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THE NEURONAL ORIGIN OF PROSTAGLANDIN RELEASED FROM THE RABBIT PORTAL VEIN IN RESPONSE TO ELECTRICAL STIMULATION

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1 Transmural electrical stimulation of the isolated portal vein of the rabbit was accompanied by the release of a prostaglandin-like substance (PLS). Thin layer chromatography coupled with bioassay indicated that this substance was probably prostaglandin E_2 (PGE_2).

2 Indomethacin potentiated the response of the portal vein to electrical stimulation at 2 Hz and abolished the release of the PLS.

3 There was no significant change in the amount of PLS released from the portal vein in response to electrical stimulation at 2 Hz when the contractile response of the portal vein was prevented by pretreatment with phentolamine or guanethidine.

4 *In vitro* denervation of the portal vein with 6-hydroxydopamine or the omission of Ca^{2+} from the bathing solution caused a significant reduction in the amount of PLS released from the portal vein in response to electrical stimulation at 2 hertz.

5 It is concluded that electrical stimulation of the isolated portal vein of the rabbit is accompanied by the release of a PLS, probably PGE_2, from a neuronal source. The synthesis and release of the PLS is Ca^{2+}-dependent.

Introduction

The role of prostaglandins in sympathetic neurotransmission is not completely clear. However, it is well established that in certain organs of several species, prostaglandins of the E type inhibit the release of noradrenaline in response to sympathetic nerve stimulation and that inhibition of protaglandin synthesis facilitates the release of noradrenaline. In addition it has been reported that the E prostaglandins are released from various tissues during periods of sympathetic nerve stimulation. On the basis of this evidence it has been postulated that the E type prostaglandins play a regulatory role in sympathetic neurotransmission (Hedqvist, 1973). However, the origin of the prostaglandins released in response to sympathetic nerve stimulation is not clear.

Nerve stimulation caused contraction of the dog spleen and release of prostaglandins. Adrenergic blockade prevented both the contraction and the release of prostaglandin. It was therefore suggested that prostaglandin release in response to nerve stimulation in dog spleen is derived from the muscle and is released as a result of its contraction (Gilmore, Vane & Wyllie, 1968). However, in the guinea-pig vas deferens, indomethacin increased the release of noradrenaline in response to electrical stimulation in absence of muscle contraction. These results suggested that the prostaglandins released from the vas deferens are neuronal in origin (Stjärne, 1972).

Recently it was shown that the contractile response of the rabbit portal vein to electrical stimulation is inhibited by prostaglandin E_2 (PGE_2) and potentiated by indomethacin and eicosa-5,8,11,14-tetraynoic acid (ETA) (Greenberg, 1974). The present study was done, therefore, to see if the indomethacin potentiation is accompanied by a reduction in the release of prostaglandin and whether the origin is neuronal.

Methods

Male albino rabbits (1.5–3 kg) were killed by a blow on the head. The portal vein was dissected out as described by Hughes & Vane (1967). Each vein was suspended in an organ bath containing 2.5 ml of Krebs-Henseleit solution (mM: NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.1, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.0) kept at 37.5°C and bubbled with

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a gas mixture of 95% O_2 and 5% CO_2. In experiments where the tissues were to be bathed in a medium without calcium, a 'Ca-free' solution was prepared by the omission of $CaCl_2$ from the standard Krebs-Henseleit formula. A tension of 3 g was applied to the veins which were then allowed to equilibrate for 1 h before they were stimulated electrically. A Grass S4 stimulator was used for field stimulation; a current was applied between 2 platinum electrodes, one placed at each side of the organ bath. The veins were stimulated with 240 pulses at a frequency of 2 Hz, with a pulse duration of 1 ms at supramaximal voltage. The release of prostaglandins was detected by passing the fluid from the organ bath over a rat stomach strip. This superfusion technique is similar to the one described for the bioassay of noradrenaline (Hughes, 1972). Contractions of both the portal vein and rat stomach strip were measured with Grass FT03 force displacement transducers and recorded on a model 7 Grass polygraph.

Prostaglandin-like substances (PLS) were extracted from the fluid bathing the portal vein. The fluid was acidified to pH 3 with HCl and extracted twice with equal volumes of ethyl acetate. The ethyl acetate phase was evaported to dryness under vacuum and the residue resuspended in 0.5 ml ethanol. Thin-layer chromatography was carried out on Silica Gel G coated with 1% phosphoric acid and with a solvent system consisting of di-isopropyl ether, ethyl acetate, acetone and isopropyl alcohol (70, 10, 10, 10, % v/v).

PGE_2 and $PGF_{2\alpha}$ standards were chromatographed simultaneously with the extracts and made visible by spraying the plates with sulphuric acid followed by heating at 250°C. The position of authentic PGE_2 and $PGF_{2\alpha}$ was visualized and the R_F values determined. The corresponding zones on the plates to which the extract was applied as well as other zones were scraped off the plates and extracted into 1 ml Krebs solution and assayed on rat stomach strips.

The *in vitro* denervation of the portal vein was done with 6-hydroxydopamine (6-OHDA). Each portal vein was exposed to 6-OHDA at a concentration of 25 μg/ml over a period of one hour. In order to reduce the rate of oxidation of 6-OHDA during exposure the bath fluid was changed and the portal veins re-exposed to 6-OHDA every 10 minutes. Loss of the contractile response to electrical stimulation at 2 Hz and to tyramine (10 μg/ml) and potentiation of the contractile response to noradrenaline (1 μg/ml) were taken to indicate *in vitro* denervation.

The drugs used in this study were: indomethacin, guanethidine sulphate, phentolamine hydrochloride, tyramine hydrochloride, 6-hydroxydopamine, prostaglandins E_2 and $F_{2\alpha}$, noradrenaline bitartrate. Indomethacin was dissolved in a 2% sodium carbonate solution and the pH was adjusted to 7.6 with HCl. A stock solution of PGE_2 was prepared by dissolving 10 mg in 9 ml of a solution of 0.2% sodium carbonate and 1 ml of 95% ethanol. Noradrenaline bitartrate was dissolved in 0.01 N HCl. 6-Hydroxydopamine was dissolved in ice cold 1% ascorbic acid. Drug concentrations are expressed as final concentrations of free base. Drugs were added to the organ bath in volumes not exceeding 0.1 ml. The data were analyzed by Student's t test for paired and unpaired data.

Results

Characterization of a prostaglandin-like substance released from the portal vein

Four portal veins were stimulated with a train of 240 pulses at a frequency of 8 Hz at 30 min intervals. The bath fluid was collected and extracted after each stimulation. The extracts from 8 stimulation periods from each vein were pooled and subjected to thin layer chromatography. Bioassay revealed that all detectable activity resided in the zone which corresponded to the authentic PGE_2, having an R_F value of 0.59.

In 6 additional experiments, the portal vein was stimulated at 2 Hz in the absence and presence of mepyramine (1 μg/ml) and methysergide (1 μg/ml). The subsequent bioassay of the effluent showed that 3.28 ± 0.73 μg of PLS was released in the absence of the antagonists and 3.68 ± 1.06 μg in the presence of the antagonists. The difference was not significant ($P > 0.4$). These results indicate that electrical stimulation of the portal vein is accompanied by the release of a substance which is not 5-hydroxytryptamine or histamine.

The effect of indomethacin on the release of a prostaglandin-like substance from the portal vein

Eight experiments were done to see if the potentiation of the response of the portal vein to electrical stimulation by indomethacin was accompanied by a reduction in the release of PLS. Each portal vein was stimulated at 2 Hz before and 60 min after the addition of indomethacin (10 μg/ml) to the organ bath. The effluent was bioassayed on the rat stomach strip after each stimulation. A typical experiment is illustrated in Figure 1. Indomethacin significantly increased the response of the portal vein by 1.7 ± 0.5 g tension ($P < 0.01$). The subsequent bioassay of the effluent showed that there was a significant concomitant reduction in the amount of PLS released (Table 1).

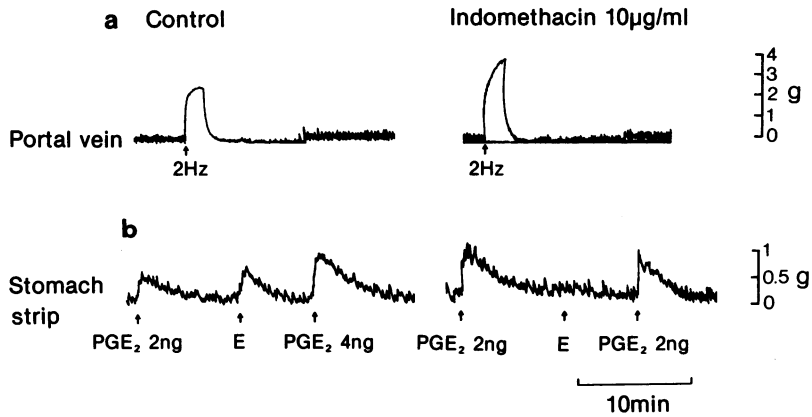

Figure 1 The effect of indomethacin on the release of a prostaglandin-like substance from the rabbit portal vein. (a) Responses of the portal vein to electrical stimulation at 2 Hz before and after indomethacin (10 μg/ml). (b) Contractions of the rat stomach strip in response to the effluent (E) from the portal vein after electrical stimulation bracketed by standard amounts of prostaglandin E_2 (PGE_2).

The effect of phentolamine and guanethidine on the release of a prostagladin-like substance from the portal vein in response to electrical stimulation

Experiments were done to see if the release of the PLS from the portal vein was due to contraction of the smooth muscle. The contractile response to electrical stimulation was prevented by pretreatment of the portal vein with either phentolamine or guanethidine.

In 7 portal veins electrical stimulation at 2 Hz caused a contraction of 1.7 ± 0.3 g tension which was accompanied by the release of 2.7 ± 0.4 ng of PLS. The subsequent electrical stimulation of the portal vein at 2 Hz in the presence of phentolamine, 10 μg/ml, did not cause a contraction but was accompanied by the release of 3.0 ± 0.5 ng of PLS. Further treatment with indomethacin (10 μg/ml) in the presence of phentolamine (10 μg/ml) significantly reduced this release of PLS to 0.6 ± 0.2 ng ($P < 0.01$). A typical experiment is illustrated in Figure 2 and the results are summarized in Table 1.

Similar results were obtained in six portal veins that were treated with guanethidine (10 μg/ml) for one hour. Guanethidine completely abolished the contraction of the portal vein in response to electrical stimulation at 2 Hz but there was no reduction in the release of prostaglandins. Further treatment with indomethacin, (10 μg/ml) completely abolished the release of PLS in response to electrical stimulation. The results are summarized in Table 1.

Table 1 The effect of various treatments on the release of prostaglandin-like substance from the portal vein in response to electrical stimulation at 2 hertz

Treatment	*No. veins*	*Control output of PLS (ng)*	*Output of PLS after treatment (ng)*	*% change in output of PLS*
Indomethacin	8	4.8 ± 1.5	0.7 ± 0.2*	−85.5
Phentolamine	7	2.7 ± 0.4	3.0 ± 0.5 NS	+11.1
Guanethidine	6	2.6 ± 0.4	2.9 ± 0.4 NS	+11.5
6-Hydroxydopamine	8	5.5 ± 1.1	1.7 ± 0.4**	−69.1
Ascorbic acid	4	5.9 ± 3.2	5.9 ± 3.5 NS	+0.0
Ca^{2+}-free Krebs-Henseleit	6	4.2 ± 1.1	0.2 ± 0.2*	−95.2

Figures show means with s.e. mean.
Significance of the difference from controls (*t* test for paired data): *$P < 0.05$; **$P < 0.01$; NS = not significant.

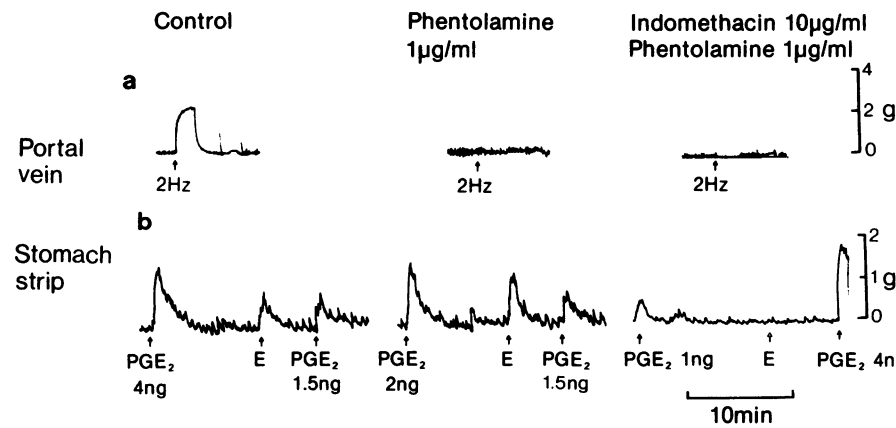

Figure 2 The effect of phentolamine on the release of a prostaglandin-like substance from the rabbit portal vein (a) Responses of the portal vein to electrical stimulation at 2 Hz before and after phentolamine (1 μg/ml) and phentolamine (1 μg/ml) plus indomethacin (10 μg/ml). (b) Contractions of the rat stomach strip in response to the effluent (E) from the portal vein after electrical stimulation bracketed by standard amounts of prostaglandin E_2 (PGE_2).

The effect of in vitro *6-hydroxydopamine treatment on the release of a prostaglandin-like substance from the portal vein in response to electrical stimulation*

Experiments were done to see if *in vitro* denervation of the portal vein with 6-OHDA would alter the release of PLS in response to electrical stimulation. In 8 portal veins electrical stimulation at 2 Hz caused a contraction of 0.8 ± 0.2 g tension which was accompanied by the release of 5.5 ± 1.1 ng of PLS. Treatment with 6-OHDA (25 μg/ml) completely abolished the response to electrical stimulation and reduced the release of PLS (3.8 ± 0.8 ng; $P < 0.01$). When these same portal veins were exposed to noradrenaline (1 μg/ml) and tyramine, (10 μg/ml) to test for completeness of denervation, the initial response to noradrenaline was significantly potentiated by 1.2 ± 0.3 g tension ($P < 0.01$) and the response to tyramine was completely abolished. A typical experiment is illustrated in Figure 3.

In four additional experiments there was no significant difference in the responses of the portal vein to electrical stimulation at 2 Hz, noradrenaline (1 μg/ml) or tyramine (10 μg/ml) before or after treatment with ascorbic acid (0.2 mg/ml). The release of PLS in response to electrical stimulation was also unaltered by the ascorbic acid treatment. The results are summarized in Table 1.

The effect of calcium on the release of a prostaglandin-like substance from the portal vein in response to electrical stimulation.

Experiments were done to see if the release of PLS from the portal vein in response to electrical stimulation was calcium-dependent. Six portal veins were bathed in normal Krebs-Henseleit solution. Stimulation at 2 Hz caused a contraction of 1.5 ± 0.4 g tension and the release of 4.2 ± 1.1 ng PLS. The portal veins were then bathed in a Ca-free solution for one hour. Electrical stimulation at 2 Hz failed to elicit contraction and the amount of PLS released was reduced to 0.2 ± 0.2 nanogram. The portal veins were then bathed in standard Krebs-Henseleit solution to replace some of the calcium. After 30 min electrical stimulation caused a contraction of the portal vein of 1.8 ± 0.6 g tension and the release of 2.5 ± 0.6 ng PLS. A typical experiment is illustrated in Figure 4 and the results summarized in Table 1.

Discussion

The results show that the indomethacin-induced potentiation of the response of the portal vein to electrical stimulation is accompanied by a reduction in the release of PLS. The data also indicate that the PLS is neuronal in origin, and its synthesis and release is calcium-dependent.

Prostaglandins have been shown to be released from various tissues in response to electrical stimulation (Piper & Vane, 1971). The present study shows that electrical stimulation of the isolated portal vein of the rabbit causes the release of a PLS. Thin layer chromatography coupled with bioassay indicated that the PLS was probably PGE_2. It was further shown that the prostaglandin synthesis inhibitor, indomethacin, prevents the release of the PLS.

Inhibition of prostaglandin synthesis with either indomethacin or ETA has been shown to decrease

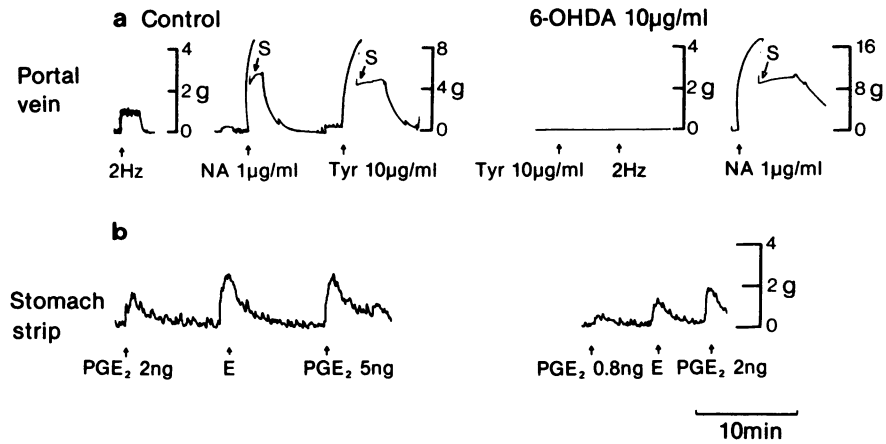

Figure 3 The effect of 6-hydroxydopamine (6-OHDA) on the release of a prostaglandin-like substance from the rabbit portal vein. (a) Responses of the portal vein to electrical stimulation at 2 Hz, noradrenaline (1 μg/ml, NA) and tyramine (10 μg/ml, Tyr) before and after treatment with 6-OHDA (25 μg/ml). The sensitivity of the amplifier was reduced by one half at (S). (b) Contractions of the rat stomach strip in response to the effluent (E) from the portal vein after electrical stimulation bracketed by standard prostaglandin E_2 (PGE_2).

simultaneously the efflux of prostaglandins and increase the contractile response or the outflow of noradrenaline in response to electrical stimulation in rabbit heart (Samuelsson & Wennmalm, 1971; Chanh, Junstad & Wennmalm, 1972), cat spleen (Hedqvist, Stjärne & Wennmalm, 1971), and guinea-pig vas deferens (Hedqvist & Von Euler, 1972; Fredholm & Hedqvist, 1972). Recently, it was found that the responses of the isolated portal vein of the rabbit to electrical stimulation are inhibited by prostaglandin E_2 and potentiated by indomethacin and ETA (Greenberg, 1974). The present results extend these findings and show that the indomethacin-induced potentiation is accompanied by a decrease in the release of PLS. This decrease reflects a decrease in the synthesis of PLS since tissues generally do not store prostaglandins and biosynthesis is immediately followed by release (Piper & Vane, 1971; Kunze & Vogt, 1971).

The origin of prostaglandins released in response to sympathetic nerve stimulation is not clear. In both the spleen and kidney the α-adrenoceptor blocking drug, phenoxybenzamine, abolished the contraction and the release of prostaglandins induced by nerve

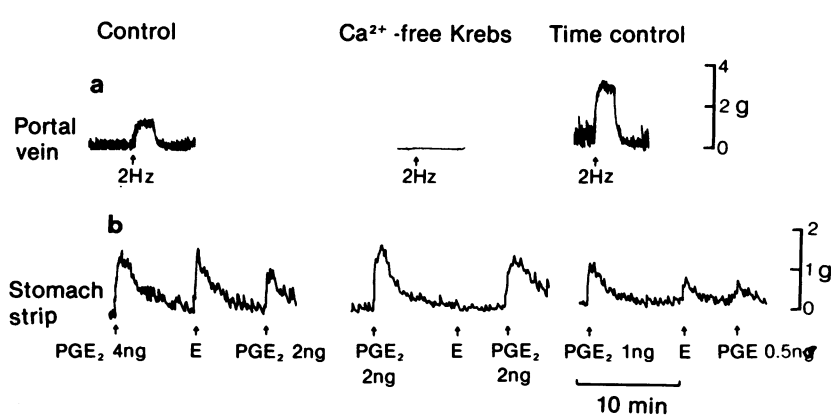

Figure 4 The effect of calcium on the release of a prostaglandin-like substance from the rabbit portal vein. (a) Responses of the portal vein to electrical stimulation at 2 Hz in normal, Ca^{2+}-free, and normal Krebs-Henseleit solution. (b) Contractions of the rat stomach strip in response to the effluent (E) from the portal vein after electrical stimulation bracketed by standard prostaglandin E_2 (PGE_2).

stimulation (Gilmore *et al.*, 1968; Davies, Horton & Withrington, 1968; Needleman, Douglas, Jakschik, Stocklein & Johnson, 1974). It was therefore concluded that the prostaglandins were not derived from the nerve endings but were released as a result of smooth muscle contraction. In contrast, Stjärne (1972) found that indomethacin increased the release of noradrenaline from the isolated vas deferens of the guinea-pig in response to electrical stimulation in the absence of contraction. These results indirectly support the view that prostaglandins can be released from a neuronal source.

The results of this study show that a PLS is released from the rabbit portal vein in response to electrical stimulation when the contractile response is prevented by pretreatment with the α-adrenoceptor blocking drug, phentolamine, or by preventing noradrenaline release with guanethidine. These results indicate that the prostaglandins are not released from the portal vein as a result of smooth muscle contraction, and may be neuronal in origin.

In vitro denervation of the rabbit portal vein with 6-OHDA was evidenced by a lack of response to electrical stimulation or tyramine, and potentiation of the response to noradrenaline. These results are similar to those reported for the *in vitro* denervation of the rat portal vein (Aprigliano & Hermsmeyer, 1976; Aprigliano, Rybarczyk, Hermsmeyer & van Orden, 1976). In this study the 6-OHDA treatment of the portal vein caused a significant reduction in the amount of PLS released in response to electrical stimulation. These results coupled with those showing no reduction in PLS released in the absence of the contractile response indicate a neuronal source of the PLS.

Calcium is necessary for both the contraction of smooth muscle (Daniel, 1965) and the release of noradrenaline from sympathetic nerve endings (Smith, 1973; Hedqvist, 1977). Activation of a calcium-sensitive phospholipase may be a requirement for the formation of arachidonic acid and the synthesis of prostaglandins (Kunze & Vogt, 1971; Coceani & Pace-Asciak, 1976). The results of the present study show that when calcium was omitted from the bathing solution it abolished the release of PLS from the portal vein in response to electrical stimulation. The absence of calcium in the bathing solution also contributes to the absence of a contraction in response to electrical stimulation. However, there was no reduction in the amount of PLS released in response to electrical stimulation when contraction was abolished by α-adrenoceptor blockade with phentolamine, or adrenergic neurone blockade with guanethidine. These results, therefore, indicate that calcium is necessary for the synthesis and release of PLS which may be related to the activation of a calcium-dependent phospholipase.

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