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Effects of (−)-nicotine on the release and inactivation of prostaglandins in the perfused rat lung

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Several recent studies have shown that nicotine and other biologically active constituents in tobacco smoke have effects on the prostaglandin (PG) system. Nicotine causes the efflux of PGE-like substance from the perfused rabbit heart (Wennmalm & Junstad 1976; Wennmalm 1977) and rat lung (Berry et al 1979), but inhibits the release of prostacyclin from rabbit and human vascular tissue (Sonnenfeld & Wennmalm 1980). Furthermore, the conversion of arachidonic acid to PGE_2 and $PGF_{2\alpha}$ in hamster isolated lungs was enhanced by cigarette smoke (Uotila et al 1980).

With regard to the catabolism of PGs, exposure of rats to cigarette smoke caused a decrease in the pulmonary inactivation ex vivo of PGE_2 but not of 5-hydroxytryptamine or bradykinin (Bakhle et al 1979). However, controlled exposure of the rabbit artificially ventilated isolated lung to cigarette smoke did not affect pulmonary $PGF_{2\alpha}$ uptake or metabolism (Hagedorn & Kostenbauder 1977).

With these differences between studies in mind, we have investigated the effect of nicotine on both prostaglandin synthesis and inactivation using lungs taken from animals exposed to a nicotine aerosol for up to 10 days, and from sham-treated or control animals.

Male Sprague-Dawley rats (150–200 g) were housed normally ('control' animals) or in specially constructed inhalation chambers (Littleton & Umney 1977), in which they were exposed to an aerosol spray for 5 min every 30 min for 21 h per day for up to 15 days. 'Sham-treated' animals received pulses from an aerosol containing sodium hydrogen tartrate (6% w/v), whereas for the 'nicotine-treated' animals the aerosol contained 6% w/v (−)-nicotine hydrogen tartrate (BDH). This treatment afforded peak blood concentrations of (−)-nicotine of 80–100 ng ml^{-1}.

The release of PG-like substances from isolated perfused lungs was estimated by multiple organ bioassay. Lungs were removed rapidly from decapitated rats, cannulated via the pulmonary artery and perfused with well-oxygenated Krebs solution (37 °C) at 6 ml min^{-1}. This solution contained a mixture of antagonists to other spasmogens (phentolamine 0·2, mepyramine 0·2, methysergide 0·2, atropine 0·6 and practolol 2·0 μg ml^{-1}). The lung effluent was superfused over a cascade of assay tissues (rat colon and two rat fundus strips) over which hexamethonium (10 μg ml^{-1}) and indomethacin (20 μg ml^{-1}) were also perfused from a separate line. Contractions of the assay tissues were recorded isometrically using Grass FT10 transducers connected to a Grass Model 7B polygraph. The contractions of the assay tissues produced by PG-like substances in the effluent released by bolus injections of nicotine (2–30 μg) were matched by infusions of PGE_2 over the tissues (15–480 ng over 3 min).

In experiments on prostaglandin inactivation, 100 ng $PGF_{2\alpha}$ containing 0·5 μCi [9β-^{3}H]$PGF_{2\alpha}$ (Radiochemical Centre, Amersham; specific activity 14·4 Ci $mmol^{-1}$) was injected through the lung as a bolus and the perfusate collected for 90 s, evaporated and the residue resuspended in 0·5 ml 1 M formic acid and 0·5 ml ethanol. The prostaglandins were extracted into ethyl acetate and, after removal of solvent, resuspended in 20 μl methanol and then subjected to radio-thin layer chromatography to determine the extent of metabolism (Hoult & Moore 1977). PGE_2 metabolism was also measured by superfusion cascade bioassay. The responses to PGE_2 injected directly over the assay tissues were compared or matched with responses obtained after PGE_2 had been injected via the lungs. Direct effects of (−)-nicotine on the enzymes of PG metabolism in rat lung, caecum and kidney 100 000 *g* cytosolic supernatants were studied using radiolabelled $PGF_{2\alpha}$ as substrate as previously described (Hoult & Moore 1977).

Injection of nicotine through the isolated perfused lung produced slow prostaglandin-like contractions of the rat fundus strip preparation which were not observed when the drug was applied directly (Fig. 1). PGE_2 produced similar responses on the rat fundus strip preparations (Fig. 1). The size of the contractions elicited by nicotine challenge of the lung depended on the dose of nicotine (Fig. 1) and were quantified in terms of PGE_2 equivalents using the rat fundus strip responses (Table 1).

Three types of response were observed using the rat colon preparations: 'sustained' contractions (similar to the fundus strip responses), single 'spike' contractions and 'bursts' of contractions. Nicotine applied directly to the tissues (34 challenges) produced a spike 33 times (as in Fig. 1) and a burst once. PGE_2 applied directly (91 challenges) produced bursts 56 times (as in Fig. 1) and sustained contractions 27 times. When prostaglandin-like material was released after nicotine challenge through the lung (137 times according to the fundus strip contractions), this was observed on the rat colon as sustained contractions 21 times (as in Fig. 1), bursts 55 times and a spike 7 times (probably due to the nicotine itself). Thus the pattern of responses on the rat

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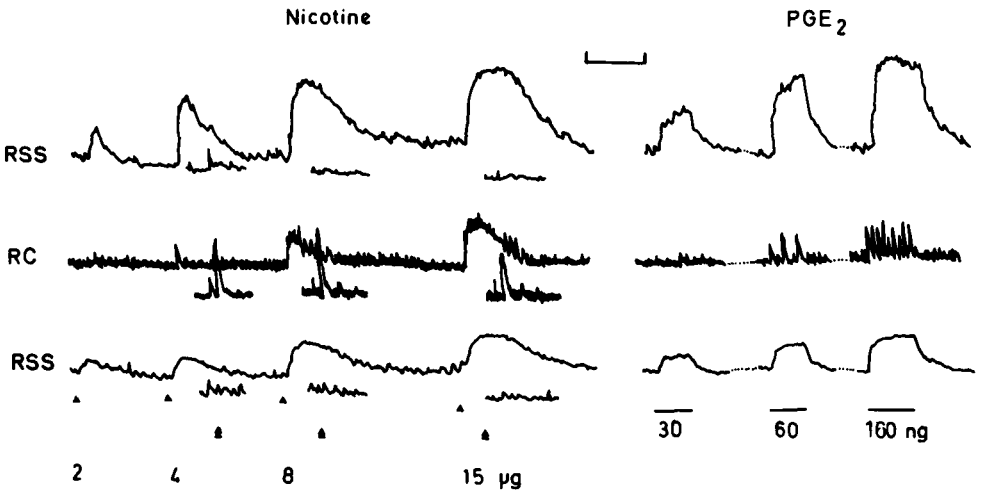

FIG. 1. Release of prostaglandin E_2-like spasmogenic material from isolated perfused rat lung challenged with (—)-nicotine at the doses indicated. The pulmonary effluent was passed over a series of assay tissues: RSS = rat stomach, strip, RC = rat colon. Similar doses of nicotine injected directly over the tissues (▲) did not elicit prostaglandin-like responses. Representative effects of three minute infusions of prostaglandin E_2 directly over the tissues are also shown. Time bar = 5 min.

colon after nicotine challenges is also indicative of release of prostaglandin E_2-like material, although this tissue cannot be used for quantitative purposes.

Similar biological activity was recovered after ethyl acetate extraction of the perfusates and reinjection; furthermore, in four experiments the tissue responses to this PG-like material were greatly reduced when portions of the extracts were injected through the lungs (as expected for classical PGs and verified by injection of authentic PGs through the lungs). In addition, release of PG-like material by (−)-nicotine was prevented by prior perfusion of indomethacin (7 μg min^{-1}) through the lung (4 tests) and 87% of the biological activity in the pooled extract from one experiment was recovered from the same zone as authentic PGE_2 after thin layer chromatography. We therefore believe these contractions are due to the presence in the effluent of stable PGs, particularly PGE_2.

Lungs from control, sham-exposed and nicotine-exposed animals were challenged in vitro with nicotine; all released PGE_2-like material in a dose-dependent fashion (Table 1), and dose for dose the amounts released were always least in the nicotine-treated groups when compared with control, sham-treated or pooled data (Table 1). Variability was considerable, especially in the sham-treated group where marked tachyphylaxis to the higher doses was evident (Table 1), and thus the differences are not statistically significant. In some cases we failed to detect any PGE_2-like material released at all from challenged lungs (notably in the sham-treated group), but these data have been included in the calculations of Table 1 despite our uncertainty about whether this is caused by genuine insensitivity of the lung, inadequate pulmonary perfusion or vagaries of the bioassay technique. Nevertheless we conclude that release by nicotine of PGE_2-like material was reduced in lungs taken from animals which had been exposed to nicotine aerosol.

Preliminary evidence suggests that the nicotine-induced release of PG-like material is receptor-mediated because it was reversibly blocked by 2 μg ml^{-1} hexamethonium (97·3±3·2% inhibition, 4 experi-

Table 1. Dose-dependent efflux of PGE_2-like material from isolated perfused rat lungs after nicotine challenge using normal, sham-exposed and nicotine-exposed animals.

Nicotine dose	PGE_2 released, as ng equivalents according to rat stomach strip bioassay			
	Control lungs	Sham-exposed	Pooled values (Control + sham)	Nicotine-exposed 10–15 days
μg	n = 4 or 10	n = 6–10	n = 10–20	n = 9 or 10
2	18·0 ± 4·5	16·7 ± 16·1	17·2 ± 9·1	3·7 ± 2·0
4	21·1 ± 5·6	60·7 ± 33·9	39·8 ± 16·1	7·4 ± 4·5
8	74·5 ± 16·2	60·1 ± 25·0	68·1 ± 13·5	32·0 ± 13·7
15	86·5 ± 19·4	75·1 ± 29·6	80·8 ± 16·8	35·0 ± 12·5
30	156·2 ± 35·2[a]	55·9 ± 29·3[a]	107·1 ± 24·7[b]	31·7 ± 11·2[b]

Significarce of differences by Student's paired t-test in those pairs denoted by [a], [b] $P < 0.05$.

Table 2. Effect of (−)-nicotine on $PGF_{2\alpha}$ inactivation in 100 000 *g* supernatants prepared from rat organs.

(−)-Nicotine μM	Percentage inactivation* Lung	Caecum	Kidney
0	12·0 ± 0·7	93·0 ± 0·5	44·3 ± 1·4
50	18·3 ± 2·6	92·0 ± 0·8	49·0 ± 4·3
100	14·8 ± 2·2	87·3 ± 3·7	44·8 ± 1·2
200	18·3 ± 2·0	89·0 ± 0·8	41·0 ± 0·5

* Results show mean % inactivation (± s.e.m., 4 determinations) of 10 μg ml^{-1} $PGF_{2\alpha}$ labelled with 0·05 μCi [9β-^{3}H]$PGF_{2\alpha}$ incubated at 37 °C for 15 min (caecum) or 60 min (lung, kidney).

ments). The response also showed tachyphylaxis after 4–6 challenges and was not elicited by similar doses of (+)-nicotine.

The presence of up to 400 μM (−)-nicotine (65 μg ml^{-1}) in the perfusing medium had no direct inhibitory effect on the metabolism of 100 ng $PGF_{2\alpha}$ in perfused lungs taken from normal or sham-treated rats (e.g. inactivation in lungs perfused with Krebs' solution alone was 66·8 ± 2·7%, and in the presence of 200 or 400 mM (−)-nicotine was 60·2 ± 6·2% and 60·5 ± 5·9% respectively, n = 15, lungs from 4 rats: differences not significant). Similarly, (−)-nicotine did not inhibit $PGF_{2\alpha}$ metabolism in vitro in rat lung, caecum or kidney 100 000 *g* cytosolic supernatants at concentrations of up to 200 μM (Table 2).

Exposure of rats to (−)-nicotine aerosols for 2 days or 10 days did not affect pulmonary breakdown of $PGF_{2\alpha}$ ex vivo (e.g. inactivation of 100 ng $PGF_{2\alpha}$ was 63·3 ± 4·7% or 58·6 ± 5·1% in lungs from sham- or nicotine-exposed rats, respectively; 20 tests from 5 rats in each group: differences not significant). This result was confirmed using PGE_2 as substrate and estimating inactivation by bioassay; lungs from control animals inactivated 200 ng PGE_2 by 93·0 ± 1·3% and those from nicotine-treated animals by 90·0 ± 2·0% (n = 6–8).

We have shown that nicotine causes the release of a PG-like substance from the perfused rat lung and presented preliminary evidence that this could be mediated by activation of a nicotinic receptor. The actual mechanism for release could be due to increased activity either of the membrane-bound 'PG synthetase' complex or of the phospholipases presumed to be responsible for mobilizing arachidonic acid from membrane phospholipids (Vogt 1978; van den Bosch 1980). However, the methods used here cannot distinguish between these possibilities. From studies with platelets, Wennmalm (1978) has suggested a third possibility, namely that nicotine redirects the arachidonic cascade away from PGI_2 formation towards PGE_2. If this were so here, a concomitant decrease in the release of 6 keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) from the perfused rat lung accompanying the increased efflux of PGE_2 would be expected.

The reduction we observed in the efflux of PGE-like substance after nicotine treatment might be due to the development of cellular or membrane adaptation to nicotine or to a direct reduction in the amount or activity of the PG synthetase enzyme, or any combination of these effects. Whatever the mechanism, these effects of (−)-nicotine could be of importance in relation to the long-term cardiovascular effects of tobacco smoking (Royal College of Physicians 1978), because a PG deficiency, especially of prostacyclin, might promote thrombosis (Gryglewski et al 1976; Moncada et al 1976).

The lack of effect of nicotine on pulmonary prostaglandin inactivation and absence of differences between normal and nicotine-exposed animals is of interest in the light of a previous finding that relatively brief exposure to cigarette smoke reduces the ability of the lung to inactivate PGE_2 (Bakhle et al 1979). Our results suggest that the inhibition of PG breakdown observed after exposure to smoke cannot be due to the nicotine component but might rather be due to one or more of the other biologically active ingredients.

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