NICOTINE STIMULATES PROSTA-GLANDIN FORMATION IN THE RABBIT HEART

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1 Rabbit isolated hearts were perfused according to Langendorff with Tyrode solution. Prostaglandin-like substances (PLS) in the effluent were purified and assayed on the rat stomach strip. Noradrenaline (NA) in the effluent was assayed fluorimetrically.

2 Infusion of nicotine (1 μM–50 μM) caused a dose-dependent, brief increase, from 1.2 ± 0.4 to maximally 8.3 ± 2.1 ng/min, in the outflow of PLS from the heart. The increase was abolished by pretreatment of the heart with indomethacin.

3 Activation of nicotinic receptors in the heart with acetylcholine (ACh, 200 μM) in the presence of atropine (1 μM) also elicited an increase in the release of PLS. This release was smaller than that caused by nicotine.

4 Nicotine (50 μM) and ACh (200 μM) in the presence of atropine (1 μM) each caused a pronounced but brief release of NA into the effluent. There was no evident correlation between the ability of the drugs to cause release of PLS on the one hand, and NA on the other.

5 It is concluded that nicotine acts as a direct stimulus for the synthesis of prostaglandins in the rabbit heart.

Introduction

The synthesis of prostaglandins in tissues, as evidenced by their appearance in the venous effluent, can be raised above the basal level by a variety of agents. Not only do drugs like adrenaline, noradrenaline and angiotensin stimulate prostaglandin synthesis (Davies, Horton & Withrington, 1968; Gilmore, Vane & Wyllie, 1968; McGiff, Crowshaw, Terragno & Lonigro, 1970a; Junstad & Wennmalm, 1973; Minkes, Douglas & Needleman, 1973), but also metabolic intervention such as hypoxia and ischaemia (McGiff, Crowshaw, Terragno, Lonigro, Strand, Williamson, Lee & Ng, 1970b; Block & Vane, 1973; Kent, Alexander, Pisano, Keiser & Cooper, 1973; Wennmalm, Pham-Huu-Chanh & Junstad, 1974).

Recently it has been demonstrated, in the rabbit isolated heart and in human forearm muscle, that nicotinic acid is a potent stimulator of prostaglandin synthesis (Kaijser & Wennmalm, 1976; Kaijser, Eklund & Wennmalm, 1976). It was therefore of interest to study whether nicotine, which has certain structural similarities with nicotinic acid, is likewise able to stimulate prostaglandin synthesis. In a preliminary report (Wennmalm & Junstad, 1976) such an action was in fact demonstrated. In the present paper further results are described concerning the stimulatory effect of nicotine on rabbit myocardial prostaglandin formation.

Methods

Rabbit heart perfusion

Rabbits of mixed strains and either sex were used for the study. The weight of the animals varied from 1.2 to 2.4 kg. 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 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. The pH of the solution was 7.4–7.5. The perfusion pressure was 6 kPa and the temperature was kept at 37°C. The apex of the heart was connected to a strain gauge transducer, and heart rate and contractile force were recorded on a Grass Model 5 D Polygraph.

Analysis of prostaglandin-like substances and noradrenaline

During the experiments the effluent from the heart was collected in 5 min periods. It was immediately chilled and acidified to pH 3–4. A 40 ml portion was separated for subsequent NA analysis (see below). The remaining effluent was passed through a column containing Amberlite XAD–2 and the lipids were
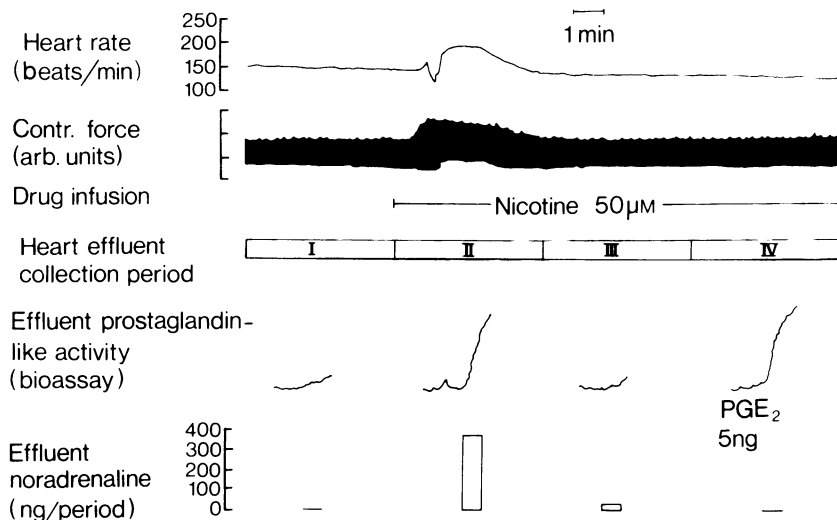
Figure 1   Perfused rabbit heart. The upper two tracings show the heart rate and the contractile force at rest and during infusion of nicotine. The lower tracing demonstrates the contractions of the rat stomach strip exposed to lipid extracts of the effluent from the heart, collected in 5 min periods as indicated by the blocks I–IV. The last bioassay tracing shows the contraction of the bioassay organ when a standard amount of prostaglandin E_2 (PGE_2) is added. The lower panel demonstrates the content of noradrenaline in the effluent, collected in 5 min periods as indicated by the blocks I–IV.

eluted in ethanol (Keirse & Turnbull, 1973). After evaporation the residue was dissolved in 0.9% w/v NaCl solution (saline) and assayed on the rat stomach strip (Vane, 1957) against known amounts of prostaglandin E_2. The assay tissue was kept in a 5 ml organ bath containing gassed and warmed Tyrode solution as above. To the solution were 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 NA, adrenaline, acetylcholine, 5-hydroxytryptamine, or histamine in the lipid extract from the heart effluent. The method permits outflow rates of less than 1 ng/min of prostaglandin E_2 equivalents to be assayed accurately. The recovery of external standards of prostaglandin E_2 was $53 \pm 4\%$ ($n = 20$). The outflow figures for PLS given here are corrected for losses during purification by the use of the present recovery method.

NA was adsorbed on alumina and assayed fluorimetrically by the trihydroxy-indole method as described by Euler & Lishajko (1961). The recovery of external standards of NA was $96 \pm 4\%$ ($n = 22$). The outflow figures for NA are not corrected for losses during purification.

Drugs

Nicotine was obtained in pure liquid form from the chemical analysis laboratory at the Swedish Tobacco

Company. It was kept in the dark and under nitrogen until used. It was dissolved in Tyrode solution just before the experiment and infused into the perfusion system via a cannula close to the aorta.

Acetylcholine (Hoffman-La Roche & Co.) was dissolved in Tyrode solution before the experiment and infused via the aortic cannula to produce a final concentration of 200 μM.

Atropine sulphate (Sigma Chemical Co.) was added directly to the Tyrode solution, at a concentration of 1 μM.

Indomethacin (Merck, Sharpe & Dohme) (as the pure substance) was dissolved in ethanol. Immediately before the experiment it was added to the Tyrode solution to produce a final concentration of 50 μM.

Calculations

Values in the text and table are given as mean $\pm$ s.e. mean. Figures in brackets indicate the number of experiments. Student's t-test has been used for statistical analysis of differences between the outflow figures of PLS obtained in the different series.

Results

The spontaneous beating frequency of the hearts in the perfusion apparatus ranged from 120 to 160 beats/minute. During sustained perfusion in the

absence of drug administration, this spontaneous rate generally fell slightly. The contractile force also faded slightly during the course of the experiments. The perfusion flow ranged from 30 to 45 ml/min and changed insignificantly during the experiments. The basal effluent content of PLS ranged from 1.2 to 2.8 ng/minute. No NA was found in the effluent during basal conditions.

Infusion of nicotine (1–50 μM)

The lowest concentration of nicotine (1 μM) failed to affect the mechanical performance of the heart. The overflow of PLS in the effluent remained at the basal level. No NA release was observed.

At a concentration of 10 μM, nicotine induced increases in the heart rate (by 24 ± 6 beats/min) and the contractile force (by 20–30%). The PLS concentration in the effluent remained at the basal level (2.2 ± 0.5 ng/min). There was a substantial release of NA into the effluent, amounting to 238 ± 29 ng during the 5 min collection of effluent.

The highest nicotine concentration (50 μM) elicited marked changes in the mechanical performance of the heart. After a brief initial decrease (Figure 1), heart rate increased 61 ± 6 beats/minute. The contractile force was almost doubled. The outflow of PLS increased markedly, to 8.3 ± 2.1 ng/minute. There was also a release of NA into the effluent, amounting to 337 ± 43 ng during the infusion period. In spite of sustained nicotine exposure, the mechanical performance and the effluent's content of PLS and NA gradually returned towards the basal level, complete return to base-line conditions being obtained after about 5 min of drug exposure.

When indomethacin was infused before and during the exposure to nicotine (50 μM), the increases in heart rate and contractile force were mainly the same as in the absence of indomethacin. The release of NA (872 ± 166 ng) during nicotine exposure was signifi-
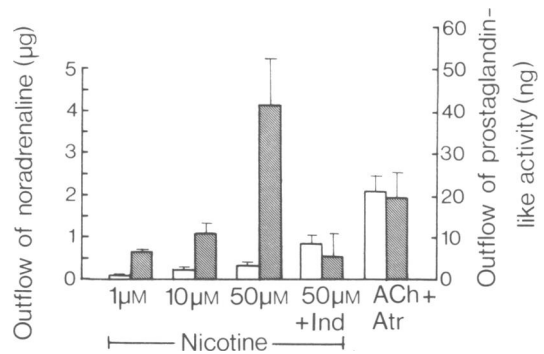

Figure 2 Perfused rabbit heart. The columns represent the outflow of noradrenaline (NA, open columns) and prostaglandin-like substance (PLS, hatched columns) during the first 5 min following exposure to various concentrations of nicotine, in the absence or presence of indomethacin (50 μM), or to acetylcholine (200 μM) in the presence of atropine (1 μM). Note the marked difference between the ability of nicotine (50 μM) and acetylcholine plus atropine to induce liberation of PLS on the one hand and NA on the other.

cantly ($P < 0.01$) higher than in the absence of indomethacin (Figure 2). The basal outflow of PLS was completely abolished, and only insignificant amounts were found in the effluent during nicotine exposure.

Infusion of acetylcholine (200 μM) in the presence of atropine (1 μM)

The mechanical response of the heart to exposure to ACh (200 μM) in the presence of atropine (1 μM) differed from that elicited by nicotine both quantitatively and qualitatively. Only in some

Table 1   Outflow of prostaglandin-like substances, expressed as equivalents of prostaglandin E_2, in ng/min during four consecutive 5 min periods of effluent collection from the perfused rabbit heart.

	I	II	III	IV
A. Nicotine				
1. II–IV: 1 μM	1.4 ± 0.2 (3)	1.3 ± 0.2 (3)	0.9 ± 0.5 (3)	0.6 ± 0.6 (3)
2. II–IV: 50 μM	1.2 ± 0.4 (7)	8.3 ± 2.1 (7)**	3.1 ± 1.0 (7)	1.0 ± 0.4 (7)
3. I: 10 μM; II–IV: 50 μM	2.2 ± 0.5 (5)	6.8 ± 0.7 (5)**	2.9 ± 1.1 (5)	2.5 ± 1.4 (5)
4. II–IV: 50 μM				
I–IV: indomethacin 50 μM	0 (2)	1.1 ± 1.1 (2)	0.4 ± 0.4 (2)	0 (2)
B. Acetylcholine				
(+ atropine 1 μM)				
1. II–IV: 200 μM)	2.8 ± 0.7 (5)	3.9 ± 1.2 (5)	2.0 ± 0.5 (5)	2.6 ± 0.9 (5)

Values are expressed as mean $\pm$ s.e.; figures in parentheses indicate number of experiments. ** indicates that the value is significantly ($P < 0.01$) higher than the corresponding value in the preceding effluent collection period.

experiments was an increase observed in the heart rate and this was both small and brief. The inotropic response differed between the experiments, being absent in some and very pronounced in others. Roughly, the increase in contractile force was correlated to the effluent's content of NA. There was a moderate but insignificant and only initial increase in the effluent's content of PLS (Table 1). The most prominent effect of exposing the heart to ACh and atropine was that large quantities of NA appeared in the effluent (2091 ± 380 ng) (Figure 2). The outflow of NA from the organ during exposure to this drug combination by far exceeded that induced by nicotine, in the absence as well as in the presence of indomethacin. The release of NA was brief and decreased exponentially during sustained exposure of the heart to ACh plus atropine.

Discussion

The present experiments clearly demonstrate that the addition of nicotine to the medium perfusing the isolated heart of the rabbit elicits a release of PLS into the venous effluent of the organ. As it is well known that tissues do not store more than minute amounts of PLS, the increased concentration in the effluent seems to reflect an increased formation in the heart. Nicotine's stimulation of PLS synthesis was dose-dependent, rising steeply in the drug concentration range 1 μM to 50 μM. The mechanism behind the increased formation of PLS might be either an augmented mobilization of the precursor fatty acid from the phospholipid stores, or an increased synthetase activity. It was beyond the scope of the present investigation to elucidate which of these mechanisms in fact operates during nicotine-stimulated formation of PLS, but by analogy with other tissues an increased availability of precursor seems likely (cf. Oates, Seyberth, Oelz, Danon & Sweetman, 1975). During the sustained administration of nicotine, the effluent's concentration of PLS gradually returned to the basal level. It has been shown earlier (Wennmalm, 1975) that larger amounts of PLS than those observed here may be released into the effluent of the rabbit isolated heart. Exhaustion of the precursor stores should consequently not have limited the continued formation of PLS during sustained myocardial exposure to nicotine. The decline of PLS in the effluent therefore probably reflects a decreased synthetase activity, although the possibility of an increased degradation cannot be ruled out completely. These experiments consequently demonstrate a dual action of nicotine on the myocardial PLS synthesis, stimulatory as well as inhibitory.

In the mammalian heart, the post-ganglionic adrenergic neurones contain nicotine receptors which on activation mediate a release of NA from the intra-neuronal transmitter stores. Such a release of NA was observed in the present experiments during administration of nicotine in the dose range of 10 μM–50 μM, as reflected by the appearance of NA in the effluent. The positive chronotropic and inotropic response of the heart upon drug exposure was probably due to activation of adrenoceptors by the transmitter released. As shown by Löffelholz (1970), the NA release in the rabbit heart by nicotine is 'explosive', and subject to sudden auto-inhibition during continued drug administration. This was confirmed in the present study. Since the effluent's concentration of PLS also decreased, in spite of sustained exposure to nicotine, it seemed that the releases of NA and PLS from the heart might be causally related. A logical basis for such a relationship exists, since it has been shown that sympathetic nerve stimulation elicits parallel NA and prostaglandin E releases from the rabbit heart (Wennmalm & Stjärne, 1971); furthermore, noradrenaline infused into the rabbit isolated heart elicits a release of prostaglandin E into the venous effluent of the organ (Junstad & Wennmalm, 1973). Such a causal relationship would imply that the stimulation of PLS synthesis in the present experiments was not primarily due to the action of nicotine, but a consequence of the vast amounts of NA released by the drug. In order to test this hypothesis, a series of experiments was performed in which NA release was elicited via the activation of nicotine receptors by the infusion of ACh (200 μM) in the presence of atropine (1 μM) (cf. Löffelholz, 1970). It was found that this drug combination causes a more pronounced release of NA than nicotine. Even in these experiments a sudden auto-inhibition of the release appeared, indicating a common mechanism behind the NA release by nicotine and by ACh in the presence of atropine. However, the release of PLS was stimulated only moderately by ACh in the presence of atropine, in spite of the marked NA release. The low PLS release with ACh plus atropine, compared with that caused by nicotine, suggests that the NA released was responsible only in part, if at all, for the increased formation of PLS. This leads to the conclusion that nicotine as such stimulates the synthesis and subsequent release of PLS in the rabbit myocardium. Furthermore, this effect of nicotine seems to be distinct from the drug's pharmacological actions, which are generally referred to the activation of 'nicotinic' receptors.

Although the PLS in the myocardial effluent was not identified completely, there are several reasons for regarding it as prostaglandin E. Firstly, prostaglandins are almost the only smooth muscle stimulating compounds which follow the purification scheme used. Secondly, the rat stomach strip, suspended in a medium containing inhibitors for the activity of adrenaline, NA, ACh, 5-hydroxytryptamine, and histamine, seems to be specific for the assay of prosta-

glandin E. Thirdly, the increase in PLS caused by nicotine was inhibited by pretreatment of the heart with indomethacin, a well-known prostaglandin synthesis inhibitor (Vane, 1971). Finally, the PLS in the effluent had the same biochemical and biological properties as the prostaglandin E which has been shown to be released from this preparation (Wennmalm, 1971; 1975).

A side observation of some interest was made in the present investigation, namely that the nicotine-induced release of NA was greater in the presence than in the absence of indomethacin. This suggests that inhibition of PLS formation leads to facilitation of the nicotine-induced release of NA. It is well-known that the release of NA induced by sympathetic nerve depolarization is inhibited by exogenous prostaglandin E in several tissues and species (cf. Hedqvist, 1970). Furthermore, it has been shown that inhibition of the endogenous synthesis of prostaglandin in the rabbit heart leads to facilitation of the transmitter release induced by sympathetic nerve stimulation (Samuelsson & Wennmalm, 1971). The current observation, that inhibition of the endogenous synthesis of PLS is followed by an increased nicotine-

induced release of NA, suggests that this drug also elicits transmitter liberation by a process that is inhibited by endogenous prostaglandins.

To summarize, the current investigation has demonstrated a stimulatory effect of nicotine on the myocardial prostaglandin synthesis. The effect is brief and appears to be separated from activation of the conventional 'nicotinic' receptors.

Nicotine is probably the most common of the drugs used by man. In view of the current observations, it seems of interest to elucidate whether the concentration of nicotine in plasma from smoking subjects may become sufficiently high to stimulate the synthesis of prostaglandins. If so, the synthesis of the potentially hazardous, recently discovered prostaglandin analogue, thromboxane (Hamberg, Svensson & Samuelsson, 1975) may be increased as well. Thromboxane induces platelet aggregation; it therefore seems essential to study whether a relation exists between nicotine and myocardial thrombus formation.

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