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NICOTINE INHIBITS HYPOXIA- AND ARACHIDONATE-INDUCED RELEASE OF PROSTACYCLIN-LIKE ACTIVITY IN RABBIT HEARTS

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1 Rabbit hearts were perfused by the Langendorff technique and the interstitial effluent content of platelet anti-aggregatory activity (prostacyclin-like activity) was assayed at regular intervals.

2 Perfusion was performed with a solution containing 5% CO_2 in O_2. At regular intervals it was changed to a solution containing 12% O_2 and 5% CO_2 in N_2. Alternatively, perfusion with 5% CO_2 in O_2 was maintained during the entire experiment and sodium arachidonate was infused (5 to 15 μg/min) at intervals. Under the basal conditions no efflux of prostacyclin-like activity was observed in the interstitial cardiac effluent, but both perfusion with a hypoxic solution and infusion of arachidonate induced such release.

3 Nicotine (5×10^{-5} M) in the solution perfusing the heart markedly inhibited the efflux of prostacyclin-like activity into the cardiac interstitial effluent, induced by hypoxia or by infusion of arachidonate.

4 It is suggested that nicotine counteracts the formation of prostacyclin-like activity in the rabbit heart by interfering with the enzymatic conversion of arachidonate to prostacyclin.

Introduction

It has been shown in our laboratory (Wennmalm & Junstad 1976) that nicotine elicits release of prostaglandin E(PGE)-like substances from rabbit isolated hearts. Furthermore, this effect of nicotine is distinct from the noradrenaline liberating effect of the drug (Wennmalm, 1977) described earlier (Löffelholz, 1970). Recent data from this laboratory (Wennmalm, 1978a, b) indicate that nicotine inhibits the conversion of prostaglandin endoperoxides to prostacyclin and at the same time facilitates their conversion into PGE. If so, nicotine, rather than eliciting an overall stimulatory effect on the cardiac formation of prostaglandins, would act by changing the relative amounts of prostaglandins formed in the organ.

In a previous study (Wennmalm, 1978b) only tracer amounts of labelled prostaglandin precursor were infused, and the results therefore probably mainly reflect the effect of nicotine on the basal cardiac formation of prostaglandins. It is clearly important to determine whether nicotine acts in a state of accelerated cardiac prostaglandin formation. In the current investigation the rabbit heart formation of prostaglandin was stimulated in two, principally different, ways (by perfusion with a hypoxic solution, and by infusion of prostaglandin precursor) and the effect of nicotine was studied on this formation.

Methods

Heart perfusion

Rabbits of either sex and mixed strains, weighing 2.5 to 4.5 kg, were used for the study. They were killed by a blow on the head and exsanguinated via the carotid artery. The hearts were prepared according to Langendorff and perfused at a constant pressure of 60 cmH_2O and 37°C with a solution containing (mM): NaCl 137, KCl 2.7, CaCl 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 12, NaH_2PO_4 0.4, and glucose 5.6. In addition, the hearts were prepared according to De Deckere, Nugteren & Ten Hoor (1977). This method is based on the observation that in the isolated perfused heart a small part (1 to 3%) of the perfusion fluid reaches the surface of the heart through the interstitial space and the lymphatics. This smaller flow is separated from the main flow by tying off the veins of the right and left atrium and cannulating the pulmonary artery. Most of the perfusion fluid is ejected by the right ventricle through the cannula (Q_{rv}), but a small amount passes the interstitium and drips from the heart (Q_i). As the flow rate of Q_i is small, the concentrations of substances (e.g. prostacyclin) released into Q_i are higher than in Q_{rv} and in the case of prostacyclin, are high enough to be assessed directly with respect to platelet anti-aggregatory activity.

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The formation of prostacyclin-like activity in the heart was stimulated either by perfusion of the heart with a hypoxic Tyrode solution or by infusion of sodium arachidonate into the organ. Hypoxia in the heart was induced by changing perfusion from the normal Tyrode solution (aerated by a gas mixture containing 5% CO_2 in O_2) to a hypoxic Tyrode solution (aerated by 5% CO_2 and 12% O_2 in N_2). Arachidonic acid (Sigma Chemicals) was prepared as sodium salt and infused at a rate of 5 to 15 μg/min through a cannula close to the aorta.

Nicotine, in pure liquid form, obtained from the Swedish Tobacco Company, was dissolved directly in the Tyrode solution, to give a final concentration of 5×10^{-5} M.

Biological assessment of cardiac efflux of prostacyclin-like activity

Venous blood was obtained from human donors, who had not taken aspirin-like drugs for at least 1 week. It was collected in test tubes containing 1/10 vol of 0.13 M sodium citrate. Platelet-rich plasma (PRP) was obtained by centrifugation of the blood at 200 *g* for 15 min. Aggregation was induced by addition of 2.5 μg of adenosine diphosphate (ADP, Sigma Chemicals) to 0.5 ml PRP in 0.1 ml 0.1 M Tris buffer, pH 7.4. Aggregation was monitored in a Vitatron UC 200 photometer connected to a W + W 1100 ink recorder.

The Q_i effluent from the heart was collected for 1 min periods continuously during the experiments. Representative effluents were tested for platelet anti-aggregatory (prostacyclin-like) activity in the following way. Half the volume (usually 0.5 to 0.7 ml) of a 1 min Q_i effluent was added to the PRP-Tris buffer mixture and the final volume was adjusted to 1.5 ml. ADP (2.5 μg) was added and aggregation was monitored.

Calculations

Prostacyclin-like activity in the Q_i effluents was estimated from the aggregation recordings and expressed as the change in light transmission amplitude of the aggregation curve 60 s after addition of ADP, compared with control aggregation curves obtained after addition of fresh Tyrode solution to the PRP-Tris buffer mixture before addition of ADP. Values are presented as mean ± s.e. mean. Student's *t* test for paired differences has been used when applicable.

Results

After an initial equilibration period in the perfusion apparatus (15 to 30 min), the mechanical performance of the heart was stable and remained so during the rest of the experiment. The basal coronary flow (Q_{rv}), which was 44 ± 4 ml/min ($n = 9$) initially, declined with time, the reduction by the end of the experiment usually amounting to 30 to 50%. The initial flow rate of the interstitial effluent (Q_i) was 1.17 ± 0.11 ml/min ($n = 6$). The Q_i efflux rate did not change significantly during the course of the experiment. No prostacyclin-like activity was present in the Q_i effluents collected in the basal state.

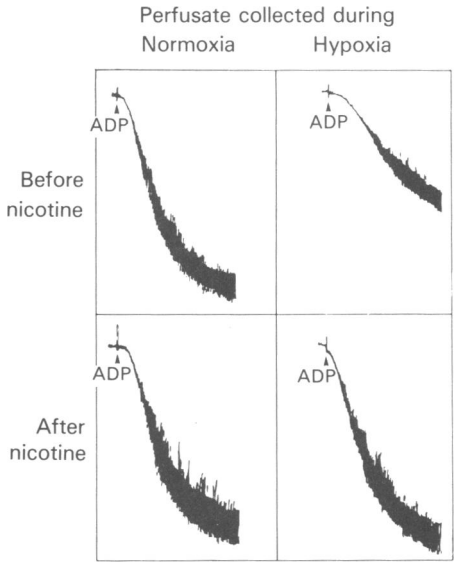

Figure 1 Effect of cardiac interstitial effluent (Q_i, cf. text) on ADP-induced aggregation in human platelet-rich plasma. As seen from the figure, Q_i-effluent collected during normoxic perfusion did not display anti-aggregatory activity, while effluent collected during hypoxia was able to counteract platelet aggregation. Nicotine abolished the efflux of anti-aggregatory activity induced by hypoxia.

Effect of nicotine on hypoxia-induced efflux of prostacyclin-like activity

When the perfusion was changed from normal Tyrode solution (approx. $Po_2 = 85$ kPa) to hypoxic (approx. $Po_2 = 15$ kPa) solution for 8 min, the visible contractile force of the heart decreased and in some cases moderate arrhythmias developed. The coronary flow increased in most cases. These changes were reversed when perfusion was changed back to normoxic solution. The Q_i efflux rate was not affected during the hypoxic period. The Q_i effluent collected during the 2nd and 7th minutes of the perfusion with hypoxic solution regularly displayed anti-aggregatory activity

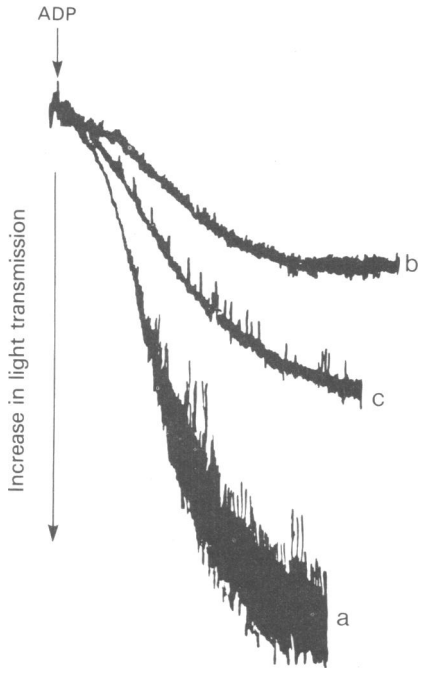

Figure 2 Effect of cardiac interstitial effluent (Q_i, cf. text) on ADP-induced aggregation of platelets in human platelet-rich plasma. The three recordings are superimposed on each other and display the increase in light transmission developing in the plasma as a consequence of the platelet aggregation. In recording (a) a portion of basal Q_i was added to PRP before induction of platelet aggregation with ADP. In recording (b) the same volume of Q_i collected during infusion of sodium arachidonate into the heart was added to PRP before induction of platelet aggregation. In recording (c) Q_i was collected as in (b), but nicotine was added to the solution perfusing the heart. As seen from the figure, infusion of sodium arachidonate into the heart elicited efflux of platelet anti-aggregatory activity in the Q_i. This efflux was in part inhibited by nicotine.

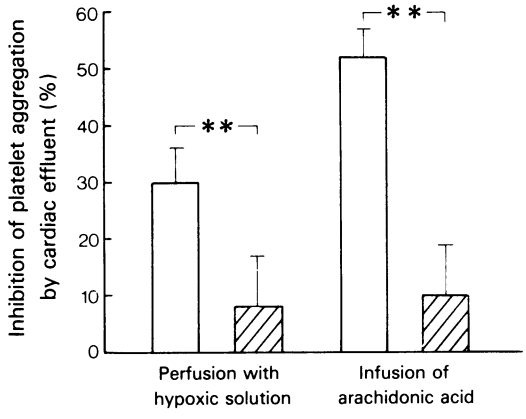

Figure 3 Inhibition of ADP-induced platelet aggregation (for calculation see text) induced by rabbit heart interstitial effluents (Q_i) collected during hypoxic perfusion (a) or during infusion of sodium arachidonate (b). As seen from the figure, the efflux of platelet anti-aggregatory activity, induced either by hypoxia or by arachidonate (open columns in (a) and (b)), is considerably diminished by the addition of nicotine to the perfusing solution (hatched columns in (a) and (b)).

(Figure 1). The efflux of such activity rapidly ceased when normoxic perfusion was restored (not shown). The mean inhibitory effect of the Q_i effluents on the ADP-induced platelet aggregation was $30 \pm 6\%$ ($n = 15$, Figure 3).

Addition of nicotine to the perfusion solution neither changed the Q_{rv} or Q_i efflux rate, nor induced release of anti-aggregatory activity. However, a brief (2 to 3 min) increase in heart rate and contractile force was always observed. This increase was probably due to release of endogenous noradrenaline in the organ (Löffelholz, 1970). No efflux of anti-aggregatory activity appeared in the Q_i effluent during this period. Changing to hypoxic solution after the end of the period of increased mechanical activity was again paralleled by a decrease in contractile force and occasionally also by arrhythmias. Efflux of anti-aggregatory activity in the Q_i also occurred but never to the same extent as in the hypoxic perfusions before nicotine (Figure 1). The mean inhibitory effect of the Q_i effluents, collected during hypoxia in the presence of nicotine in the perfusion solution, was $8 \pm 9\%$ ($n = 14$). The difference in efflux of anti-aggregatory activity during the hypoxic periods, in the absence versus the presence of nicotine in the perfusion solution, is significant ($P < 0.01$) (Figure 3).

Effect of nicotine on arachidonate-induced efflux of prostacyclin-like activity

Infusion of arachidonate (5 to 15 μg/min) induced a moderate increase in coronary (Q_{rv}) flow, while the interstitial (Q_i) flow was unaffected (1.17 ± 0.16 ml/min, $n = 6$). No change was detected in the mechanical performance of the heart. The Q_i effluent, collected during the 5th minute of infusion of arachidonate, markedly inhibited ADP-induced platelet aggregation (Figure 2). Boiling of the Q_i effluent for 2 min completely destroyed its anti-aggregatory capacity. The mean inhibitory effect of the Q_i effluents on platelet aggregation was $52 \pm 5\%$ ($n = 7$, Figure 2). Following cessation of the infusion of arachidonate, the efflux of anti-aggregatory activity in the Q_i effluent rapidly disappeared.

Addition of nicotine again induced a brief increase in mechanical activity in the heart but did not affect the coronary or interstitial flow rates. No efflux of anti-aggregatory activity appeared in the Q_i effluent. Infusion of arachidonate, was again followed by efflux of anti-aggregatory activity in the Q_i effluent, but this efflux was considerably smaller than that observed during infusion of arachidonate in the absence of nicotine (Figure 3). The mean inhibitory effect of the Q_i effluents collected during infusion of arachidonate in the presence of nicotine, on ADP-induced platelet aggregation, was only $10 \pm 9\%$ ($n = 9$, Figure 3). The inhibitory effect of nicotine on the efflux of anti-aggregatory activity from the heart during infusion of arachidonate was significant ($P < 0.01$).

Discussion

The purpose of this investigation was to study the effect of nicotine on the release of the anti-aggregatory activity that appears in the effluent of the heart when exposed to hypoxia or arachidonate. There is good reason to assume that this activity is due to the presence of prostacyclin in the effluent. Firstly, arachidonic acid is the natural precursor of prostacyclin (Gryglewski, Bunting, Moncada, Flower & Vane, 1976) and its synthesis in the heart is stimulated by hypoxia (DeDeckere *et al.*, 1977). Secondly, prostacyclin is the main prostaglandin formed in the rabbit isolated heart (Isakson, Raz, Denny, Pure & Needleman, 1977; DeDeckere *et al.*, 1977), and by far the most potent among the prostaglandins with respect to inhibition of platelet aggregation (Gryglewski *et al.*, 1976). Thirdly, the current anti-aggregatory activity in the Q_i effluents was destroyed by boiling, as is authentic prostacyclin (Gryglewski *et al.*, 1976). Finally the efflux of anti-aggregatory activity induced by hypoxia was recently shown, in a similar series in this laboratory, to be inhibited almost completely by indomethacin (Wennmalm, 1979).

When nicotine was added to the solution perfusing the heart, the efflux of prostacyclin-like activity in response to hypoxia or infusion of arachidonate was greatly diminished. This reduction was probably not a consequence of an impaired washout, since the interstitial (Q_i) efflux rate was unchanged by nicotine. Both the formation of prostacyclin-like activity from the endogenous precursor stores (stimulated by hypoxia) and that induced by administration of precursor (arachidonate) were inhibited. This strongly suggests that nicotine interfered with the enzymatic conversion of arachidonate to prostacyclin, and not with, say, the mobilization of precursor or release of prostaglandin from the cells, mechanisms that appear to apply to prostaglandin-inhibiting drugs like steroids (Herbaczynska-Cedro & Staszewska-Barczak, 1974; Lewis & Piper, 1975) or certain antimalarial drugs (Vagaftig & Dao Hai, 1972).

The current results thus accord with those from an earlier study in this laboratory in which the conversion of tracer amounts of labelled arachidonate to 6-keto-$PGF_{1\alpha}$ (metabolite of PGI_2) was decreased by nicotine in a dose-dependent manner (Wennmalm, 1978b).

Recent data from this laboratory indicate that nicotine may affect prostaglandin formation by rabbit kidney microsomes by the same mechanism as indomethacin, i.e. by interference with cyclo-oxygenase (Alster & Wennmalm, 1980). The data from the present investigation do not permit any conclusions as to which of the enzymes involved in the conversion of arachidonate to PGI_2 (cyclo-oxygenase or PGI_2 synthetase) is inhibited by nicotine. According to the two investigations mentioned, nicotine is capable of inhibiting both these enzymes. However, these studies were performed in different tissues and with different types of preparations. Further studies are required to verify whether nicotine is capable of inhibiting both cyclo-oxygenase and PGI_2 synthetase in the rabbit heart.

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