Nicotine causes prostaglandin efflux from isolated perfused rat lung

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Bakhle, Hartiala, Toivonen & Uotila (1978) have recently shown that exposure to cigarette smoke reduces the inactivation of prostaglandin (PG) E_2 by rat lung. Furthermore (–)-nicotine, the principal pharmacologically active constituent of cigarette smoke, stimulates PG release from the isolated rabbit heart (Wennmalm, 1977). It seemed important to establish the effect of (–)-nicotine on PG release from the lung.

Male Sprague-Dawley rats (150–250 g) were housed normally ('control') or in inhalation chambers (Littleton & Umney, 1977) in which they were exposed to an aerosol spray for 5 min every 30 min for periods of 10 days or more. The aerosol contained sodium hydrogen tartrate ('sham-exposed') or 6% (–)-nicotine hydrogen tartrate ('nicotine-treated'). After decapitation the lungs were rapidly removed and perfused via the pulmonary artery at a rate of 6 ml/min with well oxygenated Krebs solution at 37° containing combined antagonists (phentolamine 0.2, mepyramine 0.2, methysergide 0.2, atropine 0.6, and practolol 2.0 μg/ml). For assay of PG-like substances the effluent was superfused over a rat colon and two rat fundus strips over which hexamethonium (10 μg/ml) and indomethacin (20 μg/ml) were also perfused.

In 21 experiments, perfusion of (–)-nicotine (2–30 μg through the lung resulted in slow contraction of all 3 assay tissues, but this was not observed if the drug was applied direct to the tissues at the same doses. We attribute these contractions to the presence of stable PGs in the effluent, since (a) they could be replicated by suitable infusions of PGE_2 (15–480 ng over 3 min), (b) the biological activity in the perfusate was recovered from ethyl acetate extracts, (c) 87% of the biological activity was recovered from the same zone as PGE_2 after thin layer chromatography, and (d) this material was inactivated when injected through the lungs.

The amount of PG-like material released after (–)-nicotine perfusion varied considerably between lungs particularly from the 'nicotine-treated' rats. For example, (–)-nicotine (2 μg) released 18.0 ± 4.5, 20.0 ± 19.6 and 45.3 ± 27.4 ng PGE_2 equivalent from lungs of 'control', 'sham-treated' and 'nicotine-treated' rats respectively, and (–)-nicotine (15 μg) released 102.0 ± 26.0, 114.0 ± 43.5 and 154.0 ± 72.3 ng (mean ± s.e. mean, n = 6–8). The increase in PG efflux from the 'nicotine-treated' lungs is not significant at the 5% level and further experiments are in progress.

The mechanism whereby (–)-nicotine releases PG-like material from the rat lung is not known. However, the response shows tachyphylaxis after 4–6 challenges, is blocked by prior infusion of 12 μg hexamethonium or 7 μg indomethacin, and is not elicited by similar doses of (+)-nicotine. These findings suggest that the response may be caused by a receptor-mediated increase in PG synthesis in the lung. (–)-Nicotine may have other effects on PG metabolism. Preliminary experiments suggest that *in vitro* it inhibits $PGF_{2\alpha}$ breakdown by rat lung homogenates ($ID_{50} \simeq 100$ μM). It is possible that these effects of nicotine on PG metabolism may contribute to some of the pathological changes in lung associated with cigarette smoking.

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References

BAKHLE, Y.S., HARTIALA, J., TOIVONEN, H. & UOTILA, P. (1978). Effects of cigarette smoking on metabolism of vasoactive hormones in rat isolated lungs. *Br. J. Pharmac.*, **63**, 355P–356P.

LITTLETON, J.M. & UMNEY, N.D. (1977). The chronic administration of 1 (–)-nicotine by aerosol to rats. *J. Physiol. (Lond.)*, **266**, 11P.

WENNMALM, A. (1977). Nicotine stimulates prostaglandin formation in the rabbit heart. *Br. J. Pharmac.*, **59**, 95–100.

Sulphasalazine inhibits the pulmonary inactivation of prostaglandins in the rat *in vivo*

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Prostaglandins (PG) of the E and F series are efficiently metabolised in the pulmonary circulation of many species (Piper, Vane & Wyllie, 1970). We have previously shown that sulphasalazine is a potent inhibitor of PG breakdown *in vitro* in 100,000 g supernatants of several organs including rat, guinea-pig, and chick lungs (Moore, Hoult & Laurie, 1978). We have now studied the effect of sulphasalazine on pulmonary PG breakdown in the anaesthetized rat by comparing the vasodepressor potency of prostaglandins injected intravenously (i.v.) and intra-arterially (i.a.).

Male Sprague-Dawley rats weighing 150–250 g were anaesthetized with urethane (650 mg/kg i.p. followed after 5 min by the same dose s.c.).

Polyethylene cannulae were inserted into a femoral vein and retrogradely into the right carotid artery for i.v. and i.a. injection respectively. Blood pressure was recorded from a femoral artery. Sulphasalazine was administered by continuous slow infusion (1 ml every 30 minutes).

Prostaglandins E_1, E_2 and A_2 were vasodepressor in the anaesthetized rat, and the potencies of PGE_2 and PGA_2 relative to PGE_1 (set as 100%) were 40% and 1.6% respectively. PGD_2 produced inconsistent effects, being either pressor (3 experiments) or depressor (2 experiments). However, as a depressor PGD_2 was of equivalent potency to PGA_2. PGs of the E series had much greater effect if injected i.a. than i.v., whereas vasodepressor responses to PGA_2 were similar regardless of the route of administration. The difference between the i.a. and i.v. responses is a measure of the pulmonary metabolism of these prostaglandins and indicates that PGA_2 is not inactivated in the lungs. Our data show that there is extensive pulmonary metabolism of both PGE_1 (97.0 $\pm$ 8.2%, $n = 5$) and PGE_2 (92.3 $\pm$ 6.8%, $n = 5$).

Sulphasalazine (5–50 μg) had no direct effect on blood pressure when injected into the femoral vein, and did not influence the vasodepressor response to injected PGE_1 (4 experiments). During continuous infusion of sulphasalazine (3.1–16.3 μg $kg^{-1}min^{-1}$) the vasodepressor effect of PGE_1 injected i.v. was potentiated, causing a shift in the i.v. dose-response curve to the left towards the i.a. dose-response curve (5 experiments). The response to i.a. administration of PGE_1 was unchanged. The potentiation of i.v. PGE_1 observed in these experiments must be due to decreased inactivation of the prostaglandin in the pulmonary circulation. When the sulphasalazine infusion was stopped the i.v. response to PGE_1 declined rapidly to reach pre-treatment values some 20–40 min later, indicating that the effect of the drug on pulmonary PG breakdown is readily reversible, or that it is itself rapidly metabolized in the body.

These experiments show that sulphasalazine inhibits PG metabolism *in vivo* and, together with previous *in vitro* evidence showing the same effect, support our proposal that this action may underly the therapeutic benefit of sulphasalazine in ulcerative colitis.

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References

MOORE, P.K., HOULT, J.R.S. & LAURIE, A.S. (1978). Prostaglandins and mechanism of action of sulphasalazine in ulcerative colitis. *Lancet*, **ii**, 98.

PIPER, P.J., VANE, J.R. & WYLLIE, J.H. (1970). Inactivation of prostaglandins by the lung. *Nature, Lond.*, **225**, 600–604.

Prostaglandin and noradrenaline interactions in rat brain synaptosomes

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In the peripheral nervous system, the suggestion that prostaglandins of the E series, released as a consequence of noradrenergic action, operate a negative feedback control of noradrenaline release, has become generally accepted (see Brody & Kadowitz, 1974). It is tempting to assume a similar action in the central nervous system, but evidence for this is far less convincing. We have, therefore, studied the effects of noradrenaline on prostaglandin synthesis, and of prostaglandin E_2 (PGE_2) on noradrenaline release from rat brain synaptosomes.

Synaptosomes were prepared from whole brains of 250 g female Wistar rats by the method of Gray & Whittaker (1962). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and PGE_2 release was estimated by radioimmunoassay. As reported previously (Hillier, Roberts & Woollard, 1976) addition of noradrenaline (0.1 mM–1 mM) to incubates increased the release of PGE_2 and $PGF_{2\alpha}$ in a dose dependent manner. Typically, noradrenaline, (0.5 mM) increased PGE_2 levels from approximately 40 pg mg $protein^{-1}$ 10 min^{-1} to approximately 70 pg mg $protein^{-1}$ 10 min^{-1} and $PGF_{2\alpha}$ levels from approximately 225 pg mg $protein^{-1}$ 10 min^{-1} to approximately 340 pg mg $protein^{-1}$ 10 $minutes^{-1}$.

Noradrenaline release was studied in synaptosomes preloaded with [^{3}H]-noradrenaline. After 20–30 min perfusion, at 37°C with Krebs, spontaneous overflow of [^{3}H]-noradrenaline was relatively stable. An increased release could be obtained with 30 mM K^+ Krebs; as expected, this release was calcium dependent. We could not reproduce previous work of Roberts & Hillier (1976) in which PGE_2 (1 μg/ml) was shown to increase [^{3}H]-noradrenaline overflow. To the contrary, PGE_2 (0.5–4 μg/ml) had no effect on the spontaneous outflow or on the K^+stimulated release of [^{3}H]-noradrenaline. The reasons for this discrepancy are not readily apparent. Further, in low calcium (0.25 mM) conditions (normal was 2.57 mM), which render PGE_2 more effective in peripheral systems (Hedqvist, 1974), PGE_2 (1 μg/ml) was still without effect on [^{3}H]-noradrenaline release. The prostaglandin synthesis, inhibitor, indomethacin (10 μg/ml), also did not alter [^{3}H]-noradrenaline overflow.

We conclude that if prostaglandins do possess a