Effect of nicotine on production of prostacyclin in human umbilical artery

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SUMMARY The influence of nicotine on the production of prostacyclin was studied in umbilical arteries from newborn infants of mothers who were smokers and those who were non-smokers. Thirteen umbilical cords were obtained from the non-smokers and 10 from the smokers. After their ability to produce prostacyclin was shown, the arteries were perfused during a 20 minute period with nicotine 10^{-7} mol in Krebs-Henseleit buffer and then again during 20 minutes with pure buffer. Nicotine led to a decline in prostacyclin production in all but one artery. In five of 12 arteries from mothers who did not smoke the production recovered during buffer reperfusion, while in nine of 10 arteries from mothers who smoked, prostacyclin production declined to lower levels and did not increase during 20 minutes buffer reperfusion. It is concluded that nicotine has a direct depressive effect on the prostacyclin production in the vascular wall, which in turn may lead to increased platelet aggregation and thus be a precursor of vascular lesions.

Since the discovery of the prostaglandins by Goldblatt¹ and Von Euler,² much research has been done on this group of substances. Prostaglandins are formed by the enzymatic oxygenation of polyunsaturated fatty acids, primarily arachidonic acid. Many publications in recent years have shown the potentially important actions of the unstable intermediates of arachidonic acid metabolism.³⁻⁹ Platelet aggregation, vasoconstriction, thrombus formation, and vascular occlusion may all be fundamentally influenced by the interplay between two of these intermediates: thromboxane A_2 and prostacyclin (also known as prostaglandin I_2).^{7,8,10-12} Thromboxane A_2 is released by platelets when they aggregate. This induces further aggregation and leads to the formation of thrombi accompanied by vasoconstriction.^{4,5,10,11} These events can be counteracted by prostacyclin released by the vessel wall, which in turn inhibits platelet aggregation and produces vasodilatation. Prostacyclin is predominantly produced in the endothelial layer; there is progressively less prostacyclin production towards the outside of the vessel wall.¹³ Thus, prostacyclin might be described as a defensive local hormone against thrombocyte aggregation. Its release can be influenced by hormones, nerve stimulation, mechanical damage,

decreased oxygen tension, and several other stimuli. In 1978 Wennmalm¹⁴ showed that nicotine perfusion of isolated rabbit hearts depressed the release of 6-keto-PGF 1 alpha, a stable metabolite of prostacyclin. The present study deals with the question as to whether a similar effect could be shown on the production of prostacyclin in the human arterial wall. Because of their obvious availability from the placenta, human umbilical arteries were isolated immediately after birth. Two groups of newborn infants were studied: (i) those of mothers who did not smoke and (ii) those of mothers who smoked at least 10 cigarettes a day during pregnancy.

Subjects and methods

Segments of about 5 cm were taken within five minutes after delivery from 51 umbilical cords. One artery from each umbilical cord was then perfused for 20 minutes, in the direction of the physiological flow, with 95% O_2 , and 5% CO_2 saturated Krebs-Henseleit buffer at 37°C.

In 30 of the 51 umbilical arteries production of prostacyclin like substance could be shown. In the 21 arteries not producing this substance longer periods of buffer perfusion made no difference and these were therefore excluded from the study. The non-producing group was similar to the producing group with respect to age, gestational age, birthweight, sex, and smoking habits.

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This study was supported in part by a grant from the Dutch Heart Foundation.

Accepted for publication 15 June 1982

Seven producing arteries were perfused with buffer only during 60 minutes. The effluent was sampled for measurement of the prostacyclin like substance at 20 minute intervals, which showed that there was steady production of prostacyclin in all seven arteries during the one hour perfusion time. The other 23 arteries producing prostacyclin like substance were perfused during 20 minutes with nicotine 10^{-7} mol in buffer. This was followed by a 20 minute reperfusion with buffer only. A pulsatile flow of 30 ml per hour at a pulse frequency of 120 per minute was employed.* After the 20 minute perfusion periods, samples were taken of the effluent for measurement of the anti-aggregation effect. The extent of adenosine diphosphate-induced aggregation of rat platelets was studied because, contrary to human platelets, these have a monophasic aggregation pattern with a circumscribed peak value. Platelet rich plasma was diluted with saline- CaCl_2 solution in which the final calcium concentration was 4×10^{-4} mol. After addition of the effluent (within 15 seconds after it was sampled), aggregation was induced by adenosine diphosphate. The aggregation measurements were done with a Minigator I (Payton Associates Ltd, no 401) at 37°C . The prostacyclin like substance in the effluent was quantified by comparison with standard prostacyclin (Unilever Research) concentrations. To evaluate whether the main part of the produced aggregation inhibiting substance was indeed prostacyclin, the total effluent from four consecutive umbilical arteries was sampled during the first buffer perfusion and during nicotine perfusion. From these pooled samples gaschromatographic analysis was carried out to measure the amount of 6-keto-PGF 1α .¹⁵

Pertinent data are shown in the Table for non-smokers (group 1) and smokers (group 2). None of the women used any platelet aggregation inhibiting drug in the week before delivery.

*This is to be published by J F A Quadri, R Vos, F ten Hoor in the *J Pharmacol Methods*.

Table Data of mothers and newborns included in study

	Group 1 Non-smokers	Group 2 Smokers
Number	13	10
Family history of atherosclerosis	2	2
Median age (y)	26	25
Range	19-44	20-31
Median gestational age (wk)	39	39
Range	36-42	37-41
Median birthweight (g)	3150	3200
Range	2400-3815	2655-3650
Sex newborn (M/F)	6/7	5/5
Race white	11	9
black	1	1
other	1	

Results

Thirteen of the prostacyclin like substance producing arteries came from non-smokers (group 1) and 10 from smokers (group 2). To rule out a possible direct role of nicotine in the inhibition of platelet aggregation, nicotine 10^{-7} mol was administered to the platelet suspension in a test tube. This showed no influence on platelet aggregation.

Group 1 showed a decline in production of prostacyclin like substance after perfusion of nicotine for 20 minutes in 12 of the 13 arteries (Fig. 1). After 20

Fig. 1 Effect of nicotine perfusion on the production of prostacyclin (pc) in ng/min compared with the production of prostacyclin during first buffer perfusion.

minutes' reperfusion with buffer the production of prostacyclin like substance in five of the 12 arteries increased again (Fig. 2). In one artery a small rise during nicotine perfusion was noted; it was not reperfused with buffer. The Friedman test, a non-parametric significance test,¹⁶ of the differences

Fig. 2 Effect of buffer reperfusion on the production of prostacyclin (pc) in ng/min compared with the production of prostacyclin during nicotine perfusion.

between the three periods (that is control buffer, nicotine, and buffer reperfusion) was highly significant ($p < 0.001$).

Group 2 showed a decline in the release of prostacyclin like substance in 10 of 10 arteries (Fig. 1). After 20 minutes' reperfusion with buffer, only one of the 10 arteries showed increased production (Fig. 2). The Friedman test of the differences between the three periods also proved significant ($p < 0.001$).

The mean values of group 1 and 2 are summarised in Fig. 3. The Wilcoxon rank sum test¹⁶ yielded a significant difference in production of prostacyclin like substance during the first buffer perfusion between arteries of the newborn of mothers who smoked and of those who did not smoke ($p < 0.05$).

The amount of 6-keto-PGF 1 alpha, the stable metabolite of prostacyclin, in the four pooled samples was 353 ng before and 160 ng after nicotine perfusion, which suggests that the substance is prostacyclin.

Fig. 3 Mean values of prostacyclin production (pc) in ng/min during the three perfusion periods in umbilical arteries from non-smokers and smokers.

Discussion

Chronic intermittent cigarette smoke inhalation is, among other factors, strongly correlated with vascular diseases in humans.¹⁷ Furthermore, children of mothers who smoke show significantly lower birthweights.¹⁸ Our results indicate that nicotine directly inhibits umbilical vessel wall production of prostacyclin in vitro. When fresh umbilical arteries

were perfused during 20 minutes with a solution containing amounts of nicotine comparable to those found in the plasma of an individual after smoking one cigarette,¹⁹ in all but one there was a fall in the production of prostacyclin. Control umbilical arteries perfused without a nicotine solution showed persistent production of prostacyclin. Five of 12 umbilical arteries from non-smokers showed a rise in prostacyclin level during 20 minutes of buffer reperfusion, whereas in only one of 10 reperfed arteries from smokers could such a rise be shown. The baseline production of prostacyclin in the arteries from the mothers who smoked, however, was significantly higher than in those who did not smoke. It is postulated that a diseased endothelial layer builds up its maximum defence during nicotine free intervals, while its last reserves fall away during the nicotine "attack" and need more time to recover.

By means of a scanning electron microscope, Asmussen and Kjeldsen²⁰ have shown the deleterious effects of cigarette smoking during pregnancy on the endothelium of the umbilical artery. In our laboratory these findings have been corroborated in the same type of arteries as were used in the present investigation. These results may explain the lower growth rate and birthweight of children from mothers who smoked.¹⁸ In addition, the transitional thrombocytopenia in low weight infants of this category of mothers²¹ could result from this interaction. In the light of current evidence, it is likely that arteriosclerosis in part may be caused by increased platelet aggregability during cigarette smoking.²²⁻²⁴ This was originally thought to be caused by a rise in adrenergic tone²⁵ and/or by a rise in plasma free fatty acid level²⁶ during cigarette smoke inhalation. We found, however, a direct in vitro interference of nicotine with vessel wall prostacyclin production. In our model the influence of catecholamines and free fatty acids can be excluded. Though the exact nature of nicotine interaction with prostacyclin generation remains unclear, the mechanisms may form the basis of nicotine-induced human vascular disease.

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