Br. J. Pharmac. (1974), 52, 375-379

RELEASE OF PROSTAGLANDIN FROM THE RABBIT ISOLATED HEART FOLLOWING VAGAL NERVE STIMULATION OR ACETYLCHOLINE INFUSION

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1 Rabbit hearts were perfused by the Langendorff technique. The lipid fraction in the perfusate from the heart was isolated and analysed for prostaglandins by thin layer chromatography and quantitative assay on the rat isolated stomach strip.

2 Infusion of acetylcholine at a rate of 8 μg/min significantly increased the outflow of prostaglandins from the heart, from 1.8 to 6.2 ng/minute.

3 Addition of atropine (1 μg/ml) to the perfusing medium completely abolished not only the mechanical response but also the increase in outflow of prostaglandins caused by infusion of acetylcholine.

4 Bilateral stimulation of the parasympathetic nerves to the heart at 5 Hz also significantly increased the outflow of prostaglandins from the organ from 5.2 to 8.3 ng/minute.

5 Both prostaglandin E_1 and E_2 were isolated from the lipid fraction of the perfusate.

6 The role of prostaglandins in relation to autonomic neurotransmission in the heart is discussed.

Introduction

Adrenergic and cholinergic nerve endings have many features in common. The transmitter is stored in vesicles (Del Castillo & Katz, 1955; Euler & Hillarp, 1956) and probably released from these vesicles upon depolarization of the nerve terminal (Del Castillo & Katz, 1957; *cf.* Geffen & Livett, 1971). The release of transmitter upon depolarization is Ca^{++}-dependent in both types of neurones (Del Castillo & Stark, 1952; Huković & Muscholl, 1962; Vincenzi & West, 1965).

The release of both the sympathetic and parasympathetic transmitter from the rabbit heart following nerve stimulation has been shown to be inhibited by exogenously administered prostaglandins of the E series (Hedqvist, Stjärne & Wennmalm, 1970; Hedqvist & Wennmalm, 1971; Wennmalm & Hedqvist, 1971). The sensitivity to prostaglandins of the process of release of the sympathetic neurotransmitter is one of the prerequisites for the endogenous regulation of the release of noradrenaline from the adrenergic nerve terminals, as initially shown in the perfused rabbit heart (Samuelsson & Wennmalm, 1971; Wennmalm, 1971). The similarities in morphology and function between the two types of autonomic nerve terminals, especially in their sensitivity to exogenous prostaglandins, made it of interest to study whether the release of acetylcholine, in analogy with the liberation of noradrenaline, is restricted by a prostaglandin mediated endogenous feed-back mechanism. In the present paper we describe some observations which support this concept, namely release of prostaglandins from the rabbit heart following vagal nerve stimulation or acetylcholine infusion.

Methods

Rabbits of mixed strains and either sex were used for the study. They were killed by a blow on the head and bled from the left carotid artery. The heart with intact bilateral parasympathetic nerve supply was dissected out according to a modification of the technique described for dissection of the sympathetic nerve supply (Huković & Muscholl, 1962). The sympathetic trunk was removed from about 10 mm cranially down to the level of the stellate ganglion. The heart was perfused by the Langendorff technique at 37° C and at a pressure of about 60 cm H_2O with Tyrode solution (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.6) aerated with 6.5% CO_2 in O_2.

Right and left vagal nerves were pulled through separate plastic tubes with platinum rings in their walls serving as electrodes and connected to Grass Model S 44 stimulators. The part of the nerve stimulated had a predissection distance to the heart of about 15 mm. In the electrodes the nerves were continuously superfused with aerated Tyrode solution. They were stimulated by rectangular pulse trains of supramaximal strength (20 V, 20 ms) at a frequency of 5 Hz for 10 minutes. 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. Acetylcholine was infused at a rate of 8 μg/min during 10 min through a cannula immediately above the aorta.

During the experiment, the perfusate from the heart was collected in consecutive 10 min periods, starting 10-15 min after the organ had been applied to the perfusion apparatus. The first, fourth and sometimes the seventh of these 10 min periods were 'rest' periods, during which the heart remained un-exposed to acetylcholine or vagal nerve stimulation. During the second and sometimes the fifth perfusate collection period, the heart was stimulated, either by acetylcholine, infused at a rate of 8 μg/min, or by bilateral vagal nerve stimulation at a frequency of 5 Hz. The third and sometimes the sixth perfusate collection periods were 'washout' intervals, during which the heart was allowed to return to the basal level of mechanical activity. The perfusates from these two periods were discarded. In some experiments atropine (1 μg/ml) was present in the Tyrode solution during the entire experiment.

The perfusates collected were tested for prostaglandins in the following way. The perfusate was acidified to pH 3 with HCl. Lipids were extracted twice with an equal amount of ethyl acetate. The ethyl acetate was washed once with 1/20 volume of 0.5 M acetate buffer pH 6.5 and once with 1/20 volume of water and subsequently evaporated to dryness. The residue was dissolved and subjected to thin layer chromatography in system A II (Gréen & Samuelsson, 1964) or to quantitative assay on rat isolated superfused stomach muscle strip. This organ was superfused by Tyrode solution to which was added atropine (10^{-7} M), phentolamine (7×10^{-7} M), propranolol (7×10^{-7} M), methysergide (6×10^{-7} M) and diphenhydramine (7×10^{-7} M). Prostaglandin E_2 served as a reference substance.

All values are given as mean with s.e. For analysis of the increase in outflow of prostaglandins during periods of acetylcholine infusion or vagal nerve stimulation, the overflow of prostaglandins during these periods was compared to the mean outflow during the periods preceding and following the stimulation period. The paired differences were analysed by Student's *t* test.

Results

The frequency of the spontaneously beating hearts ranged from 110 to 150/minute. Infusion of acetylcholine at a rate of 8 μg/min or vagal nerve stimulation at 5 Hz decreased the frequency by approximately 50%, and in parallel, diminished the contractile force (Figure 1). A small but definite outflow of prostaglandin E into the effluent was observed from the spontaneously beating hearts, ranging from less than 2 ng/min in series of hearts where the dissection period was short (1-2 min, no

Table 1 Outflow of prostaglandin (ng) from rabbit isolated perfused hearts during successive 10 min periods

Treatment	*I 0-10 min*	*II 10-20 min*	*III 30-40 min*	*IV 40-50 min*	*V 60-70 min*
Infusion of ACh during II and IV. No atropine	19.8 ± 2.6 (8)	62.0 ± 10.2 (8)**	16.1 ± 5.5 (8)	53.0 ± 2.0 (2)*	24.5 ± 9.5 (2)
Infusion of ACh during II. Atropine (1 mg/l)	11.3 ± 1.0 (4)	17.8 ± 7.9 (4)	19.3 ± 9.0 (4)	–	–
Vagal nerve stimulation during II. No atropine	49.2 ± 13.5 (5)	82.8 ± 19.4 (5)*	53.6 ± 20.9 (5)	–	–
No stimulation	15.3 ± 3.6 (4)	14.5 ± 7.0 (4)	14.8 ± 8.3 (4)	–	–

Values are given as mean with s.e. Figures in parentheses indicate number of experiments.
* Significantly ($P < 0.05$) different from the mean of the preceding and the following observation.
** Significantly ($P < 0.01$) different from the mean of the preceding and the following observation.

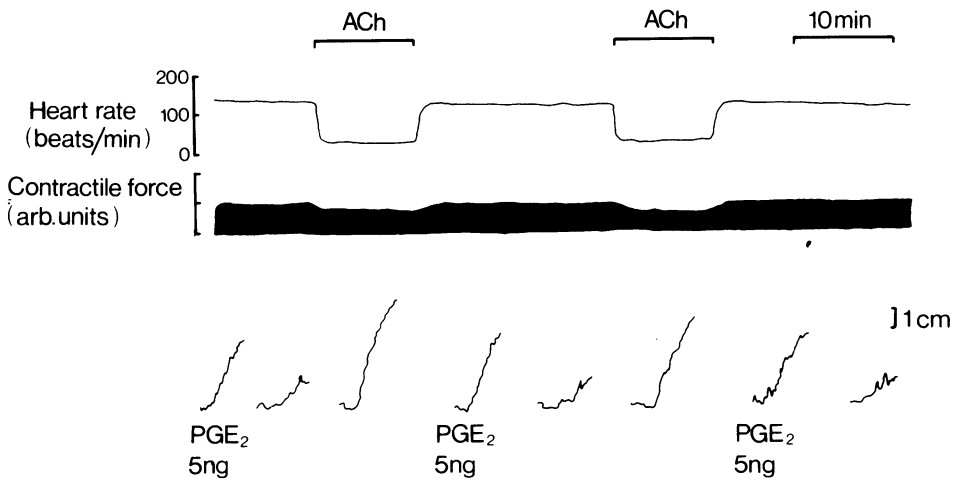

Fig. 1 Infusion of acetylcholine (ACh) in the rabbit isolated heart increases the outflow of prostaglandin E from the organ. The upper two tracings show heart rate and contractile force at rest and during two infusions of acetylcholine (80 μg during 10 minutes). The lower tracing shows the contractions of the bioassay organ (rat stomach strip) when exposed to known amounts of prostaglandin E_2 (PGE_2), or to extracts of the effluent from the heart, collected during 10 min periods corresponding in time to the bioassay tracing.

nerve dissection) to about 5 ng/min in series where the dissection was longer (12-15 min, bilateral vagal nerve dissection). The basal outflow of prostaglandin E was thus correlated to the time the heart was manipulated before being set up in the perfusion apparatus. The basal outflow of prostaglandin was also found to increase slightly during the course of the experiments (Table 1).

Infusion of acetylcholine at a rate of 8 μg/min significantly ($P < 0.01$) increased the outflow of prostaglandin E, from 1.8 to 6.2 ng/minute. Expressed in relation to the amount of acetylcholine infused, the increase in overflow of prostaglandin E was 0.5 ng per μg acetylcholine. Addition of atropine (1 μg/ml) to the Tyrode solution completely abolished not only the acetylcholine induced chronotropic and inotropic response of the heart but also the increase in overflow of prostaglandin E caused by acetylcholine (Table 1).

Vagal nerve stimulation at 5 Hz for 10 min also significantly ($P < 0.05$) increased the outflow of prostaglandin E in the effluent from the heart. In this series the outflow was 4.9-5.3 ng/min at rest and increased to 8.3 ng/min during the period of nerve stimulation. Expressed in relation to the nerve stimulation, the increase in prostaglandin overflow was 11 pg/impulse.

Thin layer chromatography of the biologically active compound in the effluent from parasympathetically stimulated hearts, or hearts infused with acetylcholine, revealed that the activity was due to the presence of both prostaglandins E_1 and E_2 in the effluent.

Discussion

Prostaglandin release can be evoked by electrical, pharmacological or mechanical stimuli in various tissues and species (*cf.* Gilmore, Vane & Wyllie, 1968; Minkes, Douglas & Needleman, 1973).

Since prostaglandins are stored in tissues in small amounts, the type of stimuli mentioned initiate the endogenous synthesis and subsequent release of prostaglandins rather than cause liberation from preformed stores.

Prostaglandin release in connection with cholinergic nerve stimulation has only been studied in experiments on the gastro-intestinal tract (Coceani, Pace-Asciak, Volta & Wolfe, 1967; Shaw & Ramwell, 1968), where an increased liberation of prostaglandins from the rat stomach in response to vagal nerve stimulation was reported.

In the present study a small but consistent overflow of prostaglandin into the effluent from the rabbit isolated perfused heart was observed. In experiments lasting more than 1 h a slight increase in the basal overflow of prostaglandin was observed, which confirms in the rabbit heart earlier observations in the isolated spleen (Ferreira, Moncada & Vane, 1973). Not only the duration of the experiment influenced the basal outflow of

prostaglandin from the heart, but also the length of the dissection which preceded setting up of the heart in the perfusion apparatus. Thus, in those experiments where the bilateral parasympathetic nerve supply to the heart was dissected prior to transferring the heart to the perfusion apparatus a considerably higher basal overflow of prostaglandin was obtained compared to the experiments where no such manipulation of the heart was performed. It has earlier been shown that mechanical vibration elicits increased overflow of prostaglandin-like substances from the rabbit spleen (Gryglewski & Vane, 1972). The current observation that extended mechanical handling elevates the basal overflow of prostaglandin from the rabbit heart points in the same direction.

Infusion of acetylcholine caused a marked and reversible increase in the overflow of prostaglandin from the heart. Repeated infusions caused outflow increments of the same magnitude. It has recently been reported that infusion of the sympathetic neurotransmitter, noradrenaline, elicits release of prostaglandin from the rabbit heart at a rate of about 15 ng per μg noradrenaline infused (Junstad & Wennmalm, 1973). In the present study the parasympathetic neurotransmitter, acetylcholine, increased the liberation of prostaglandin by about 0.5 ng per μg acetylcholine infused. The apparent difference between the two neurotransmitters in ability to elicit increased prostaglandin liberation from the heart is probably not explained in terms of variations in animal strains or in perfusion technique. It rather seems to reflect a true difference between noradrenaline and acetylcholine in their capacity to liberate prostaglandins.

Prostaglandin release from the perfused rabbit heart can be evoked by inducing hypoxia in the organ (Wennmalm, Pham-Huu-Chanh & Junstad, 1974). That this mechanism is not acting when prostaglandin is liberated from the heart by acetylcholine infusion can be seen from the unchanged perfusion flow during these periods. This release seems to be specifically evoked via cholinergic receptors and because the release was inhibited by atropine, they are by definition muscarinic in character. This parallels the observation that the sympathetic transmitter, noradrenaline, liberates prostaglandin from the rabbit heart by activating receptors which are blocked by α- or β-adrenoceptor antagonists (Wennmalm, 1974).

Vagal nerve stimulation also increased the overflow of prostaglandin E from the heart. In this series, the basal outflow of prostaglandin E was considerably higher than in the hearts where there was no dissection of the nerves, which has to be taken into account when estimating the increased outflow. Expressed in relation to the number of stimuli given, vagal nerve stimulation liberated about 11 pg per impulse at the frequency used. This figure is only slightly lower than those obtained when the sympathetic nerves of the rabbit heart are stimulated at 2 or 10 Hz (17 pg/impulse and 25 pg/impulse respectively; Wennmalm, unpublished), and does not correspond to the striking difference between noradrenaline and acetylcholine in ability to liberate prostaglandin E from the heart on infusion. A possible explanation for this difference might be that the cholinergic 'prostaglandin-release' receptors are densely concentrated in the part of the myocardium where cholinergic nerve endings are present, while the adrenergic 'prostaglandin-release' receptors are dispersed and not closely related to the sympathetic nerve endings. Such a morphological difference might explain the fact that cholinergic nerve stimulation, in spite of the fairly low number of nerve endings present in the heart, liberates almost the same amount of prostaglandin E per impulse as sympathetic nerve stimulation.

The prostaglandin released in response to adrenergic nerve stimulation in the rabbit heart has been shown to inhibit the release of the sympathetic transmitter noradrenaline, thus exerting the negative link in a feed-back regulation of the liberation of the sympathetic transmitter (Samuelsson & Wennmalm, 1971; Wennmalm, 1971). Since it has earlier been shown that exogenous prostaglandin E_1 inhibits the release of acetylcholine in response to parasympathetic nerve stimulation (Wennmalm & Hedqvist, 1971) the current findings that endogenous prostaglandin is released following nerve stimulation might indicate that a similar feed-back mechanism for regulation of transmitter release is operating at the parasympathetic nerve endings as well. However, further experiments are required to show the existence of such a mechanism inhibiting the release of the parasympathetic transmitter.

This work was supported by the Swedish Medical Research Council, project 14X-3027, and by Magnus Bergvalls stiftelse. The skilful technical assistance of Miss Gun Lindgren is gratefully acknowledged.

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(Received April 15, 1974)