Halbach, H. (1972) Bull. World Health Org. 47: 21–29
League of Nations (1936) Document O.C. 1617
Maitai, C. K. (1981) J. Pharm. Pharmacol. 33: 195
McDonald, R. H., Jr. Goldberg, L. I. (1963) J. Pharmacol. Exp. Ther. 140: 60–66
Meyer, M. B., Goldberg, L. I. (1966) Cardiologia Suppl. 49: 1–10
United Nations Narcotics Laboratory (1979) MNAR/3/1979

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The influence of ethanol on the synthesis of prostaglandin-like material by resident rat peritoneal cells from exogenous arachidonic acid

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During the course of initial investigations into the effect of different parameters on the uptake and metabolism of arachidonic acid (AA) in rat peritoneal cells it was found that if ethanol was added to the AA solution before mixing with the cell suspension (but not after) there was a stimulation of prostaglandin-like (PGL) synthesis and a change in the cell membrane as shown by trypan blue uptake. This phenomenon was investigated further and the results of the experiments are presented in this communication.

Resident rat peritoneal cells were isolated from a 0·9% NaCl (saline) wash of a Wistar rat peritoneum by centrifugation at 4 °C and 500 *g* for 10 min. The different cell samples were pooled and the number of viable cells measured using trypan blue exclusion. The cells were recentrifuged and a final suspension of 5×10^6 viable cells ml^{-1} was prepared in saline. AA (10 μl volume) and 1 ml of the cell suspension were added to 1 ml plastic reaction vials, vortex mixed and incubated for 20 min at 37 °C in a water bath. After the incubation period a few drops of the medium were taken to measure cell viability and the rest of the cells centrifuged at 8000 *g* for 30 s. PGL activity in the supernatant was assayed by superperfusion of rat stomach strips as described by Vane (1964) and modified by Bult & Bonta (1976). PGE_2 was used as a reference compound and the activity of PGL material in the supernatant is given as equivalent to ngPGE_2/20 min/5×10^6 cells.

The level of PGL material in the medium of saline incubated cells was below the limit of detection of the bioassay used (10–100 pg PGE_2/50 μl sample). There was significant formation of PGL material if the cells were incubated with 10 μg ml^{-1} AA, however, if the stock solution (100 μg/10 μl) was diluted 1/10 with saline (to give a concentration of 10 μg/10 μl) there was less activity (6·7 ± 0·6 ng PGE_2) than if it was diluted in ethanol (27·2 ± 2 ng PGE_2). Addition of 10 μl of ethanol to incubation tubes after the cells and saline diluted AA had been mixed (to give the same final concentration of ethanol) did not stimulate PGL formation to any great extent (7·7 ± 0·5 ng PGE_2). Control incubations of AA in saline had no PGL activity after 20 min. Cells incubated with AA diluted in ethanol showed permeability changes as shown by trypan blue uptake (0% viable) whilst cells incubated with saline diluted AA showed no alteration (93 ± 6% viable). Addition of ethanol after mixing the cells and saline siluted AA resulted in a small decrease in viability (86 ± 4%) (Fig. 1). When different volumes of ethanol were added to 10 μg (10 μl volumes) AA before mixing with the cell suspension, a dose-dependent increase in PGL formation and decrease in cell viability was seen. If the ethanol was added after mixing, no effect of ethanol was observed (Fig. 2).

These experiments showed that the initial concentration of ethanol in the solution used to dissolve the AA was important, not the end concentration in the incubation mixture. They also excluded the possibility that ethanol stimulated cell metabolism in general as the same effect would then have been expected when the ethanol was added after the cells and AA had been mixed. Similarly, the permeability changes would appear to be due to the AA and not the ethanol.

In order to investigate the effect of ethanol at different AA concentrations 10 μl volumes of an ethanolic solution of AA (for each concentration) were added to the 1 ml reaction tubes and the ethanol evaporated off. Saline or ethanol (10 μl) was then added and the tubes left for 10 min, with occasional vortex mixing, to allow the AA to redissolve. If the AA was presented in ethanol there was a log/linear increase in PGL synthesis over the range 1–50 μg ml^{-1} AA but at 100 μg ml^{-1} there was a decrease in formation to 20% of the 10 μg ml^{-1} level (Fig. 3). No PGL activity could be detected using 10 ng or 100 ng AA. Permeability changes were seen in all cells incubated with greater than 1 μg ml^{-1} AA. If saline was the solvent there was a similar log/linear increase which was seen over the range 1–100 μg ml^{-1} AA with an absolute increase of half the ethanol group at each AA concentration (up to 50 μg ml^{-1} AA). There was little change in cell permeability up to 10 μg ml^{-1}, but at higher concentrations viability decreased rapidly to less than 10% at 50 μg ml^{-1}. AA samples treated as above but incubated without cells

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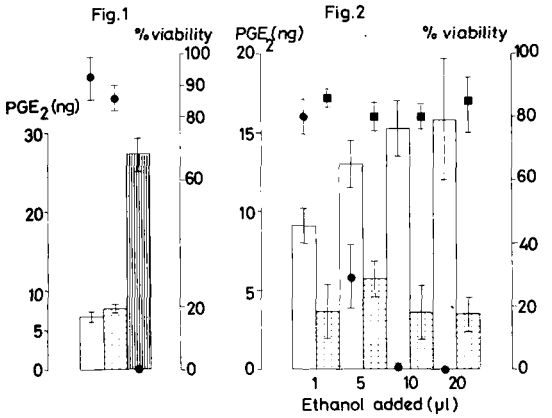

FIG. 1. The influence of ethanol on PGL production and cell viability of resident rat peritoneal cells incubated with 10 µg ml^{-1} AA. Open column: cells were incubated with 10 µl of saline-diluted AA (1/10 dilution of a stock solution in ethanol, 10 mg ml^{-1}). Total volume of ethanol present, 1 µl. Dotted column: cells were mixed with 10 µl of saline-diluted AA before the addition of 10 µl of ethanol. Total volume of ethanol was 11 µl. Ruled column: cells were incubated with 10 µl of ethanol-diluted AA. PGL formation, standardized against PGE_2, is represented by the histogram and cell viability, measured by trypan blue uptake, at the end of each treatment by the symbol ●. All values are the mean with s.d. of triplicate samples.

FIG. 2. The influence of ethanol concentration on PGL production and cell viability of resident rat peritoneal cells incubated with 10 µg ml^{-1} AA. Open column: the required volume of ethanol was added to 10 µl saline-diluted AA (1/10 dilution of a stock solution, 10 mg ml^{-1}, in ethanol), and mixed before addition of the cells. The total volume of ethanol present ranged from 2–21 µl. Dotted column: ethanol was added after the cells had been mixed with 10 µl saline-diluted AA. The total volume of ethanol present ranged from 2–21 µl. PGL production, standardized against PGE_2, is represented by the histogram and cell viability, as measured by trypan blue exclusion, at the end of each treatment by the symbol ● for ethanol added before the cells and ■ for ethanol added after. All values are the mean with s.d. of triplicate samples.

contained no detectable PGL activity. Incubation of the cells in a buffered medium containing Ca^{2+} (Dulbecco's modification of Eagles medium), did not prevent the stimulation of PGL synthesis or the drop in cell viability when the AA was dissolved in ethanol (data not given).

It would appear that ethanol, besides stimulating AA metabolism also enhances expression of adverse side effects associated with the fatty acid. These were seen first as a change in cell permeability and then, at higher concentrations, disruption of enzyme activity. Ethanol is most likely to act by increasing the availability of the AA to the cell, or more specifically, the cell membrane, for example by preventing micelle formation. This would allow a greater dispersion of the molecules in solution and facilitate penetration of the lipid bilayers of the membrane. Recently Chin & Goldstein (1981) reported that ethanol can disrupt the structure of biological membranes in a specific manner. It is possible therefore that ethanol also has a direct action on the cell membrane which enhances the uptake and metabolism of AA.

These results have implications not only for the in vitro and in vivo use of AA but also suggest a mechanism by which ethanol could exert apparently beneficial and toxic actions. It is clear that, at least over fairly short periods of time, there will be differences in the kinetic parameters between AA dissolved in saline and ethanol. It is also conceivable that the distribution of the fatty acid will be different in each case. At levels of 1 µg AA, cell permeability changes were seen and it is likely that these could result in a leakage of cAMP from the cells. These considerations may be of particular interest to users of radio-labelled AA which is often delivered dissolved in ethanol and to workers interested in the measurement of cell cAMP levels in AA dependent processes. Ethanol has been reported to effect a number of processes associated with aspects of AA metabolism. It inhibited aggregation in human, rat and rabbit platelets (Renaud et al 1979; Hwang 1981; McGregor & Renaud 1978) and decreased the incidence of atherosclerosis in rabbits (Renaud et al 1979). Karppanen & Puurenen (1976) reported that ethanol reduced gastric acid secretion by a PG dependent mechanism; Thomas et al (1980) that it stimulated PGE_2 and $PGF_{2\alpha}$ release in the rat isolated perfused lung, and Collier et al (1975) that it stimulated AA metabolism in bull seminal vesicle homogenates. Ethanol is capable of not only stimulating metabolism of exogenous AA (this report and Collier et al 1975), but it also stimulates release of metabolites from endogenous sources (Thomas et al 1981).

We postulate that ethanol acts by causing the mobilization of AA from a specific pool within the cell membrane. If the cell is capable of metabolizing AA then these products are released into the medium. An example of this could be the release of PGE_2 and PGF_2 from the rat isolated

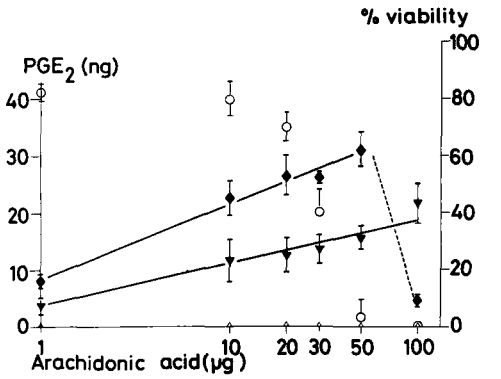

FIG. 3. The effect of AA concentration on PGL production and cell viability of resident rat peritoneal cells: its modification by ethanol. Ethanol diluted AA was placed into incubation tubes and the ethanol evaporated off. 10 µl of ethanol (♦ and △) or saline (▼ and ○) was added and the tubes stoppered and left for 10 min. The incubation was then carried out as normal. PGL production, standardized against PGE_2, is shown by the closed symbols and cell viability, at the end of each treatment, by the open symbols. All values are the mean with s.d. of triplicate samples.

perfused lung (Thomas et al 1981). If the cell is not capable of metabolizing AA then the free fatty acid will be released and any AA-dependent process will be impaired. This suggestion could account for the inhibition of platelet aggregation reported by Hwang (1981). The net result of imbiding alcohol in vivo will depend on the relative importance of AA-dependent processes in the tissue concerned and the effect of any of its metabolites produced.

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REFERENCES

Bult, H., Bonta, I. L. (1976) Agents Actions 6: 712–719
Chin, J. H., Goldstein, D. B. (1981) Mol. Pharmacol. 19: 425–431
Collier, H. O. J., McDonald-Gibson, W. J., Saeed, S. A. (1975) Lancet, March 22nd, 702
Hwang, D. H. (1981) Prostaglandins Medicine 7: 511–513
Karppanen, H., Puurenen, J. (1976) Eur. J. Pharmacol. 35: 221–223
McGregor, L. Renaud, S. (1978) Thrombosis. Res. 12: 921–927
Renaud, S., Morazain, R., McGregor, L., Baudier, F. (1979) Haemostasis 8: 234–251
Thomas, M., Boura, A. L. A., Vijayakumar, R. (1980) Clin. Exp. Pharmacol. Physiol. 7: 373–381
Vane, J. R. (1964) Br. J. Pharmacol. 23: 360–373

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In vitro effect of reproterol upon pulmonary, cardiac, vascular and intestinal 3′,5′-monophosphate phosphodiesterase nucleoside

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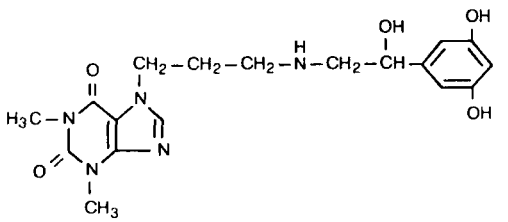
Reproterol [7-(3(β,3,5-trihydroxyphenethyl)amino-1-)-propyl]-theophylline hydrochloride], (I), is a water-soluble theophylline phenethylamine derivative with a catecholamine component linked to dimethyl-xanthine through a propyl group (Habersang et al 1977a; Klingler 1977); it has a potent bronchospasmolytic action which has been demonstrated experimentally (Habersang et al 1977b) and clinically (Diewitz 1977; Konietzko 1977; Mándi et al 1977a,b; Nolte et al 1977; Tabori et al 1977; Zečević et al 1977).



Reproterol activates the tracheo-bronchial adenylcyclase (β_2-adrenergic effect) to a greater extent than the cardiac and hypothalamic enzyme (Marmo et al 1981).

The methylated xanthines, such as theophylline, also have a tracheobronchodilator effect by blocking the phosphodiesterases, which are enzymes responsible for the degradation of cyclic 3′5′-(c)AMP. As a result, there is an increase in cAMP and relaxation of the smooth muscle in the trachea and bronchi (Robinson et al 1968). In view of the presence of a methylxanthine in reproterol, it was decided with this study to assess the possible inhibitory effect of reproterol upon pulmonary, cardiac, vascular (abdominal aorta) and intestinal (duodenum) phosphodiesterase (PDE) in the guinea-pig. The drug was compared with theophylline.

Phosphodiesterase activity was measured in lung, heart, abdominal aorta and duodenum of male guinea-pigs, 400–500 g, as described by Brooker et al (1968), Lippmann (1974), Somerville et al (1970), Tateson & Trist (1976) and by Weinryb et al (1973). The IC50 was determined (inhibitory concentration 50, probit method of Finney 1952). The drugs used were reproterol hydrochloride (Farmades SpA Rome) and theophylline monoethanolamine. All doses are stated as theophylline base.

In vitro, reproterol had a dose-dependent inhibitory effect upon pulmonary, cardiac, vascular and intestinal phosphodiesterase (Tables 1, 2). This effect corresponds with that of its theophylline content (Tables 1, 2) which

Table 1. Effects upon cAMP phosphodiesterase (PED) activity. Each value is the mean ± s.e.m. of 5 preparations. Reproterol concentrations indicated as theophylline base.

Drug μM	Inhibition % ± s.e. of PDE in Lung	Heart	Aorta	Intestine
Reproterol				
200	12 ± 2	27 ± 3	10 ± 1	4 ± 1
500	36 ± 4	48 ± 5	22 ± 3	16 ± 2
1000	60 ± 7	90 ± 9	38 ± 4	27 ± 4
2000	97 ± 8		86 ± 7	75 ± 8
Theophylline				
250	11 ± 2	26 ± 5	8 ± 1	3 ± 1
500	30 ± 4	49 ± 6	21 ± 7	14 ± 3
1000	58 ± 6	86 ± 9	35 ± 8	22 ± 4
2000	91 ± 9		90 ± 9	80 ± 9

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