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EFFECTS OF NICOTINE ON CARDIAC PROSTAGLANDIN AND PLATELET THROMBOXANE SYNTHESIS

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1 Rabbit hearts were perfused with a solution containing [^{14}C]-arachidonic acid (AA) and various concentrations of nicotine (3×10^{-8} to 3×10^{-5} M). The venous effluent was collected and extracted for lipid acid material, which was subsequently subjected to thin layer radiochromatography.

2 Human platelets were incubated with nicotine (10^{-8} to 10^{-4} M), in the absence or presence of unlabelled AA. The amount of smooth muscle stimulating activity resulting from 30 s of incubation was tested on a rabbit aortic strip.

3 In hearts perfused with [^{14}C]-AA; nicotine induced a dose-related depression of the release of [^{14}C]-6-keto-prostaglandin $F_{1\alpha}$, and a parallel increase in the release of [^{14}C]-prostaglandin E_2.

4 Nicotine neither induced synthesis of thromboxane in human platelets, nor affected the platelet synthesis of thromboxane induced by AA.

5 It is suggested that nicotine affects the metabolism of prostaglandin endoperoxides in the heart by inhibiting their conversion to prostacyclin and facilitating, directly or indirectly, the formation of prostaglandin E_2.

Introduction

The synthesis of prostaglandins in tissues, as indicated by their appearance in the venous effluent, can be raised above the basal level by a variety of agents, such as adrenaline, noradrenaline and angiotensin (Davies, Horton & Withrington, 1968; Gilmore, Vane & Wyllie, 1968; McGiff, Crowshaw, Terragno & Lonigro, 1970; Junstad & Wennmalm, 1973; Minkes, Douglas & Needleman, 1973). Recent studies in this laboratory have demonstrated that nicotine also increases the outflow of prostaglandin E (PGE)-like material from the rabbit isolated heart (Wennmalm & Junstad 1976), probably by an action dissociated from the noradrenaline-liberating effect of this compound (Wennmalm 1977).

The mechanism behind the stimulating effect of nicotine on the release of PGE in the rabbit heart has not been clarified hitherto. Agents that stimulate the synthesis of prostaglandins in tissues usually operate by activating phospholipase A, thereby increasing the availability of the prostaglandin precursor arachidonic acid (AA). In the current study some evidence is presented favouring a different mechanism behind the effect of nicotine on the tissue synthesis of prostaglandins.

Methods

Perfused rabbit hearts

Male rabbits of mixed strains, weighing from 1.2 to 2.4 kg, were used for the study. After a blow on the head, the animal was exsanguinated by cutting the left carotid artery. The heart was rapidly removed and transferred to the perfusion apparatus, where it was perfused according to Langendorff with 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. The solution was aerated continuously with 5% CO_2 in O_2.

After a 10 min basal period of perfusion during which the heart was allowed to stabilize in the perfusion apparatus, a priming dose of [^{14}C]-AA (New England Nuclear, sp. act. 40 to 60 Ci/mole, 2 μCi) was infused over a 10 min period. Subsequently perfusion was changed to a Tyrode solution containing nicotine (3×10^{-8} to 3×10^{-5} M) which was maintained for 15 min. After the first 5 min of perfusion with nicotine, a second dose of [^{14}C]-AA was unfused as above. During this infusion of [^{14}C]-AA the effluent from

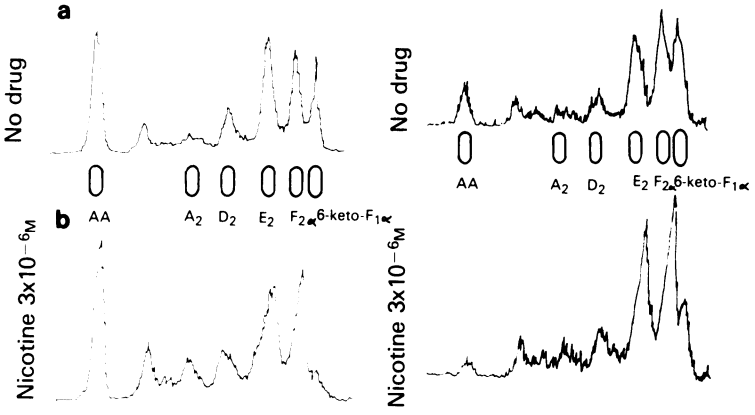

Figure 1 (a) Radio-scans (thin layer chromatography) of the ethyl acetate extracted cardiac effluent collected during infusion of [^{14}C]-arachidonate (AA) (0.2 μCi/min). No drug added to the solution perfusing the heart. The radioactivity appears in three major peaks, corresponding to 6-keto-prostaglandin $F_{1\alpha}$($PGF_{1\alpha}$), $PGF_{2\alpha}$, and PGE_2. (b) Radio-scans of the cardiac effluents as above. Nicotine (3×10^{-6} M) was added to the solution perfusing the heart 5 min before the infusion of isotope. Note that the peak corresponding to 6-keto-$PGF_{1\alpha}$ is almost absent (left chromatogram) or markedly decreased (right chromatogram).

the heart was collected for subsequent analysis of [^{14}C]-AA metabolites. In most hearts only one concentration of nicotine was tested, but in some cases, after a further perfusion for 20 min with drug-free Tyrode solution, a second concentration of nicotine was administered to the heart during concomitant infusion of [^{14}C]-AA and collection of perfusate as above. In the latter experiments the higher dose of nicotine was always given during the last infusion of [^{14}C]-AA. Control experiments, in which no nicotine was given before or during the second and third [^{14}C]-AA infusions, were also performed.

Effluents collected from the hearts during the second (or third) infusion of [^{14}C]-AA were treated as follows. Neutral lipids were extracted at pH 7 with an equal amount of petrol (40 to 60°). Prostaglandins were extracted twice with equal amounts of ethyl acetate at pH 3 to 3.5. The combined ethyl acetate portions were washed once with 1/10 volume of 0.5 M acetate buffer pH 6.5, and twice with 1/10 volume of water. They were subsequently evaporated to dryness and the residue was dissolved in a small amount of ethanol. Thin layer chromatography of the lipid residue was performed by the use of 0.25 mm DC Fertigplatten Kieselgel-F 254 (Merck) in solvent ethyl acetate:acetic acid:2,2,4-trimethyl-pentane:water (90:20:50:100, v/v) (A IX, Hamberg & Samuelsson 1966), against standards of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2, PGD_2, PGA_2 and sodium arachidonate. Radioscans of the chromatograms were developed with a Berthold Dünnshickt-Scanner. The relative amounts of the various ^{14}C-labelled prostaglandins formed were calculated from the areas under the different radiopeaks in relation to the total area of the identified peaks in the chromatogram.

Platelet incubation experiments

Peripheral venous blood was obtained from healthy donors who had not taken drugs of the aspirin type for at least one week. It was collected in 1/10 volume of 0.077 M disodium edetate (EDTA) and centrifuged at 260 *g* for 15 min. The plasma thus obtained was centrifuged at 720 *g* for 15 min. The platelet pellet from 10 ml of plasma was washed in 5 ml of the following medium: 0.15 M NaCl:0.15 M Tris–HCl buffer (pH 7.4):0.77 M EDTA (90:8:2, v/v) and recentrifuged. The pellet of the washed platelets was suspended in 4 ml of calcium-free Tyrode solution; 0.4 ml portions of the platelet suspension were incubated at 37°C during 30 s in the absence or presence of 1 to 2 μg of sodium arachidonate (Sigma Chemical Co.) and nicotine (10^{-8} to 10^{-4} M). The smooth muscle stimulating activity of the incubate was tested on a rabbit aortic strip, mounted in a 5 ml organ bath, heated to 37°C and containing aerated Tyrode solution of the composition given above. To the Tyrode solution was added phentolamine (0.7 μM), propranolol (0.8 μM), atropine (0.1 μM), methysergide (0.6 μM), and diphenhydramine (0.7 μM), in order to block activity in the strip due to the presence of noradrenaline, adrenaline, acetylcholine, 5-hydroxytryptamine, or histamine in the incubate. In addition indomethacin (5×10^{-5} M) was added to the solution. The isotonic contractions of the strip were recorded on the polygraph.

Results

Effects of nicotine on the outflow of ^{14}C-labelled prostaglandins from the perfused heart

During perfusion with [^{14}C]-AA, 80 ± 2% (mean ± s.e., n = 19) of the radioactivity was retained in the organ. After extraction of the effluent with petrol, 89 ± 3% (n = 16) of its content of radioactivity remained. Following extraction with ethyl acetate at pH 3 to 3.5, 54 ± 5% (n = 19) was still present in the effluent. Neither the amount of radioactivity retained in the heart nor the amount extracted into the ethyl acetate differed between controls and effluents obtained during perfusion with nicotine. Radiochromatographic separation of the evaporated effluent, collected during perfusion with nicotine-free Tyrode solution, revealed the presence of (apart from AA) three major and two or three minor peaks of radioactivity (Figure 1). The major peaks chromatographed with 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, and PGE_2, and one of the minor peaks with PGD_2. The remaining activity appeared in one or two small peaks between PGD_2 and AA. Of these less polar [^{14}C]-AA metabolites, one chromatographed with PGA_2 while the other was unidentified. In Table 1 and Figure 2 the sum of these two peaks of [^{14}C]-AA metabolites is represented by 'Me'. The relative proportions of the different ^{14}C-labelled prostaglandins in the control experiments (no nicotine) are presented in Table 1. No change in the quantitative relation between the different ^{14}C-prostaglandins in the cardiac effluent was seen during three repeated infusions of [^{14}C]-AA in the absence of nicotine. The data given in Table 1 are therefore based on chromatograms of consecutive cardiac effluents obtained in four control experiments.

Nicotine, at the lowest tested dose (3×10^{-8} M), did not affect the synthesis of ^{14}C-prostaglandins, as reflected by their distribution in the chromatogram of the cardiac effluent. However, at 10^{-6} M nicotine markedly depressed the formation of [^{14}C]-6-keto-$PGF_{1\alpha}$, with a parallel increase in the formation of [^{14}C]-PGE_2. Apart from this redistribution, the metabolism of [^{14}C]-AA was unchanged (Figure 2). At 3×10^{-5} M, this effect of nicotine was still more pronounced. inasmuch as the formation of [^{14}C]-6-keto-$PGF_{1\alpha}$ remained low, while the synthesis of [^{14}C]-PGE_2 was further accelerated (Figure 2).

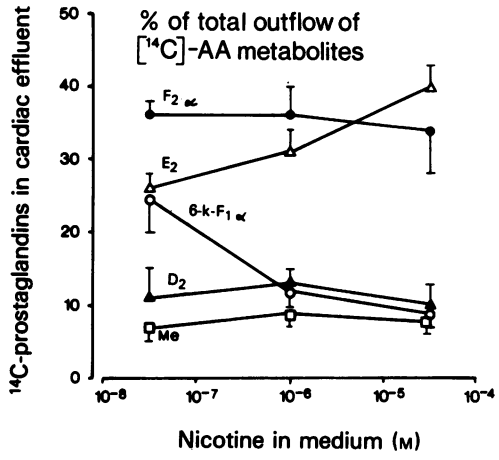

Figure 2 Effect of nicotine on the relative amounts of different ^{14}C-labelled prostaglandins in the cardiac effluent from hearts infused with [^{14}C]-arachidonic acid (AA). The values are calculated from the area under the respective radiopeaks in relation to the sum of the areas under all the peaks. They are expressed as mean ± s.e. and are based on data obtained in 4 different heart perfusions. For definition of 'Me' see Results.

Effect of nicotine on the synthesis of thromboxane in human platelets

Incubation of a portion of the human platelet suspension with AA for 30 s resulted in the formation of a compound that strongly contracted the rabbit aortic

Table 1 ^{14}C-labelled prostaglandins in the effluent from hearts infused with [^{14}C]-arachidonic acid (AA)

	Metabolites (% of total)				
	6-keto-$PGF_{1\alpha}$	$PGF_{2\alpha}$	PGE_2	PGD_2	'Me'
Mean	21	32	28	8	12
± s.e.	2	2	2	1	1

The relative amounts of the various ^{14}C-labelled prostaglandins have been calculated from the area under the respective peaks in the radio-chromatograms and are expressed in relation to the sum of the areas of the peaks. Each value represents mean ± s.e. of data from 11 chromatograms, obtained in 4 different heart perfusions. 'Me' is sum of two small peaks running between AA and PGD_2.

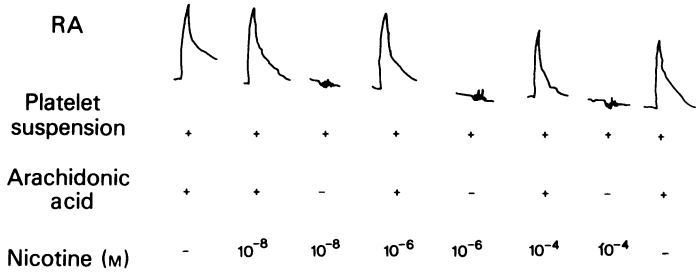

Figure 3 Contractions of the rabbit aortic strip (RA) when exposed to thromboxane A_2 (TxA_2) formed in a human platelet suspension incubated with arachidonic acid (AA, 1 μg) during 30 s in the absence or presence of nicotine. As seen from the figure, the incubates elicit reproducible contractions of the strip. Nicotine does not change the amount of bioassayed TxA_2 formed from AA. Incubation of human platelets with nicotine only is not followed by production of material contracting the strip.

strip (Figure 3). According to earlier workers' evidence, this compound consists mainly of thromboxane A_2 (TxA_2) (Hamberg, Svensson & Samuelsson 1975). Incubation of another portion of the human platelet suspension with nicotine (10^{-8} to 10^{-4} M) for 30 to 120 s did not result on any occasion in the formation of material contracting the aortic strip (Figure 3). When increasing doses of nicotine (10^{-8} to 10^{-4} M) were added to the platelet suspension before incubation with AA, the amount of TxA_2 formed was the same as without nicotine (Figure 3).

Discussion

In the present investigation nicotine at moderate doses was found to decrease the outflow of 6-keto-$PGF_{1\alpha}$, the stable metabolite of PGI_2 (Johnson, Morton, Kinner, Gorman, McGuire, Sun, Whittaker, Bunting, Salmon, Moncada & Vane, 1976), and to increase the outflow of PGE_2 into the cardiac effluent. These data might indicate that nicotine operates by selectively counteracting the conversion of prostaglandin endoperoxides to 6-keto-$PGF_{1\alpha}$, thereby elevating the level of endoperoxides available for synthesis of PGE. However, such a mechanism behind the action of nicotine would make increased amounts of endoperoxides accessible for synthesis of all prostaglandins formed in the heart, apart from PGI_2. Since only the formation of PGE_2 was increased, the possibility must be considered that nicotine, in addition to its inhibitory effect on the formation of PGI_2, facilitates the conversion of endoperoxides to PGE. The effect of nicotine on the cardiac synthesis of prostaglandins would, thus, be dual: inhibition of PGI_2 formation from endoperoxide and facilitation of PGE_2 synthesis from this compound. An inhibitory effect on the cardiac synthesis of 6-keto-$PGF_{1\alpha}$ was recently reported to occur in guinea-pig hearts treated with 15-hydro-peroxyarachidonic acid (Schrör, Moncada, Ubatuba & Vane, 1978). However, these authors found increased formation of both PGE_2 and $PGF_{2\alpha}$ concurrently with the decrease in 6-keto-$PGF_{1\alpha}$. Whether the difference between their observation and the present data is due to species differences or, alternatively, reflects separate biochemical effects of nicotine on the one hand and 15-hydro-peroxyarachidonic acid on the other, remains to be established.

In earlier reports from this laboratory (Wennmalm & Junstad, 1976; Wennmalm, 1977) an increased efflux of material bioassayed as PGE was found to occur following administration of nicotine to perfused rabbit hearts not treated with AA. On the basis of those data it was suggested that the mechanism behind the action of nicotine was identical to that elicited by most other agents capable of stimulating the formation of prostaglandins in tissues, i.e. activation of acyl hydrolases (cf. Oates, Seyberth, Oelz, Danon & Sweetman, 1975). The technique used in the current cardiac perfusion experiments does not permit any conclusions concerning a possible action of nicotine on mobilization of AA from the endogenous stores. The platelet incubation experiments, however, yielded direct evidence against an activating action of nicotine on acyl hydrolases, inasmuch as high concentrations of nicotine failed to induce formation of thromboxane. Although these data on the lack of an effect of nicotine in platelets are not directly transferable to events in the perfused rabbit heart, they provide indirect support for the assumption that nicotine differs in its mechanism of action from other agents that facilitate the formation of prostaglandin (E) in tissues. However, one cannot completely rule out the possibility that the above-mentioned observations on increased efflux of PGE-like material from nicotine-treated hearts (Wennmalm & Junstad, 1976; Wennmalm, 1977) to some extent may be related to an increased mobilization of AA from endogenous cardiac stores.

The platelet incubation experiments exclude the possibility of a facilitating action of nicotine on the conversion of AA to prostaglandin endoperoxides. Such an effect should have resulted in an increased formation of thromboxane, which was not observed. Combining the data from the two experimental systems in the present investigation, it thus appears less likely that the stimulatory effect of nicotine on cardiac PGE_2 formation can be explained to any significant extent in terms of a facilitating action of the drug on the acyl hydrolases that mobilize AA from the tissue stores, or of an effect on the cyclo-oxygenase that converts AA to prostaglandin endoperoxides. The current data rather favour the hypothesis that nicotine acts in the last step of the formation of prostaglandins, i.e. in the conversion of endoperoxides to PGE_2 and PGI_2.

An inhibitory effect of nicotine on the cardiac formation of PGI_2 may be of considerable clinical importance. It has been proposed that PGI_2 may play a role as an endogenous protector against thrombosis (Gryglewski, Bunting, Moncada, Flower & Vane, 1976). The currently observed action of nicotine is of interest in this context since it may constitute a biochemical basis for the well-known connection between the use of tobacco and increased morbidity in cardiovascular diseases.

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