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INHIBITION BY CLINICALLY USED DYES OF PROSTAGLANDIN INACTIVATION IN RAT AND HUMAN LUNG

Y.S. BAKHLE

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN

1 The effect of several clinically used dyes on prostaglandin E_2 (PGE_2) inactivation was studied in rat and human isolated lung.

2 All the dyes, given as infusions through the pulmonary circulation, inhibited the inactivation of PGE_2 as measured by bioassay. The action of the dyes was readily reversible on stopping the infusion.

3 The concentration producing 30% inhibition varied from 0.6 μM for indocyanine green to over 100 μM for methylene blue.

4 Inhibition of PGE_2 inactivation was also observed when the dyes were given as bolus injections during PGE_2 infusions through the lung.

5 These dyes are therefore not physiologically inert as they had previously been thought to be and their interaction with prostaglandins may affect the variables they are used to measure.

Introduction

In 1975 Bito & Baroody showed that bromcresol green blocked the uptake and thus the inactivation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in rat isolated lung. A few years later this and other indicator (i.e. pH sensitive) dyes were shown to inhibit PGE_2 inactivation by the same mechanism in rat lung (Bakhle, Jancar & Whittle, 1978). There was no obvious correlation between the structure of these dyes and their activity and because several dyes are used in clinical practice, for instance, to measure cardiac output or liver function, it seemed reasonable to examine particularly those dyes for their effects on prostaglandin inactivation in lung. Some of these results have been presented to the British Pharmacological Society (Bakhle, 1978).

Methods

Preparation of lungs and bioassay

Lungs were removed from anaesthetized male rats and perfused via the pulmonary circulation with Krebs solution at 8 ml/min (Bakhle, Reynard & Vane, 1969). Samples of human lung removed during surgical operations were perfused via the pulmonary vessels in the same way (Al-Ubaidi & Bakhle, 1980). The effluent from the lung superfused strips of hamster stomach (Ubatuba, 1973) for bioassay of PGE_2.

Two experimental procedures were followed. In one, injections of PGE_2 (200 to 1000 ng) were given into the pulmonary arterial cannula and the resulting contractions of the hamster stomach strip (HSS) calibrated by injecting smaller amounts of PGE_2 (10 to 50 ng) directly over the tissues. Inactivation of PGE_2 was assayed thus before and then again during an infusion of the dye (0.05 to 0.2 ml/min) into the lung perfusate to give the required final concentration of dye. In the other procedure, a constant infusion of PGE_2 was made into the perfusate entering the lung. The PGE_2 surviving passage through the pulmonary circulation sometimes caused a change in the basal tone of the assay tissue but this was maintained during the infusion. Injections of dye were then made into the perfusate in increasing amounts until a contraction of the assay tissue was seen. This response was then calibrated in terms of PGE_2 injected directly over the tissues. The dye was also injected directly over the tissues to assess its ability to contract the tissue by direct action.

Materials

The following drugs were used: indocyanine green (Cardio-green, Hynson Westcott and Dunning); bromsulphthalein (Bromsulphalein, Hynson Westcott and Dunning) were obtained from John

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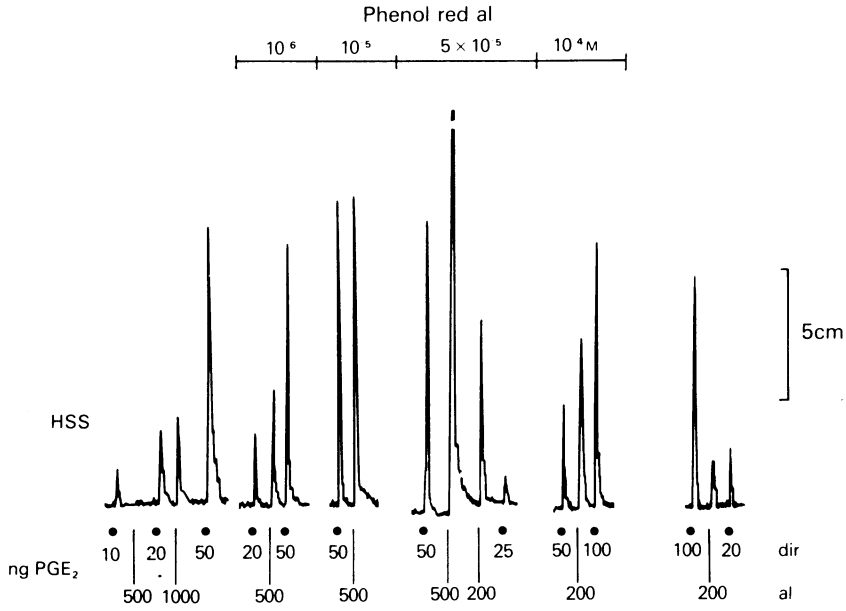

Figure 1 Inhibition by phenol red of prostaglandin E_2 (PGE_2) inactivation in rat isolated lung. The trace shows the responses of a hamster stomach strip (HSS) superfused with the effluent from rat isolated lung. All the responses are from the same experiment. In the first set of responses, less than 10 ng PGE_2 survived passage through the pulmonary circulation following the injection of 500 ng (al) but, from 1000 ng PGE_2 injected al, just over 20 ng PGE_2 survived. In the next three sets of responses, as phenol red in increasing concentration was infused through the lung, increasing amounts of PGE_2 survived (from the same 500 ng al injection) from just over 20 ng with 10^{-6}M phenol red to over 50 ng with 5×10^{-5}M phenol red. At the highest concentration of dye tested (10^{-4}M), the responses of the tissue were depressed (compare the response to 50 ng here with that in the immediately preceding set; the recorder sensitivity was constant throughout this trace). In spite of this, the inactivation was further inhibited, about 75 ng surviving from 200 ng PGE injected al. The last set of responses was obtained 10 min after the phenol red infusion was stopped and shows that the survival was already decreasing towards the control values, less than 20 ng surviving from 200 ng injected al.

Bell and Croyden. Indocyanine green was dissolved in the solvent supplied and bromsulphthalein was diluted in sterile saline (0.9%, w/v NaCl solution). Evans blue, methylene blue (Gurr) and phenol red (Hopkins and Williams) were dissolved in saline as required. Prostaglandin E_2 was obtained from Sigma and the 16,16-dimethyl PGE_2 was generously supplied by Dr J.E. Pike (Upjohn).

Statistical methods

Results unless otherwise stated are given as the mean ± s.e.mean of results from n experiments. Each experiment represents a different lung or lung sample (human lung only). Differences between means were considered significant if $P < 0.05$ using the t test (two tailed) for paired results.

Results

Rat isolated lungs

All the dyes tested inhibited PGE_2 inactivation in rat isolated lungs in a dose-related manner. This is illustrated by the trace shown in Figure 1 where, in a single lung, four concentrations of phenol red were tested. In the first set of responses, the inactivation of PGE_2 was about 98% (only 2% of 1000 ng surviving) under control conditions. Note that 500 ng PGE_2 injected 'al', i.e. through the pulmonary circulation did not produce a response on the HSS. However, as the phenol red infusion was increased from 10^{-6}M to 5×10^{-5}M, the amount of PGE_2 surviving from a 500 ng injection also increased from just over 20 ng to over 50 ng. At the highest concentration used (10^{-4}M) the

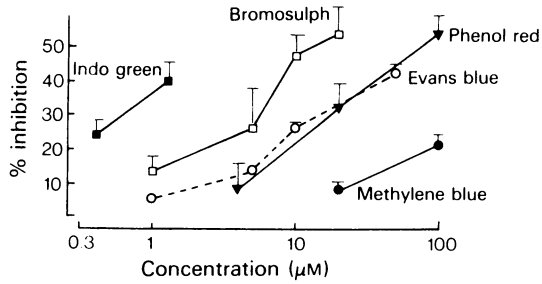

Figure 2 Variation of inhibition of prostaglandin E_2 inactivation with concentration of infused dye. The values shown are the mean (± s.e. mean) result of 3 to 6 experiments at each concentration, expressed as % inhibition of control inactivation. Indo green = indocyanine green; Bromosulph = bromsulphthalein.

Table 1 Effect of dye injections on prostaglandin inactivation in rat isolated lung

	Prostaglandin infusion (ng/ml)			
	PGE_2		*16,16 dimethyl* PGE_2	
Dye injected (1 μmol)	2	20	2	20
Bromsulphthalein	26±4	56±4*	22±4	131±33*
Methylene blue	17±3	57±18	35±6	127±32*

* Significantly different from value at 2 ng/ml ($P \leq 0.05$; *t*-test)

The values are the mean (± s.e. mean) height of responses of the hamster stomach strip to dye injected through the pulmonary circulation expressed as ng equivalents of PGE_2 or 16, 16 dimethyl PGE_2 as appropriate. Between 5 and 10 experiments were carried out for each value. The experimental procedure is illustrated in Figure 3.

responses of the assay tissues were depressed but survival of PGE_2 had increased to about 40%, a 20 fold increase over the control value. The last set of responses was obtained 10 min after stopping the phenol red infusion and shows that survival was already returning towards control values.

A series of similar experiments provided the results summarized in Figure 2 where the effect of the dyes has been expressed as inhibition of the control inactivation. It is important to realize that with control inactivation of about 98%, an apparently small inhibition of 10% means a very significant 6 fold increase in survival from 2% to 12%.

The most potent dye was indocyanine green giving 30% inhibition at about 0.6 μM compared with 6 μM for bromsulphthalein and 18 μM for phenol red and Evans blue. Methylene blue was the least potent, about one-tenth as effective as phenol red.

Because in clinical use it is more likely that there will be a bolus of dye passing through the lung rather than a prolonged infusion, experiments using dye injections superimposed on PGE_2 infusions were carried out. One such experiment using bromsulphthalein is illustrated in Figure 3. Here, in the first set of responses, the higher infusion of PGE_2 (20 ng/ml) was used and injections of 0.25 and 0.1 μmol (200 and 84 μg respectively) of the dye were given producing a fleeting inhibition of prostaglandin inactivation and the increase in surviving prostaglandin caused a contraction of the HSS. The responses to dye injection were longer lasting than those due to PGE_2 given as calibration.

The dependence of the response on the concentration of dye is shown by the responses to 0.25 and 0.1 μmol of bromsulphthalein during the 20 ng/ml PGE_2 infusion. The correlation with PGE_2 concentration is shown by the second set of responses during 2 ng/ml PGE_2 infusion where 0.25 μmol bromsulphthalein caused a response equivalent to about 10 ng PGE_2, whereas the same amount of dye caused a response equivalent to 100 ng PGE_2 during the higher PGE_2 infusion. Although not shown in this figure, direct injection of dye did not produce a contraction of the tissue (see however, Figure 4b).

Similar experiments were carried out with Evans blue, methylene blue and phenol red. In each case direct injection of dye did not contract the tissue but injection through the pulmonary circulation produced a contraction of the HSS. For two dyes, bromsulphthalein and methylene blue, the effect of changing the concentration of prostaglandin infused was studied further. In these experiments a fixed high dose of dye (1 μmol) was used as a test with 2 and 20 ng/ml infusions of either PGE_2 or 16, 16-dimethyl PGE_2. The results are shown in Table 1. Here it is clear that for a constant amount of dye injected (1 μmol), the response, i.e. the amount of prostaglandin protected from inactivation was related to the concentration of prostaglandin infused. This relationship was very obvious with the dimethyl analogue of $PGE_2$with both dyes causing a 4 to 6 fold increase in response at the higher substrate concentration. With PGE_2, only bromsulphthalein (the more potent inhibitor, see Figure 2) caused a two fold increase in response, whereas the results with methylene blue did not reach statistical significance although the mean response was greater with the 20 ng PGE_2/ml infusion.

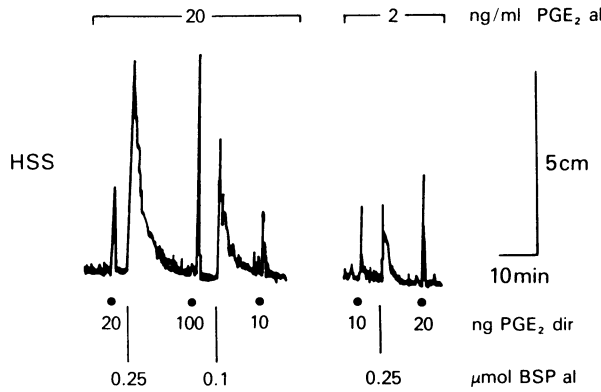

Figure 3 Effect of bromsulphthalein on inactivation of prostaglandin E_2 (PGE_2) infused through rat lung. The trace shows the responses of a hamster stomach strip (HSS) superfused with effluent from a rat lung and both sets are taken from the same experiment. In the first set of responses, a constant infusion of 20 ng PGE_2/ml was given through the pulmonary circulation. This caused a small and maintained contraction of the HSS (not shown). However when an injection of bromsulpthalein (BSP, 0.25 μmol; 200 μg) was made during the PGE_2 infusion, the HSS contacted and this contraction was equivalent in height to that caused by 100 μg PGE_2 given directly to the tissue (dir). A smaller amount of BSP (0.1 μmol; 84 μg) produced a smaller contraction, equivalent to between 10 and 20 μg PGE_2. Note that between the two injections of dye, the baseline was maintained, i.e. no significant retention of dye occurred.

In the second set of responses, a lower constant infusion of PGE_2 (2 ng/ml) was made and against this background, BSP (0.25 μmol) produced a contraction equivalent to only 10 ng PGE_2 in height. Thus the effect of BSP was related to the concentration of PGE_2 infused. In the absence of exogenous PGE_2 or when given directly to the tissue, BSP caused no contraction of the HSS. Note that in all of the BSP-induced contractions, although the heights were matched by dir injections of PGE_2, the duration of the BSP-induced contraction was greater and that the amount of PGE_2 protected from inactivation was thus greater than that calculated from the peak height alone.

Human isolated lungs

Only one dye, indocyanine green, was investigated in any detail in human lung. This dye was as effective in human lungs as it was in rat lungs and this is illustrated by Figure 4a. In this experiment, the survival of PGE_2 before infusion of indocyanine green was just over 1% (5 ng from 400 ng injected) but during the infusion survival increased many fold to about 30% (30 ng from 100 ng injected). In a total of 5 such experiments, the mean inhibition of PGE_2 inactivation by this concentration of indocyanine green (0.5 μg/ml; 0.6 μM) was 31 (±3)%. This value is very close to that interpolated from results in rat lung (see Figure 2). The effect of bolus injections of indocyanine green is illustrated in Figure 4b. Here the infusion of PGE_2 was at an intermediate concentration (12.5 ng/ml) compared with those used in rat lungs. However, injections of the dye through the lung produced marked contractions of the assay tissue in comparison with the negligible effect of a larger dose injected directly to the tissue (last injection; 80 μg indocyanine green). The different shape of the dye-induced contractions is very obvious in this trace but direct comparison with the results from rat lung (Figure 3) is not possible as a faster chart speed was used with human lung. Similar effects were observed in two other lung samples.

Discussion

The results represented here show clearly that several dyes in clinical use are inhibitors of the pulmonary inactivation of PGE_2. This effect was observed when the dye was infused constantly through the pulmonary circulation or when it was given as a bolus injection. The latter experimental procedure was adopted because it was thought to provide a better model of the way in which these dyes are administered. This produced a comparatively high concentration for a comparatively short time rather than a constant blood level attained over several minutes or hours.

These experiments showed that the contractions of the assay tissue induced by the bolus injections of dye were indeed due to a sudden and short-lasting increase in the amount of PGE_2 surviving passage through the lung. Thus, even though the contact time was short, about 2 s, significant inhibition of prosta-

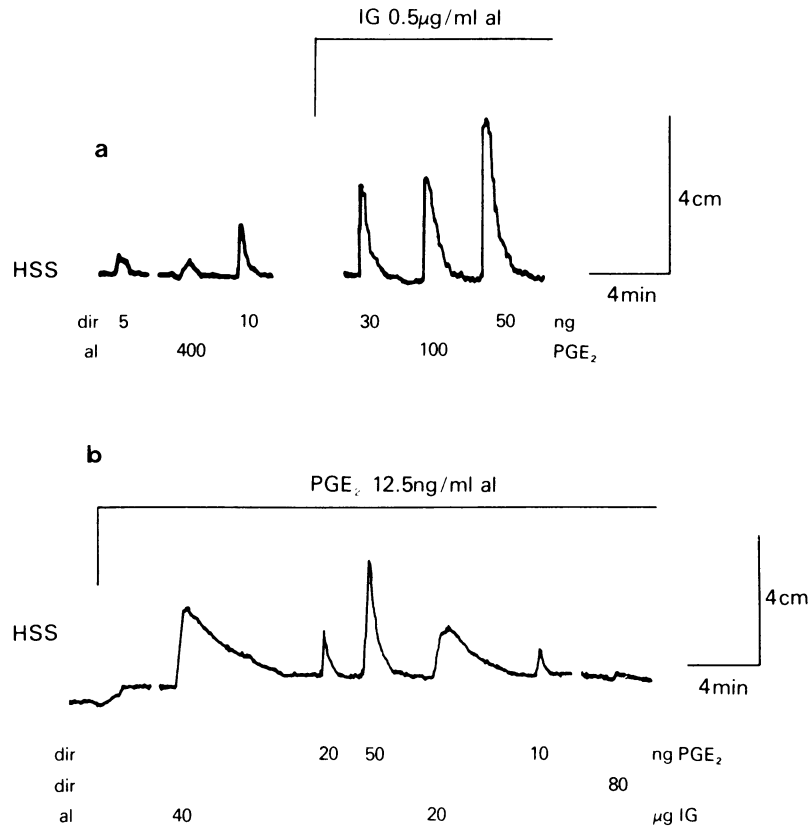

Figure 4 Inhibition of prostaglandin E_2 (PGE_2) inactivation in human isolated lung by indocyanine green. (a) Infusion of dye: the trace shows the responses of a hamster stomach strip superfused with effluent from a sample of human lung. In the first set of responses, only 5 ng survived from 400 ng PGE_2 injected through the pulmonary vessels (al), an inactivation of just under 99%. In the second set of responses, during an infusion of indocyanine green (IG, 0.5 μg/ml; 0.6 μM) through the pulmonary vessels, the inactivation was decreased to 70% (30 ng surviving from 100 ng given al). (b) In a different sample of lung, PGE_2 was infused to give a final concentration of 12.5 ng/ml in the perfusate. The start of this infusion was accompanied by a small increase in the baseline shown at the beginning of the trace. Two injections of indocyanine green (IG, 40 and 20 μg; 0.05 and 0.025 μmol) given al during the infusion produced dose-related contraction of the HSS. The last very small effect was due to a higher dose of IG (80 μg; 0.1 μmol) given directly over the tissues (dir) and confirmed that the contractions observed earlier were not due to the dye alone.

glandin inactivation occurred and that inhibition diminished over a few minutes as the dye was washed from the lung.

The rapidity of this effect is compatible with the mode of action already described for other dye-inhibitors of prostaglandin inactivation, i.e. inhibition of uptake of prostaglandin from the vascular space to the cell where it is metabolized (Bito & Baroody, 1975; Bakhle *et al.*, 1978). I have already suggested that the chemical requirements for inhibition of prostaglandin uptake are very general, i.e. a large organic molecule with a strongly ionized acidic group (Bakhle, 1979). All the dyes tested in this paper are anionic except for the weakest inhibitor, methylene blue, which is a basic dye. Another distinctive property of this dye is that it has biologically important redox characteristics and is used clinically as a reducing agent (Goodman & Gilman, 1975) to correct methaemoglobinaemia. Since the crucial enzymic step in prostaglandin inactivation is oxidation of the 15-hydroxy group catalysed by prostaglandin dehydrogenase (PGDH), it was possible that some of the effect of methylene blue could be due to an interference with the enzymic step and not the transport step

in prostaglandin inactivation. However, this possibility was made less likely by the experiments involving the dimethyl analogue of PGE_2, 16, 16-dimethyl PGE_2. The latter is not a substrate for PGDH (Magerlein, Ducharme, Magee, Miller, Robert & Weeks, 1973) and its inactivation is a reflection of uptake into lung (Bakhle *et al.*, 1978; Bakhle, 1979). Thus, inhibition of 16, 16-dimethyl PGE_2 inactivation is a strong indication of inhibition of the prostaglandin uptake process and that was shown equally by the acidic dye bromsulphthalein and by methylene blue (Table 1).

The experiments on human lung have demonstrated that for at least one dye the same relative inhibitory effect is shown in human lung as it is in rat lung. The relative scarcity of pieces of human lung large enough to perfuse has made it difficult to carry out as extensive a survey of dyes as in rat lung, but it is likely that the other dyes would have equivalent effects in human lung.

The experiments described here related to the metabolism of exogenous prostaglandin in the pulmonary circulation. However, the results have implications for the metabolism of endogenous prostaglandin in any vascular bed, as they suggest that a bolus of dye passing through a vascular bed produced in its wake a bolus of un-metabolized prostaglandin. This could have important consequences for the tone of vascular smooth muscle and for other prostaglandin-responsive tissues. For instance, the blood flow in the kidney may be regulated by locally produced prostaglandin (McGiff & Malik, 1976) and the local concentration of prostaglandin is a balance between synthesis and inactivation. Inhibition of prostaglandin synthesis has clear effects on the kidney (Kimberley, Bowden, Keiser & Plotz, 1978) and it is possible that inhibition of prostaglandin inactivation could equally influence intrarenal blood flow. The ability of kidney and liver to secrete some dyes (indocyanine green and bromsulphthalein) could lead to a concentration of these compounds in extravascular tissues with consequent extravascular affects. The implications are therefore that these dyes are not inert physiologically and that their activity is such that they may interact with the mechanism controlling the variables they are used to measure.

It is important to realise that the amounts of dye used in these experiments are not far from the clinical range. For instance, bromsulphthalein is infused as a 5% solution over 3 min to a total dose of 2.5 mg/kg body weight; for a 70 kg man, 140 mg infused over 3 min gives a concentration in blood of about 10 μM and that concentration produced 50% inhibition of PGE_2 inactivation in rat lung. However, there are other facts that would modify the simple comparison proposed above, for instance, binding of dyes to plasma protein or cells. Nevertheless, in a recent paper, the metabolism of PGE_1 in cat isolated lungs, pump-perfused with blood, was studied by giving a bolus of PGE_1 and indocyanine green together (Linehan & Dawson, 1979). It is noticeable that the percentage metabolism even for small amounts of PGE_1, 0.08 and 6 nmol (about 30 ng and 2 μg respectively) was never greater than 70% whereas previously inactivation of at least 90% had been reported (Ferreira & Vane, 1967; Horton & Jones, 1969; McGiff, Terragno, Strand, Lee, Lonigro & Ng, 1969). One reason for this discrepancy would be the inhibition by indocyanine green of prostaglandin inactivation as demonstrated in the present work.

It seems that there is now a case for the investigation of this potential interaction between dyes and prostaglandins *in vivo* in man. As the dyes are already in clinical use and prostaglandin infusions are ethically permissible, there would seem to be no procedural bar to such a study. In the absence of such direct investigation, it seems proper to consider these and perhaps other similar dyes as capable of interacting with physiologically important mediators with all the consequences that follow.

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