Br. J. Pharmac. (1974), 52, 61-68

THE EFFECTS OF INDOMETHACIN AND EICOSA-5,8,11,14-TETRAYNOIC ACID ON THE RESPONSE OF THE RABBIT PORTAL VEIN TO ELECTRICAL STIMULATION

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1 The effects of indomethacin and eicosa-5,8,11,14-tetraynoic acid (ETYA) on the contractile response of the transmurally stimulated rabbit portal vein were studied *in vitro*.

2 When the veins were stimulated for 240 pulses at 1 and 2 Hz, the responses were potentiated by indomethacin and ETYA. However, responses to 4 and 8 Hz were not potentiated. The responses to continuous electrical stimulation at 2 Hz were also potentiated by indomethacin and ETYA. This potentiating effect was attenuated when the veins were pretreated with α-methyl-*p*-tyrosine. The responses of the veins to noradrenaline were not altered by either indomethacin or ETYA.

3 Prostaglandin E_2 inhibited the responses of the portal vein to electrical stimulation. The magnitude of this inhibition was inversely related to the frequency of stimulation. The responses of the vein to noradrenaline were not altered by prostaglandin E_2.

4 It is concluded that potentiation by indomethacin and ETYA of the response of the isolated portal vein is due to an increased release of newly synthesized noradrenaline as a result of inhibition of prostaglandin synthesis.

Introduction

The prostaglandins of the E series have been shown to inhibit sympathetic neurotransmission in several tissues and species. Inhibition of the response to nerve stimulation by the E prostaglandins has been shown in the cat spleen (Hedqvist & Brundin, 1969), rabbit heart (Hedqvist & Wennmalm, 1971), guinea-pig vas deferens (Baum & Shropshire, 1971; Hedqvist & von Euler, 1972a), guinea-pig seminal vesicle (Hedqvist, 1972a), and cat hindleg (Hedqvist, 1972b). Inhibition, by the prostaglandins, of noradrenaline release in response to sympathetic nerve stimulation has been shown in cat spleen (Hedqvist, 1970a, b), rabbit heart (Hedqvist, Stjärne & Wennmalm, 1971), and guinea-pig vas deferens (Stjärne, 1972). This inhibitory effect of the prostaglandins is frequency dependent with the magnitude of the inhibition varying inversely with the frequency (Baum & Shropshire, 1971; Hedqvist & von Euler, 1972a). On the basis of this evidence together with the natural occurrence of prostaglandins and their availability for release, Hedqvist and his co-workers have postulated that endogenous prostaglandins modulate sympathetic neurotransmission. Further support for this hypothesis was obtained from studies showing that either eicosa-5,8,11,14-tetraynoic acid (ETYA) or indomethacin, both effective inhibitors of prostaglandin biosynthesis (Ahern & Downing, 1970; Vane, 1971), facilitate the release of noradrenaline in response to sympathetic nerve stimulation in the cat spleen (Hedqvist *et al.*, 1971; Ferreira & Moncada, 1971), rabbit heart (Samuelsson & Wennmalm, 1971; Chanh, Junstad & Wennmalm, 1972), and guinea-pig vas deferens (Fredholm & Hedqvist, 1973; Stjärne, 1973).

Newly synthesized noradrenaline has been shown to be involved in the maintenance of transmitter release during sympathetic nerve stimulation (Kopin, Breese, Kraus & Weise, 1968; Kupferman, Gillis & Roth, 1970; Stjärne & Wennmalm, 1970; Kalsner, 1972; Hughes & Roth, 1972; Bennett, 1973a). This study was therefore made to examine the possible role of newly synthesized noradrenaline in the frequency dependent facilitation of sympathetic neurotransmission by indomethacin and ETYA in the rabbit isolated portal vein.

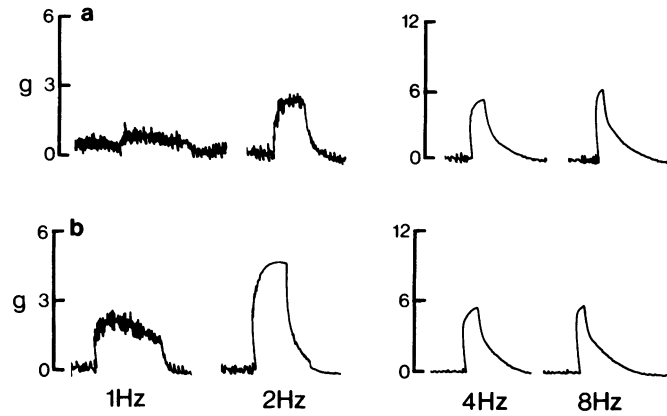

Fig. 1 The effect of indomethacin on the response of the rabbit portal vein to electrical stimulation at 1, 2, 4 and 8 Hz. (a) Control; (b) indomethacin (10 μg/ml). The sensitivity of the amplifier was reduced by one half between 2 and 4 Hz.

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 15 ml of Krebs-Henseleit solution (mM: NaCl 1.18, 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 a gas mixture of 95% O_2 and 5% CO_2. A tension of 3 g was applied to the veins which were then allowed to equilibrate for 1 h before they were stimulated electrically or before any drugs were added to the bath. Isometric contractions were measured with Grass FT03 force displacement transducers and recorded on a model 7 Grass polygraph. 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 strips were stimulated either with a train of 240 pulses at frequencies of 1-8 Hz or continuously at 2 Hz for 20-30 min, with a pulse duration of 1 msec at supramaximal voltage.

The drugs used in this study were: indomethacin; eicosa-5,8,11,14-tetraynoic acid (ETYA); prostaglandin E_2; α-methyl-*p*-tyrosine methylester (α-MPT); noradrenaline bitartrate guanethidine sulphate, and phentolamine hydrochloride. Indomethacin and ETYA were dissolved in a 2% sodium carbonate solution and the pH was adjusted to 7.6 with HCl. A stock solution of prostaglandin E_2 was prepared by dissolving 10 mg in 9 ml of a solution of 0.2% Na_2CO_3 and 1 ml of 95% C_2H_5OH. Noradrenaline bitartrate was dissolved in 0.01 N HCl. Drugs were added to the organ bath in volumes not exceeding 0.3 ml. Drug concentrations are expressed as final bath concentration of free base. The data were analysed by Student's *t* test for paired data.

Results

Response of the portal vein to electrical stimulation

Electrical field stimulation caused contraction in 30 isolated portal veins, the magnitude of which was proportional to the frequency (Figure 1). Evidence that these responses are the result of postganglionic nerve stimulation and the subsequent release of noradrenaline were obtained from experiments with guanethidine and phentolamine.

Guanethidine (1 μg/ml) in 3 experiments and phentolamine (1 μg/ml) in 6 experiments abolished the responses of the portal vein to electrical stimulation. These results are in agreement with those of Hughes & Vane (1967) and Hughes (1972) who showed that the response of the rabbit portal vein to electrical field stimulation is due to excitation of sympathetic nerve endings and the subsequent release of noradrenaline.

Frequency dependent potentiation of the response of the portal vein to electrical stimulation

Sixteen experiments were done to determine the effects of indomethacin (8 veins) and ETYA (8

veins) on the response of the portal vein to electrical stimulation. In each experiment 1 portal vein was stimulated electrically with a train of 240 pulses at 1, 2, 4 and 8 Hz. The vein was allowed to relax for 15 min between each period of stimulation. The vein was then restimulated at 1, 2, 4 and 8 Hz as in the control period but, in the presence of either indomethacin or ETYA at a concentration of 10 μg/ml. A typical experiment is shown in Figure 1. Both indomethacin and ETYA caused a significant potentiation of the responses to 1 and 2 Hz but either did not significantly change or reduced the responses to 4 and 8 Hz. The results are summarized in Table 1. In 5 additional experiments there was no significant difference in the responses obtained to repeated electrical stimulation in the absence of any drug treatment.

The effect of inhibition of noradrenaline synthesis with α-methyl-p-tyrosine on the potentiation of the response of the portal vein to electrical stimulation

(a) Electrical stimulation at 1, 2, 4 and 8 Hz. Fourteen experiments were done to determine if the release of newly synthesized noradrenaline contributed to the potentiating effects of indomethacin and ETYA. The portal vein was electrically stimulated with a train of 240 pulses at 1, 2, 4 and 8 Hz, before and 30 min after the addition of α-MPT (100 μg/ml) to the Krebs-Henseleit solution bathing the vein. The vein was then restimulated at 1, 2, 4 and 8 Hz in the presence of α-MPT and either indomethacin or ETYA at a concentration of 10 μg/ml). A typical experiment showing the effect of indomethacin on the responses of the portal vein to electrical stimulation in the presence of α-MPT is shown in Figure 2. Pretreatment of the 14 veins with α-MPT caused a small but significant increase in the responses to 1 and 2 Hz of 0.31 ± 0.06 g and 0.26 ± 0.11 g ($P < 0.001$, < 0.05), respectively, but did not significantly change the responses to 4 and 8 Hz. However, this pretreatment abolished the potentiating effects of indomethacin and ETYA, as the responses to 1 and 2 Hz in the presence of α-MPT were not significantly changed by indomethacin or ETYA. The responses to 4 and 8 Hz in the presence of α-MPT were significantly reduced both by indomethacin and ETYA. The results are summarized in Table 2.

(b) Continuous electrical stimulation. The frequency of 2 Hz was selected to assess the role of noradrenaline synthesis in the potentiation of the response to continuous electrical stimulation by indomethacin or ETYA. A typical experiment is illustrated in Figure 3. Two portal veins were used in each experiment. Both veins were stimulated continuously at 2 Hz but only one vein was pretreated with α-MPT (100 μg/ml) for 30 min and then stimulated in the presence of α-MPT throughout the entire experiment. The response to continuous electrical stimulation declined and reached a plateau after 10 min, at which time indomethacin or ETYA was added to the bath. After 10-15 min the drugs were washed out of the bath and electrical stimulation terminated. The veins were then allowed to relax for 30 min and then restimulated at 2 Hz until a maximum response was obtained. The initial responses to electrical stimulation at 2 Hz in 13 control veins and 11 veins with α-MPT present were 2.26 ± 0.26 and 2.91 ± 0.23 g, respectively, which declined to 47.89 ± 2.90% and 49.86 ± 3.54%, respectively, of the initial response after 10 minutes. There was no significant difference between the responses of the

Table 1 The effect of indomethacin and eicosa-5,8,11,14-tetraynoic acid (ETYA) on the response of the portal vein to electrical stimulation

	Tension (g)			
Frequency (Hz)	*Control response (n = 8)*	*Change in response after indomethacin (10 μg/ml)*	*Control response (n = 8)*	*Change in response after ETYA (10 μg/ml)*
1	0.63 ± 0.26	+0.60 ± 0.16**	0.53 ± 0.09	+0.53 ± 0.09***
2	2.44 ± 0.43	+1.30 ± 0.4**	1.78 ± 0.35	+1.12 ± 0.28**
4	5.55 ± 0.80	+0.20 ± 0.13 NS	4.40 ± 0.43	+0.52 ± 0.31 NS
8	6.44 ± 0.82	−0.44 ± 0.13**	5.75 ± 0.46	−0.18 ± 0.15 NS

Figures show mean with s.e. of mean. Significance of difference from controls (*t* test for paired data): * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; NS = not significant at 0.05. *n* = number of veins.

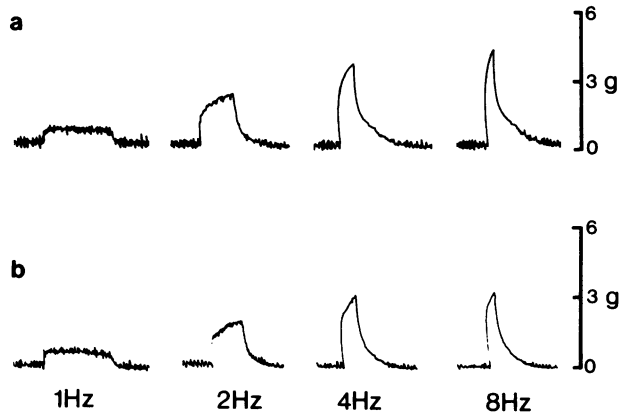

Fig. 2 The effect of α-methyl-*p*-tyrosine (α-MPT) on the potentiation by indomethacin of the response of the rabbit portal vein to electrical stimulation at 1, 2, 4 and 8 Hz, in the presence of (a) α-MPT (100 μg/ml) and (b) α-MPT (100 μg/ml) and indomethacin (10 μg/ml).

control and α-MPT treated veins ($P > 0.05$). When either indomethacin (10 μg/ml) or ETYA (10 μg/ml) was added to the bath they caused a significant increase in tension in control veins but not in α-MPT treated veins. When the veins were restimulated at 2 Hz, 30 min after indomethacin or ETYA had been washed out of the bath, the responses were significantly greater than the initial responses before drug treatment in the control veins but not in the α-MPT treated veins. The results are summarized in Table 3.

The effect of indomethacin and ETYA on the response of the portal vein to noradrenaline

Rabbit isolated portal veins were exposed to cumulative concentrations of noradrenaline (0.01 to 10.0 μg/ml) in the absence and presence of indomethacin (10 μg/ml; 16 veins) and ETYA (10 μg/ml; 5 veins). Neither indomethacin nor ETYA had any effect on the responses of the portal vein to noradrenaline. The dose-response curves are shown in Figures 4 and 5.

The effect of prostaglandin E_2 on the response of the rabbit portal vein to electrical stimulation and noradrenaline

Four portal veins were electrically stimulated with a train of 240 pulses at 1, 2, 4 and 8 Hz in the absence and presence of prostaglandin E_2 (0.2 μg/ml). In the presence of prostaglandin E_2 the responses to stimulation frequencies of 1 and 2 Hz were substantially reduced from the control

Table 2 The effect of α-methyl-*p*-tyrosine (α-MPT) on the potentiation by indomethacin and eicosa-5,8,11,14-tetraynoic acid (ETYA) of the response of the portal vein to electrical stimulation

	Tension (g)			
Frequency (Hz)	*Response in presence of α-MPT (100 μg/ml) (n = 8)*	*Change in response after treatment with indomethacin (10 μg/ml) in the presence of α-MPT*	*Response in presence of α-MPT (100 μg/ml) (n = 6)*	*Change in response after treatment with ETYA (10 μg/ml) in the presence of α-MPT*
1	1.48 ± 0.14	−0.08 ± 0.07 NS	1.19 ± 0.26	+0.08 ± 0.04 NS
2	3.70 ± 0.24	−0.14 ± 0.23 NS	3.18 ± 0.53	−0.20 ± 0.21 NS
4	5.88 ± 0.15	−1.23 ± 0.11***	4.94 ± 0.51	−0.48 ± 0.16*
8	6.70 ± 0.23	−1.58 ± 0.19***	5.84 ± 0.54	−0.99 ± 0.14***

Figures show mean with s.e. of mean. Significance of difference from controls (*t* test for paired data): * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; NS = not significant. *n* = number of veins.

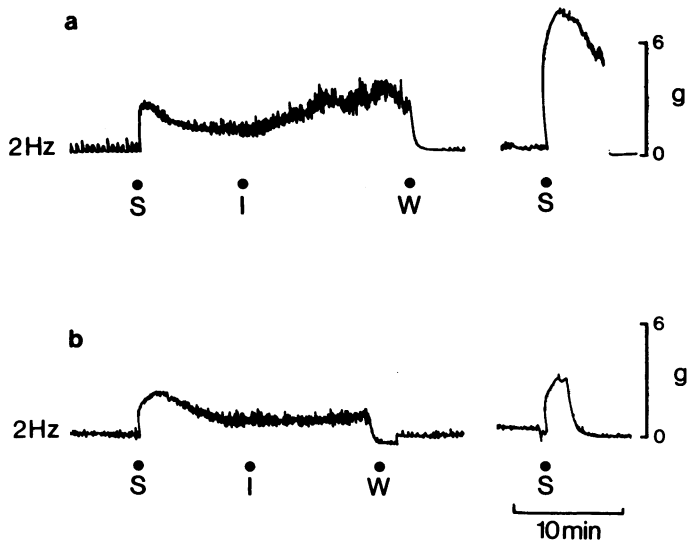

Fig. 3 The effect of α-methyl-*p*-tyrosine (α-MPT) on the potentiation by indomethacin of the response of the rabbit portal vein to continuous electrical stimulation. (a) Control, (b) response of a vein in the presence of α-MPT (100 μg/ml). Both veins stimulated continuously at 2 Hz (S) for 10 min then indomethacin (I) (10 μg/ml) was added to the bath. After 10-15 min the bath was washed (W) and electrical stimulation terminated. The vein was restimulated at 2 Hz, 30 min later (S).

responses (26.3 ± 10.5% and 44.2 ± 8.3%, respectively). However, when the frequencies were increased to 4 and 8 Hz prostaglandin E_2 only slightly reduced the responses with 82.5 ± 4.7% and 93.9 ± 1.7%, respectively, of the control response being obtained.

Six portal veins were exposed to cumulative concentrations of noradrenaline (0.01 to 10.0 μg/ml) in the absence and presence of prostaglandin E_2 (0.2 μg/ml). There was no effect of this concentration of prostaglandin E_2 on the responses of the portal veins to noradrenaline.

Table 3 The effect of indomethacin and eicosa-5,8,11,14-tetraynoic acid (ETYA) on the response of the rabbit portal vein to continuous electrical stimulation in the absence and presence of α-methyl-*p*-tyrosine (α-MPT)

	Tension (g)			
	Indomethacin (10 μg/ml)		*ETYA (10 μg/ml)*	
Treatment	*Response (n = 6)*	*Response in presence of α-MPT (100 μg/ml) (n = 6)*	*Response (n = 7)*	*Response in presence of α-MPT (100 μg/ml) (n = 5)*
Initial response to electrical stimulation at 2 Hz	+2.63 ± 0.47	+3.45 ± 0.22	+1.94 ± 0.25	+2.28 ± 0.16
Relaxation after 15 min of stimulation	−1.38 ± 0.31**	−1.57 ± 0.24**	−1.03 ± 0.16***	−1.26 ± 0.15**
Increase in response due to drug treatment	+1.03 ± 0.14***	+0.67 ± 0.34 NS	+2.67 ± 0.54**	+0.16 ± 0.08 NS
Increase from initial response 30 min after drug washed from bath	+1.88 ± 0.41**	+0.05 ± 0.18 NS	+2.10 ± 0.60*	0.0 ± 0.23 NS

Figures show mean with s.e. of mean. Significance of difference from controls (*t* test for paired data): * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; NS = not significant. *n* = number of veins.

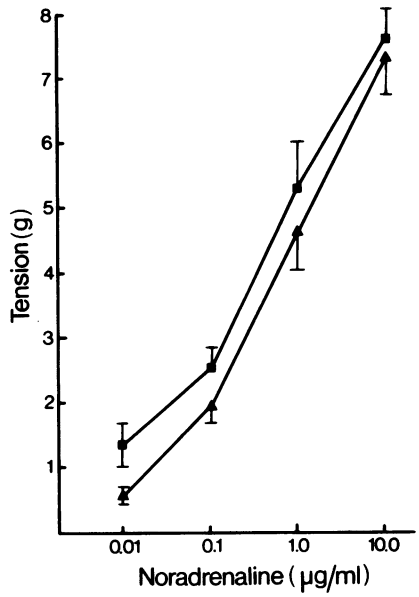

Fig. 4 The effect of indomethacin on the response of the rabbit portal vein to noradrenaline. Dose-response in the absence (▲) and presence (■) of indomethacin (10 μg/ml). Each point is the mean of the response of 16 veins. Vertical bars represent the s.e.

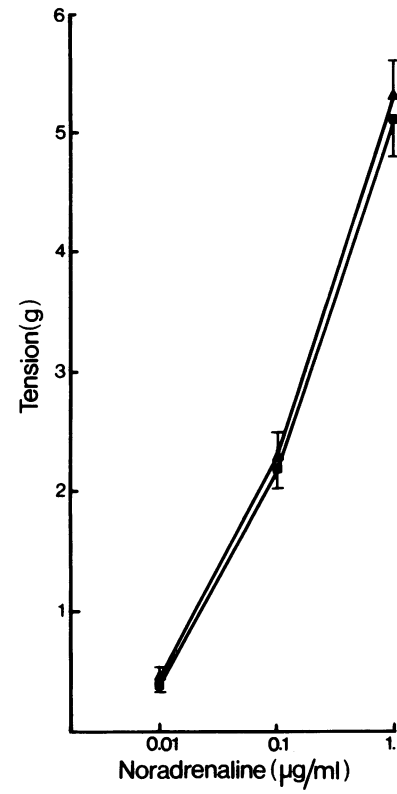

Fig. 5 The effect of eicosa-5,8,11,14-tetraynoic acid (ETYA) on the response of the rabbit portal vein to noradrenaline. Dose-response in the absence (▲) and presence (■) of ETYA (10 μg/ml). Each point is the mean of the response of five veins. Vertical bars represent the s.e.

Discussion

The results of this study show that indomethacin and ETYA cause a frequency dependent potentiation, while prostaglandin E_2 causes a frequency dependent inhibition of the response of the portal vein to electrical stimulation. Inhibition of noradrenaline synthesis with α-MPT abolished the potentiating effects of indomethacin and ETYA. These results suggest that prostaglandins may play a role in modulating the release of newly synthesized noradrenaline.

The responses of the portal vein to electrical stimulation were inhibited by prostaglandin E_2. The magnitude of this inhibition varied inversely with the frequency. The same concentration of prostaglandin E_2 had no effect on the response of the portal vein to exogenous noradrenaline. These results are in agreement with Baum & Shropshire (1971) and Hedqvist & von Euler (1972a) who showed that prostaglandins E_1 and E_2 caused a frequency dependent inhibition of the response to electrical stimulation in the guinea-pig isolated vas deferens. Frequency dependent inhibition of noradrenaline release is not unique for prostaglandins, as it has been shown that morphine causes a frequency dependent inhibition of noradrenaline release in response to nerve stimulation in the cat isolated nictitating membrane (Henderson, Hughes & Thompson, 1972).

Because the prostaglandins inhibit the release of noradrenaline and the subsequent response to sympathetic nerve stimulation, inhibition of their biosynthesis should facilitate release of noradrenaline and enhance the response to sympathetic nerve stimulation. Prostaglandin biosynthesis can be blocked either with ETYA (Ahern & Downing, 1970) or with indomethacin (Vane, 1971). ETYA has been shown to simultaneously decrease the efflux of prostaglandins and increase the outflow of noradrenaline in response to sympathetic nerve stimulation in the rabbit heart (Samuelsson & Wennmalm, 1971) and cat spleen (Hedqvist *et al.*, 1971). Similar results were obtained in the guinea-pig vas deferens where ETYA inhibited prostaglandin efflux and increased the response to

nerve stimulation (Swedin, 1971; Hedqvist & von Euler, 1972b). Subsequently, similar results have been obtained with indomethacin showing inhibition of prostaglandin release with a concomitant increase in noradrenaline outflow in rabbit heart (Chanh *et al.*, 1972) and guinea-pig vas deferens (Fredholm & Hedqvist, 1973).

The results of this study show that both ETYA and indomethacin potentiate the response of the portal vein to electrical stimulation. This potentiation is most probably due to facilitation of the release of noradrenaline and not due to an effect on its disposition, as the responses of the portal vein to exogenous noradrenaline were not altered by either indomethacin or ETYA. It has also been shown that neither ETYA nor indomethacin block noradrenaline uptake (Samuelsson & Wennmalm, 1971; Chanh *et al.*, 1972).

The results further show that the indomethacin- and ETYA-induced potentiations were dependent on the frequency of electrical stimulation. The responses of the portal vein to 1 and 2 Hz were potentiated whereas the responses to 4 and 8 Hz were not. These results are consistent with the frequency dependent inhibition of the response to electrical stimulation by prostaglandin E_2. Therefore, if endogenous prostaglandins are implicated in modulating the release of noradrenaline, it follows that blockade of their biosynthesis should cause a frequency dependent potentiation of the response to electrical stimulation.

Newly synthesized noradrenaline has been shown to play an important role in the maintenance of transmitter release during sympathetic nerve stimulation (Kopin *et al.*, 1968; Kupferman *et al.*, 1970; Gewirtz & Kopin, 1970; Stjärne & Wennmalm, 1970; Hughes & Roth, 1972; Kalsner, 1972). It has been suggested that the noradrenaline released is derived from a 'functional or available pool' located within the adrenergic nerve ending (Shore, 1972). It has been proposed that this available pool is replenished by reuptake of the released noradrenaline and newly synthesized noradrenaline (Bennett, 1973b). Recently, Hughes (1973) obtained results in the rabbit isolated vas deferens and portal vein indicating that noradrenaline may be released from different pools at different frequencies of stimulation.

In the experiments described here α-MPT abolished the indomethacin- and ETYA-induced potentiation of the responses of the portal vein to electrical stimulation at 1 and 2 Hz. α-MPT itself caused small but significant increases in the responses of the portal vein to these frequencies. α-MPT has been shown to be an effective inhibitor of noradrenaline synthesis (Udenfriend, Saltzman-Nirenberg & Nagatsu, 1965; Kupferman *et al.*, 1970). These results indicate, therefore, that the potentiation caused by indomethacin and ETYA may be due to an increased release of newly synthesized noradrenaline. They further indicate that the noradrenaline which is released in response to low frequencies of electrical stimulation in the portal vein is newly synthesized, and that endogenous prostaglandins control this release.

I wish to acknowledge the competent technical assistance of Mr G. Levesque.

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(Received December 10, 1973)