone and ASA on AA after bradykinin stimulation shows that ASA-culture values are significantly higher than those of dipyrone.

The protein values per culture dish, which were used as an estimate of the cell mass, are similar in controls and in cultures treated with ASA or dipyrone. Statistical analysis revealed no significant differences between the groups.

The uptake of [^{14}C]-AA was 82–83% in controls as well as in cultures exposed to different concentrations of the drugs.

No attempt was made to establish quantitative data for specific activity of PGE_2. However, calculations with the available data (Tables 2 and 3) show qualitatively that the specific activity is probably higher in basal release than in stimulated release. The decrease in radioactivity under the influence of a drug is accompanied by a decrease of radioimmunoassayable PGE_2; the specific activity remains of the same order of magnitude for the drug studies and controls.

Discussion

Human fibroblasts in culture have been shown to produce prostaglandins spontaneously from the arachidonic acid content of a culture medium supplemented with 10% foetal calf serum (Baenziger *et al.* 1977; 1979; Weksler *et al.* 1978). This production of protaglandins within 24 h is called basal release. Stimulated release corresponds to the prostaglandin production from arachidonic acid precursor liberated from cell membrane phospholipids during 5 min of stimulation with bradykinin.

Within basal release a drug might exert an effect on the release of arachidonic acid, on its uptake, or on prostaglandin synthesis (Flower, 1979). During a 24 h period the metabolism of released prostaglandins could also be influenced by drugs. In bradykinin-stimulated release a drug might interfere with either the release of the precursor or with the formation of prostaglandins. An influence on re-uptake and metabolism of prostaglandins might quantitatively be negligible within the first 5 min. By replacing the medium containing basal release with buffer solution without a drug, an opportunity is created to study the reversibility of effects observed in basal release.

The inhibition of prostaglandin production by ASA shows the well-known dose-dependency (Vane, 1971; Flower & Vane, 1974) in both basal and stimulated release. It is also in accordance with the concept claiming covalent binding of ASA to cyclo-oxygenase (Flower *et al.*, 1980), since this dose-dependent effect has also been observed in stimulated release, during which no drug was present in the culture medium.

A certain tendency of the arachidonic acid values to increase after exposure to ASA, seen in stimulated release, might be explained by enhanced availability of arachidonic acid. However, since no significant difference from the 100% control was found, no conclusion could be drawn about the effect of either ASA or dipyrone on arachidonic acid availability.

Since dipyrone has been shown to inhibit prostaglandin production from arachidonic acid in microsomal preparations (Szczeklik *et al.*, 1977) and since in our study basal prostaglandin production is comparably inhibited by dipyrone and by ASA, the present findings may be interpreted as an effect of dipyrone on cyclo-oxygenase. However, since the present data show clear reversibility of the effect of dipyrone on the stimulated release, it appears unlikely that dipyrone inhibits cyclo-oxygenase by binding covalently to the enzyme. The exact mechanism of its inhibition of cyclo-oxygenase remains to be clarified. The present results seem consistent with a competitive inhibition of cyclo-oxygenase by dipyrone. The elimination of dipyrone from the surrounding medium as well as the increased availability of arachidonic acid after bradykinin stimulation may change the previous equilibrium in favour of arachidonic acid and thus induce the rapid reversibility of the dipyrone effect.

The reversibility of this effect of dipyrone might be of clinical relevance in so far as cyclo-oxygenase inhibition disappears as soon as dipyrone is eliminated from the extracellular fluid.

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References

BAENZIGER, N.L., DILLENDER, M.J. & MAJERUS, P.W. (1977). Cultured human skin fibroblasts and arterial cells produce a labile platelet-inhibitory prostaglandin. *Biochem. biophys. Res. Comm.*, **78**, 294–301.

BAENZIGER, N.L., BECHERER, B.R. & MAJERUS, P.W. (1979). Characterization of prostacyclin synthesis in cultured human arterial smooth muscle cells, venous endothelial cells and skin fibroblasts. *Cell*, **16**, 967–974.

DOMSCHKE, S. & DOMSCHKE, W. (1980). Advances in prostaglandin research. *Med. Monatsschr. Pharm.*, **3**, 6–13.

ENGELMAN, L., FRANE, J.W. & JENNRICH, R.I. (1979). Analysis of variance and covariance. In *Biomedical Computer Programs, P-series*, ed. Dixon, W.J. & Brown, M.B. pp. 521–599. Berkeley, Los Angeles, London: University of California Press.

FLOWER, R.J. & VANE, J.R. (1974). Some pharmacologic and biochemical aspects of prostaglandin biosynthesis and its inhibitors. In *Prostaglandin Synthetase Inhibitors: Their Effects on Physiological Functions and Pathological States*, ed. Robinson, H.J. & Vane, J.R. pp. 9–18. New York: Raven Press.

FLOWER, R.J. (1974). Drugs which inhibit prostaglandin biosynthesis. *Pharmac. Rev.*, **26,** 33–67.

FLOWER, R.J., MONCADA, S. & VANE, J.R. (1980). Analgesic-antipyretics and antiinflammatory agents; drugs employed in the treatment of gout. In *The Pharmacological Basis of Therapeutics*, ed. Goodman, L.S. & Gilman, A. pp. 682–728. New York: Macmillan Publishing Co, Inc.

GRYGLEWSKI, R. (1974). Structure-activity relationship of some prostaglandin synthetase inhibitors. In *Prostaglandin Synthetase Inhibitors.* ed. Robinson, H.J. & Vane, J.R. pp. 33–52. New York: Raven Press.

JAFFE, B.M., BEHRMAN, H.R. & PARKER, C.W. (1973). Radioimmunoassay measurement of prostaglandins E.A and F in human plasma. *J. clin. Invest.*, **52,** 398–405.

LOWRY, O.H., ROSEBROUGH, M.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193,** 265–275.

OETLIKER, O. & MESTEL, F. (1981). An approach to the study of the role of prostaglandins. In *Pediatric Nephrology, Developments in Nephrology*, Vol. 3. pp. 443–450. ed. Gruskin, Alan B. & Norman, Michael E. The Hague, Boston, London: Martinus Nijhoff Publ.

ROTH, G.J. & SIOK, C.J. (1978). Acetylation of the NH_2-terminal serine of prostaglandin synthetase by aspirin. *J. biol. Chem.*, **253,** 3782–3784.

SZCZEKLIK, A., GRYGLEWSKI, R.J. & CZERNIAWSKA-MYSIK, G. (1977). Clinical patterns of hypersensitivity to nonsteroidal anti-inflammatory drugs and their pathogenesis. *J. Allergy Clin. Immunol.*, **60,** 276–284.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature (New Biol.)*, **231,** 232–235.

WEKSLER, B.B., LEY, Ch.W. & JAFFE, E.A. (1978). Stimulation of endothelial cell prostacyclin production by thrombin, trypsin and the ionophore A 23187. *J. clin. Invest*, **62,** 923–930.

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