Br. J. Pharmac. (1980), **71**, 609–613

INHIBITION BY NICOTINE OF THE FORMATION OF PROSTACYCLIN-LIKE ACTIVITY IN RABBIT AND HUMAN VASCULAR TISSUE

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1 Rings of vascular tissue (from rabbit aorta or human peripheral vein) were incubated at room temperature in Tyrode solution in the absence or presence of nicotine or indomethacin.

2 Addition of portions of the incubates to human platelet-rich plasma (HPRP) elicited a decrease in adenosine 5'-diphosphate (ADP)-induced platelet aggregation in this plasma. Authentic prostacyclin (PGI_2) also induced such a decrease. The decreased aggregation amplitudes that followed the addition of the vascular tissue incubates and of PGI_2 were equally potentiated by theophylline (10^{-4} M).

3 Both nicotine and indomethacin counteracted the formation of platelet anti-aggregatory activity in the vascular tissue incubates. The IC_{50}s of nicotine and of indomethacin on the formation of platelet antiaggregatory activity were 2×10^{-5} M and 6×10^{-6} M, respectively.

4 Nicotine failed to affect the platelet anti-aggregatory effect induced by authentic PGI_2 in HPRP.

5 It is concluded that nicotine counteracts the formation of platelet anti-aggregatory activity in rabbit aorta and human peripheral vein by eliciting an inhibitory effect on the bioformation of prostacyclin in these types of vascular tissue.

Introduction

Nicotine is a naturally occurring alkaloid, obtained from the dried leaves of *Nicotiana tabacum*. It is frequently used by man via tobacco smoking, chewing and snuffing. The pharmacological effects of nicotine are complex and its administration often gives rise to unpredictable changes in the body. It has been shown in our laboratory that the efflux of 6-keto-prostaglandin-$F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), the stable metabolite of prostacyclin (PGI_2), from rabbit isolated hearts perfused with sodium arachidonate was diminished when the perfusion medium contained nicotine (Wennmalm, 1978a), and that this decreased efflux of 6-keto-$PGF_{1\alpha}$ was paralleled by an increased liberation of prostaglandin E_2 (PGE_2) (Wennmalm, 1978b). Support for the concept of nicotine as an inhibitor of PGI_2 formation in tissues has been obtained in studies where the addition of nicotine (10^{-7} to 10^{-4} M) to the perfusing medium substantially reduced liberation of prostacyclin-like activity from isolated or perfused rat aortae or rabbit hearts (ten Hoor & Quadt, 1979; Wennmalm, 1980c).

In the present paper we describe dose-response relationships for the inhibitory effect of nicotine on the formation of prostacyclin-like activity in vascular tissue from rabbits and man.

Methods

Platelet aggregation

Peripheral venous blood was obtained from voluntary laboratory personnel of either sex and mixed ages who had not taken aspirin-like drugs for at least one week. It was collected into plastic tubes containing 1/10 vol. 3.8% sodium citrate. Following centrifugation at 200 *g* for 15 min, the platelet-rich plasma thus obtained was decanted and diluted with an equal volume of 0.1 M Tris buffer pH 7.4. The diluted plasma was used for the aggregation recordings.

Platelet aggregation was induced by adding 2 μg of adenosine 5'-diphosphate (ADP) (Sigma Chemicals) to 1 ml portions of the diluted plasma. It was monitored in a Vitatron DC 200 photometer connected to a linear ink recorder.

Incubation of vascular rings

Rabbits of either sex and mixed strains, weighing from 1.5 to 3.0 kg, were killed by a blow on the head and exsanguinated via the left carotid artery. The aorta was rapidly dissected free from the heart down

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to the pelvic bifurcation and rinsed carefully in saline. If not used immediately, it was kept at 4°C immersed in saline.

Samples of human peripheral veins were obtained after informed consent from patients undergoing vascular surgery. The veins were immediately cleansed of extra-vascular tissue and carefully rinsed in saline.

Incubation of vascular tissue was performed at room temperature in 2 ml of Tyrode solution for a period of 1 h (rabbit aortae) or 15 min (human veins). Just before incubation, the rabbit aorta or human vein was cut into rings approximately 1 mm wide, weighing 5 to 7 mg. Equal numbers of rings were incubated in medium containing nicotine, indomethacin or no drug (control medium), respectively. The incubation medium was Tyrode solution of the following composition (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 12, NaH_2PO_4 0.4 and glucose 5.6. Usually 30 to 60 mg (5 to 10 rings) of vascular tissue were incubated in 2 ml medium. Incubation was stopped by removing the vascular rings from the medium.

Assessment of the anti-aggregatory effect of vascular incubates and of prostacyclin

Analysis of the platelet anti-aggregatory activity in the vascular incubates, and of the inhibitory action which nicotine or indomethacin exerted on the formation of such activity, was performed as follows. A dose-response relationship was established for the anti-aggregatory effect of authentic PGI_2 on ADP-induced aggregation in the diluted human platelet-rich plasma (HPRP). The dose-response line thus obtained (Figure 1) was used to estimate the amount of platelet anti-aggregatory activity (expressed as PGI_2 equivalents) which was formed during the incubation of vascular tissue in the absence or presence of drug. Both authentic PGI_2 and portions of the vascular incubates were added to the aggregometer tube, 2 min before the addition of ADP. The volume of vascular incubate added for assessment of the amount of PGI_2-like activity formed (in the absence or presence of drugs) was chosen to result in a 50% inhibition of the aggregation amplitude. The volume required to induce such inhibition varied between 20 and 150 µl. All aggregation amplitudes were expressed as a percentage of the amplitude in control aggregations induced by the addition of ADP to the HPRP-Tris buffer mixture only. The amplitude of the aggregation curve was always measured 90 s after the addition of ADP.

One series of experiments was designed to investigate the effect of theophylline on the inhibitory activity of incubates of rabbit aortic rings and of PGI_2 on the inhibition of ADP-induced platelet aggregation in the diluted HPRP. Theophylline (Teofyllamin ACO, 10^{-4} M) was added to the HPRP-Tris buffer mixture before the addition of vascular incubates or of PGI_2. ADP was added 2 min after the addition of vascular incubate or PGI_2 and platelet aggregation was monitored. The effect of theophylline on the aggregation amplitude was calculated as described above.

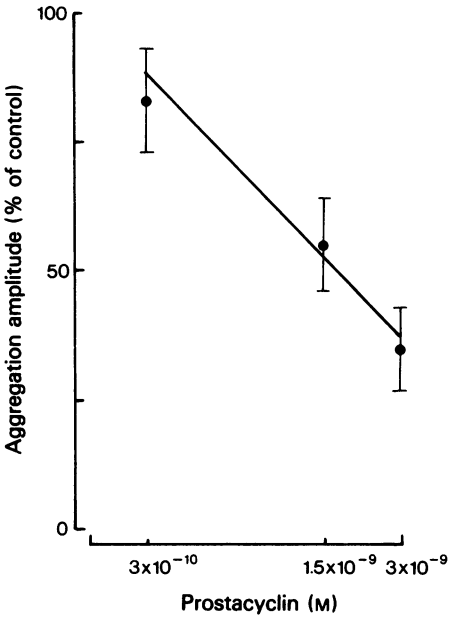

Figure 1 Effect of prostacyclin on ADP-induced platelet aggregation in an HPRP-Tris buffer mixture. All aggregation amplitudes were assessed 90 s after the addition of ADP. The aggregation amplitudes in the presence of PGI_2 are expressed as a percentage of control aggregation amplitudes in the absence of drug. The bars indicate the s.e. mean of experiments.

The inhibitory effect of authentic PGI_2 on ADP-induced aggregation in HPRP-Tris buffer mixture was also tested in the presence of various concentrations of nicotine. In this series, nicotine was added to the HPRP-Tris buffer mixture 10 to 20 min before the addition of ADP. Evaluation of the anti-aggregatory effect of PGI_2, in the absence or presence of nicotine, was performed as above.

Results

Effect of vascular incubates and of authentic prostacyclin on ADP-induced aggregation

Addition of a small volume (20 to 80 µl) of the medium resulting from incubation of 3 to 5 rings of rabbit aorta in 2 ml of Tyrode solution to the HPRP-Tris buffer mixture caused a marked inhibi-

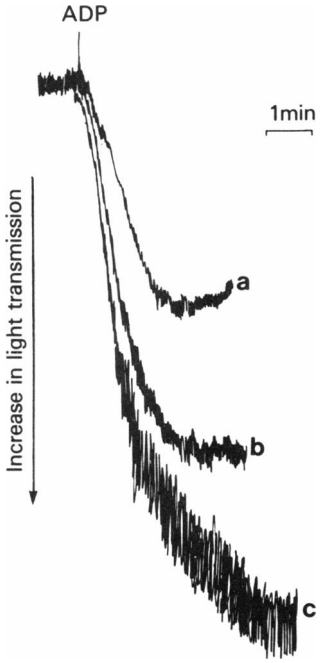

Figure 2 Typical aggregation recordings, demonstrating the inhibitory effect of vascular incubate on ADP-induced platelet aggregation in an HPRP-Tris buffer mixture and the attenuating effect of nicotine on this inhibition. In (a), 30 µl of the medium resulting from incubation for 60 min of 5 rings of rabbit aorta (approx. 30 mg) with 2 ml of Tyrode solution was added to 1 ml of an HPRP-Tris buffer mixture and 2 min later ADP was added. In (b) the procedure was identical except that nicotine (2×10^{-6} M) was present in the medium during the incubation. Tracing (c) is a control, performed without addition of incubate. As seen from the figure, the platelet anti-aggregatory activity was decreased when nicotine was present during incubation of the aortic rings.

tion of ADP-induced platelet aggregation in this mixture (Figure 2). The amplitude of the aggregation varied inversely with the volume of vascular incubate added, indicating a close relation between these parameters.

Portions (80–150 µl) of the medium resulting from incubation during 15 min of 3 to 5 rings of human vein in 2 ml of Tyrode solution also elicited a dose-related inhibition of ADP-induced platelet aggregation in the HPRP-Tris buffer mixture.

When authentic PGI_2 was added to the HPRP-Tris buffer mixture, a straight-line relationship was obtained between the log dose PGI_2 added and the resulting percentage inhibition of the amplitude of ADP-induced aggregation. A slight inhibitory effect on platelet aggregation was obtained after addition of 100 pg of PGI_2 to the HPRP-Tris buffer mixture,

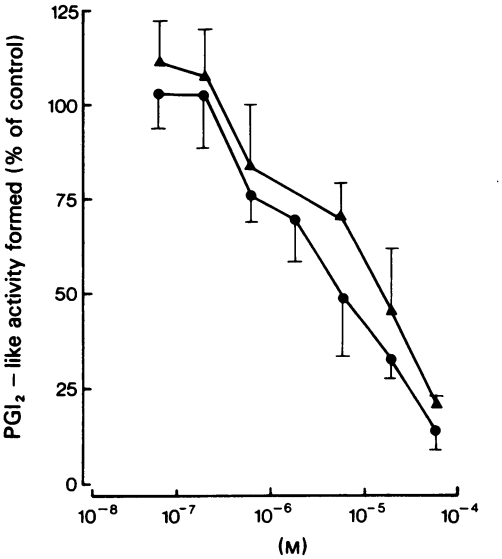

Figure 3 Effect of nicotine (▲) and of indomethacin (●) on the prostacyclin-like activity formed during 60 min incubation of rabbit aortic rings in Tyrode solution. The activity in the presence of nicotine or indomethacin is expressed as a percentage of the activity resulting from simultaneous incubations in the absence of drug. The points on the curves represent the mean of 3 to 13 such incubation pairs (nicotine curve) or 3 to 7 incubations pairs (indomethacin curve); vertical lines show s.e. mean. Further details of the calculation are given under Methods.

and larger amounts of PGI_2 regularly exerted a marked inhibitory effect. The IC_{50} of PGI_2 on the platelet aggregation amplitude was about 2×10^{-9} M (Figure 1).

Effect of nicotine and of indomethacin on the platelet anti-aggregatory activity in vascular incubates

The presence of nicotine in the medium during incubation of the rabbit aortic rings considerably diminished the resulting platelet anti-aggregatory activity of the incubate (Figure 2). The lower concentrations of nicotine studied (6×10^{-8} and 2×10^{-7} M) were found to be ineffective but higher concentrations (6×10^{-7} M to 6×10^{-5} M) caused a progressive decrease in the platelet anti-aggregatory capacity of the incubate (Figure 3). The IC_{50} of nicotine as inhibitor of platelet anti-aggregatory activity was 2×10^{-5} M.

Indomethacin added to the medium before incubation also elicited a dose-related inhibition of the incubate's platelet anti-aggregatory capacity (Figure 3). Like nicotine, indomethacin was ineffective at the lowest concentrations investigated (6×10^{-8} and 2×10^{-7} M) and dose-dependently active at higher

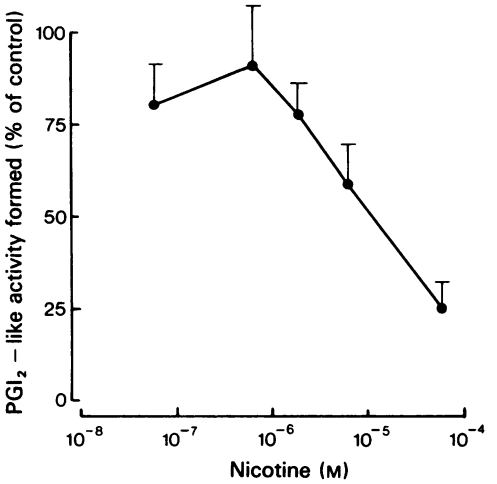

Figure 4 Effect of nicotine on the prostacyclin-like activity formed during 15 min incubations of rings of human veins in Tyrode solution. Each point represents the mean of 3 to 8 observations (cf. text to Figure 3); vertical lines show s.e. mean.

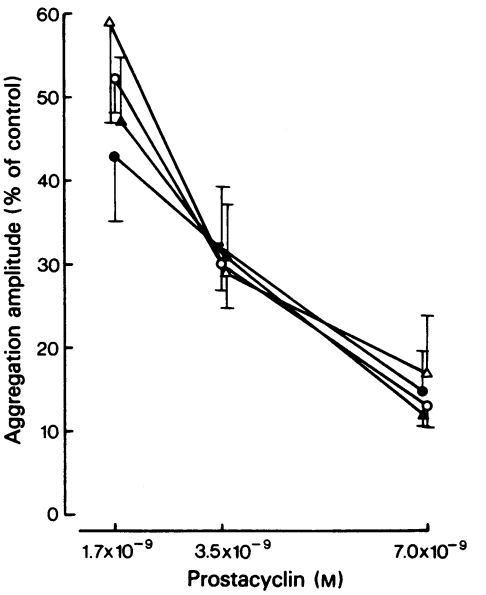

Figure 5 Effect of different concentrations of authentic prostacyclin (in the absence or presence of different concentrations of nicotine) on ADP-induced aggregation of platelets in an HPRP-Tris buffer mixture. No nicotine (control) (○); nicotine 6×10^{-5} M (△); 6×10^{-6} M (●) and 6×10^{-7} M (▲). As seen from the figure, nicotine did not affect the platelet anti-aggregatory activity of prostacyclin.

concentrations. The IC_{50} of indomethacin in counteracting the anti-aggregatory activity formed during incubation of rabbit aortic rings in Tyrode solution was 6×10^{-6} M.

Addition of nicotine to the incubation medium also decreased the platelet anti-aggregatory activity of incubates of human vascular tissue. As with rabbit aortic rings, the activity in the incubates of human vein rings was not counteracted by the lowest dose of nicotine studied (6×10^{-8} M), while higher doses (6×10^{-7} to 6×10^{-5} M) were effective. The IC_{50} of nicotine was 10^{-5} M (Figure 4).

Effect of theophylline and of nicotine on the inhibition of platelet aggregation induced by vascular incubates and by prostacyclin

Theophylline (10^{-4} M) augmented the inhibitory effect of the aortic ring incubates by 44 ± 3% (mean ± s.e., $n = 8$) compared to controls without drug. When authentic PGI_2 was used to inhibit ADP-induced platelet aggregation, theophylline (10^{-4} M) augmented the inhibitory effect by 46 ± 2% ($n = 8$). Thus, theophylline enhanced the inhibitory effect of the vascular incubates and of PGI_2 on platelet aggregation to the same extent.

Nicotine added to the HPRP-Tris buffer mixture before addition of PGI_2 did not affect the inhibitory effect of PGI_2 on ADP-induced platelet aggregation. As seen from Figure 5, the dose-response curve expressing the log conc. PGI_2-platelet aggregation amplitude was not displaced by concentrations of nicotine that counteract the anti-aggregatory activity which follows incubation of vascular tissue.

Discussion

In the present experiments, incubation of rings of rabbit aorta or human peripheral vein in a saline medium resulted in the appearance of platelet anti-aggregatory activity. There is very little doubt that this activity was due to formation of prostacyclin in the vascular tissue during the incubation, for the following reasons. Firstly, others have shown that PGI_2 is the anti-aggregatory agent formed during incubation of vascular tissue in saline media (cf. e.g. Bunting, Gryglewski, Moncada & Vane 1976; Remuzzi, Misiani, Muratore, Marchesi, Livio, Schieppati, Mecca, Gaetano & Donati, 1979). Secondly, the formation of anti-aggregatory activity was inhibited by indomethacin, as is the biosynthesis of prostaglandin in tissues (Vane 1971). Thirdly, the anti-aggregatory activity of the incubates was augmented by the phosphodiesterase inhibitor, theophylline. This indicates that the anti-aggregatory action of the incubates was mediated by a rise in platelet cyclic adenosine 3′, 5′-monophosphate (cyclic AMP), a mechanism that has been

shown to apply to PGI_2 (Tateson, Moncada & Vane, 1977). In fact, theophylline was found to facilitate the anti-aggregatory activity of the vascular incubates and of authentic PGI_2 to the same degree in the present experiments.

Nicotine dose-dependently inhibited the appearance of prostacyclin-like activity in the incubates of rabbit aortic rings. Since nicotine failed to interfere with the inhibitory effect that was elicited by authentic PGI_2 on platelet aggregation, its attenuating action in the incubates probably relates to inhibition of the synthesis of anti-aggregatory activity taking place in the vascular tissue during incubation. This crucial statement is supported by the finding that the dose-response curve of nicotine as inhibitor of the formation of anti-aggregatory activity parallels that of indomethacin, which is an established inhibitor of the cyclo-oxygenase converting arachidonate to prostaglandin endoperoxides. Nicotine has been shown to interfere with the basal efflux of 6-keto-$PGF_{1\alpha}$ from the rabbit isolated heart, as well as the liberation of PGI_2 from this organ in response to exposure to arachidonate or hypoxia (Wennmalm 1978a,b; 1980). The present results demonstrate that the inhibitory effect of the drug also operates in extra-cardiac tissue. Furthermore, they show that nicotine also affects the formation of prostacyclin-like activity in human vascular tissue.

The IC_{50} of nicotine as inhibitor of the formation of prostacyclin-like activity in human veins was 10^{-5} M. It is certainly of interest to relate this concentration to that in plasma in tobacco-smoking subjects. Few such data have been published, mainly due to the technical difficulties involved in assessing low concentrations of nicotine. Armitage, Dollery, George, Houseman, Lewis & Turner (1974) observed arterial blood concentrations of nicotine of 30 to 40 ng/ml (195 to 247 nM) during inhaling cigarette smoking and Turner, Silett & McNicol (1977), analysing nicotine concentrations in venous blood during cigar smoking, reported plasma levels of 13 to 46 ng/ml (79 to 281 nM). According to the present dose-response curve (Figure 4), the effect of such plasma levels of nicotine on the synthesis of prostacyclin-like activity in human veins would be negligible. However, there may be differences between individuals in the inhibitory effect of nicotine, as well as differences in the sensitivity of various types of vascular tissue. Such differences would certainly result in displacements of the dose-response curve, rendering tobacco smoking a significant inhibitor of vascular prostacyclin formation in man. Furthermore, the pharmacokinetics of nicotine are poorly studied, and concentration gradients may exist between e.g. plasma and vascular tissue.

In summary, the present investigation, demonstrating an inhibitory action of nicotine on the formation of prostacyclin-like activity in vascular tissue from rabbit and man, points to the possibility that tobacco smoking interferes with endothelial cyclo-oxygenase/prostacyclin synthetase activity and hence with the vascular defence against platelet deposition and thrombosis. The possibility that such an effect of nicotine may constitute the biochemical link between tobacco smoking and cardiovascular disease deserves attention.

This study was supported by the Swedish Medical Research Council, the Swedish Tobacco Company and Stiftelsen Lars Hiertas Minne. PGI_2 was kindly supplied by Dr J. Pike, Upjohn Company, U.S.A.

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(Received April 1, 1980.)