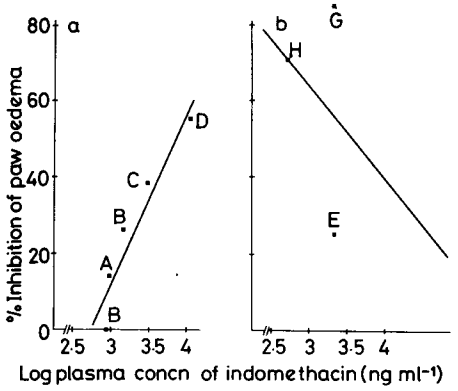

FIG. 1. Correlation between plasma concentration of indomethacin and inhibition of paw oedema in groups having pathologic mean score minor (a) and greater (b) than 0·47 (see Table 1). (a): n = 5, r = 0·968 and $P < 0{\cdot}01$. (b): n = 3, r = 0·282 and P not significant.

anti-inflammatory properties of the drug. On the other hand, the presence of marked inflammation in the intestine produced a considerable reduction in oedema development (Table 1) unrelated to the plasma concentration of drug (Fig. 1b) and therefore conceivably attributable to factor(s) other than its specific anti-inflammatory properties (Del Soldato et al 1979).

The non-specific role played by pre-existing inflammatory processes is substantiated by the observation that the plasma concentration of indomethacin that produced marked anti-oedema inhibition in presence of intestinal ulceration was below that which did not influence oedema formation in absence of intestinal pathology.

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Acetylcholine stimulates the release of prostacyclin by rabbit aorta endothelium

JOHAN R. BEETENS*, COR VAN HOVE, MARC RAMPART AND ARNOLD G. HERMAN, *Division of Pharmacology, Faculty of Medicine, University of Antwerp, B-2610 Wilrijk, Belgium*

Acetylcholine causes relaxation of isolated blood vessels in-vitro. Furchgott & Zawadski (1980) have shown that removal of the endothelium from the isolated blood vessels of different species abolishes the relaxation induced by the cholinergic transmitter. Since prostacyclin (PGI_2) is a potent vasodilator (Bunting et al 1976) and the major product of arachidonic acid metabolism in vascular endothelial cells (Weksler et al 1977), the effect of acetylcholine on the release of prostacyclin by rabbit aorta endothelium was studied. For this purpose, the 'well' or 'template' technique, originally described by Eldor et al (1981) was modified to limit the damage to the endothelial cells.

Methods

Rabbits were heparinized (100 iu kg^{-1} i.v.) before being killed by a blow on the head and exsanguination. The thorax was opened and the aorta was freed from fat and connective tissue. The aorta was cannulated and continuously perfused with oxygenated Krebs solution to remove the blood. The aorta was then removed to a petri dish, filled with Krebs solution. The remaining fat and connective tissue was further removed and the aorta was cut open longitudinally, in between the intercostal branches. During the entire procedure, the aorta was perfused with Krebs solution, to prevent the aorta from collapsing and the endothelium being damaged. Prepared in this way, the aortic intima remained completely covered with endothelium cells, as shown by silver nitrate staining. In experiments in which the aorta was not prevented from collapsing during the preparation, large areas of the aortic intima were denuded of endothelial cells.

The aorta was then placed between two lucite plates, held together with 4 lateral screws. The upper plate contained six holes (Ø 7 mm) narrower than the aortic width which served as incubation chambers in which the aortic luminal surface formed the chamber base. No leakage occurred from one chamber to another, and no diffusion out of the well of a solution of Evans blue or cyanocobalamin took place. The chambers were filled with 100 µl isotonic Hepes buffer and the aorta was allowed to equilibrate for 40 min. After the preincubation, the buffer was discarded and the chambers were refilled with 100 µl Hepes buffer. After incubation for

* Correspondence.

Table 1. Influence of ionophore A23187 and acetylcholine on the release of PGI_2 by rabbit aorta endothelium. Results are expressed as: (a) the difference of the amount PGI_2 released during the first and the second incubation period, in pg 6-oxo-$PGF_{1\alpha}$, stable metabolite (mean ± s.e.m.). (b) the absolute amount of PGI_2 formed during the first and the second incubation period, in pg 6-oxo-$PGF_{1\alpha}$ (mean ± s.e.m.).

		A23187 (μM)	Acetylcholine (nM)				
	Control	10	68	136	340	680	1340
(a) Δ	−15 ± 20	+4280 ± 775*	+67 ± 19	+95 ± 13*	+200 ± 35*	+350 ± 29*	+480 ± 40*
(b) Period 1	420 ± 40	480 ± 37	405 ± 43	450 ± 46	420 ± 42	400 ± 42	435 ± 30
Period 2	390 ± 33	4632 ± 615*	512 ± 43	589 ± 31*	659 ± 50*	793 ± 46*	945 ± 42*

* Significantly different from control (Duncan's test, $P < 0{\cdot}05$, n = 6).

15 min at room temperature (20°C), 95 μl of the incubation fluid was transferred to a test tube containing indomethacin (28 μM, final dilution) and the volume was adjusted to 1 ml with Hepes buffer. The chambers were refilled with Hepes buffer, containing the compounds to test and the incubation was continued for another 15 min. Silver nitrate staining showed that the endothelial layer was still almost intact, even after 80 min incubation. Prostacyclin production by the endothelial layer was measured as 6-oxo-$PGF_{1\alpha}$ by radioimmunoassay, as described earlier (Beetens et al 1982).

Results

The results can be evaluated either as the difference between the amount of 6-oxo-$PGF_{1\alpha}$ released during the first and the second incubation period or as the absolute amount of 6-oxo-$PGF_{1\alpha}$ released during the second incubation period.

This experimental outline has the following advantages over the method described by Eldor et al (1981). Firstly, the release of endogenously produced prostacyclin can be determined. Secondly, by measuring the difference between the first and the second incubation period, each chamber serves as its own control. Thirdly, by preventing the aorta from collapsing during the procedure no damage is afflicted upon the endothelium and the aortic intima remains completely covered with endothelial cells.

In order to evaluate the presently described modification of the technique for its suitability to measure changes in prostacyclin production, we stimulated the aorta with ionophore A23187, a substance known to increase the formation of prostacyclin in cultures of human and bovine endothelial cells (Weksler et al 1978; Goldsmith et al 1981). Ionophore A23187 (10 μM) markedly stimulated the release of prostacyclin (see Table 1). This effect, however, could not be completely reversed by removal of the drug and a second dose of ionophore was not able to further stimulate the prostacyclin release during a third incubation period (results not shown).

Acetylcholine was tested in five concentrations from 68 to 1360 nM. From 136 nM on, acetylcholine significantly stimulated the release of prostacyclin by the rabbit endothelium, in a dose-dependent manner (Table 1).

Discussion

Our experiments clearly show that acetylcholine stimulates the release of prostacyclin from the intact rabbit aorta endothelium. This might indicate that PGI_2 is a mediator of the vasodilating action of acetylcholine. However, the concentrations of acetylcholine required for PGI_2 stimulation, are higher than those which relax the rabbit aorta and the relaxing effect of acetylcholine is not abolished in the presence of indomethacin (Furchgott & Zawadzki 1980) thereby casting doubt upon the possible involvement of PGI_2 in the acetylcholine-induced vasodilatation.

Although acetylcholine has recently been shown to increase the PGI_2 production in rabbit aortic rings (Boeynaems et al 1982) our experimental set up provides direct evidence that it is the interaction of the cholinergic transmitter with the endothelial cells which results in the production of another vasoactive substance, i.e. prostacyclin. The physiological importance of this interaction however still remains to be determined.

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