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EVIDENCE AGAINST A PHYSIOLOGICAL ROLE OF PROSTAGLANDINS IN THE REGULATION OF NORADRENALINE RELEASE IN THE CAT SPLEEN

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SUMMARY

1. The effects of prostaglandins E_2 (PGE_2) and indomethacin on responses and on noradrenaline overflow elicited by nerve stimulation were studied in the perfused cat's spleen, at different calcium concentrations in the perfusion medium: 0·26, 0·65 and 2·6 mM.

2. In the presence of 0·28 μM PGE_2 there was a significant reduction in responses to nerve stimulation and in the overflow of the transmitter. PGE_2 was more effective in reducing transmitter overflow at 5 than at 30 Hz.

3. Indomethacin, 14·0 μM, prevented the release of PGE-like material in the venous effluent of the spleen elicited by either nerve stimulation or by exogenous noradrenaline.

4. During exposure to 14·0 μM indomethacin there was no increase in responses to nerve stimulation or in the overflow of noradrenaline elicited by nerve stimulation at 5 or at 30 Hz.

5. Similar results to those obtained with exogenous PGE_2 and with indomethacin in the presence of 2·6 mM calcium, were observed when the experiments were carried out in the presence of either 0·65 or 0·26 mM calcium.

6. In the presence of the α-adrenoceptor blocking agents, phenoxybenzamine (2·9 μM) or phentolamine (3·1 μM), the increase in transmitter overflow obtained during stimulation was 6·5 and 8·3-fold respectively.

7. Since inhibition of the synthesis of PGE did not increase transmitter overflow during nerve stimulation, it appears that the proposed negative feed-back mechanism mediated by endogenous prostaglandins does not play an important physiological role in the regulation of adrenergic neurotransmission in the cat spleen. In this tissue the major endogenous negative feed-back regulatory mechanism is triggered by the neurotransmitter through the activation of prejunctional α-adrenoceptors.

INTRODUCTION

The prostaglandins of the E series inhibit noradrenaline release elicited by nerve stimulation in several organs of different species (Hedqvist, 1970*a*, 1973, 1974; Wennmalm, 1971; Stjärne, 1972, 1973*a*, *b*, *c*, *d*, *e*). In addition, in some of these tissues, it was reported that prostaglandins of the E series are released during periods of adrenergic nerve stimulation (Davies, Horton & Withrington, 1968; Gilmore, Vane & Wyllie, 1968; Hedqvist, Stjärne & Wennmalm, 1971, Samuelson & Wennmalm, 1971; Chanh, Junstad & Wennmalm, 1972; Ferreira, Moncada & Vane, 1973; Junstad & Wennmalm, 1973*a*). These results led to the hypothesis that during nerve stimulation prostaglandins released either from effector cells (Gilmore *et al.* 1968; Junstad & Wennmalm, 1973*b*) or from adrenergic nerve endings (Stjärne, 1972, 1973*b*) would lead to inhibition of noradrenaline release. Consequently, according to this hypothesis, the prostaglandins would mediate an endogenous feed-back inhibitory mechanism for noradrenaline release by nerve stimulation (Hedqvist, 1970*a*, *b*, 1973, 1974; Hedqvist, Stjärne & Wennmalm, 1970; Hedqvist *et al.* 1971; Samuelson & Wennmalm, 1971; Wennmalm, 1971; Chanh *et al.* 1972; Stjärne, 1972, 1973*a*, *b*, *c*; Junstad & Wennmalm, 1973*a*).

If a feed-back mechanism mediated by endogenous prostaglandins exists, an increase in noradrenaline release by nerve stimulation should be obtained when the synthesis of prostaglandins is inhibited by drugs. However, inhibition of prostaglandin synthesis by drugs results in rather small increases (Fredholm & Hedqvist, 1973; Starke & Montel, 1973; Stjärne, 1973*a*, *b*, *c*) or no increase (Hoszowska & Panczenko, 1974) in noradrenaline release during nerve stimulation.

In recent publications it was reported that a decrease in the availability of calcium for transmitter release induced by prostaglandins would in turn enhance the inhibitory effect of prostaglandins on neurotransmission (Stjärne, 1973*d*, *e*; Hedqvist, 1973). Consequently, in the present experiments we studied in the perfused cat spleen the effects on adrenergic neurotransmission of prostaglandin E_2 and of inhibition of prostaglandin synthesis by indomethacin at different calcium concentrations. These experiments were carried out at two frequencies of stimulation, 5 and 30 Hz.

METHODS

Cats of either sex (1·5–2·5 kg) were used. After induction of anaesthesia with ether, spinal preparations were set up as described by Burn (1952); this procedure includes destruction of the brain as well as artificial respiration. After evisceration, the spleen was isolated and the splenic artery and vein were cannulated. A pair of fine platinum electrodes was hooked around the splenic artery in order to stimulate

the post-ganglionic nerve fibres. The spleen was placed in a plethysmograph filled with liquid paraffin kept at 37° C. The tissue was perfused with Krebs solution at 37° C, at a constant volume, 7·5 ml./min. Changes in perfusion pressure, as a measure of the splenic vascular resistance, were recorded with a mercury manometer. The composition of the Krebs solution was (in millimolar concentrations): NaCl, 118·0; KCl, 4·7; $CaCl_2$, 2·6; $MgCl_2$, 1·2; NaH_2PO_4, 1·0; $NaHCO_3$, 25·0; glucose, 11·1; Na_2EDTA, 0·004 and ascorbic acid, 0·11. The solution was bubbled continuously with a mixture of 95% O_2 and 5% CO_2 and kept at 37° C. For some groups of experiments the calcium concentration in the Krebs solution was reduced to 0·65 or 0·26 mM without further changes in the composition of the solution. Stimulation was carried out with an S-44 Grass stimulator. Square pulses of 0·1 msec duration and of supramaximal voltage were applied.

One hour after the supramaximal voltage was determined the nerves were stimulated at 5 Hz for 60 sec (S_5) and 21 min later at 30 Hz for 10 sec (S_{30}). A total of 300 shocks was delivered during each period of stimulation. The first period of nerve stimulation (S_5) was applied 120 min after setting up the preparation and starting the perfusion of the spleen with Krebs solution. After these two control stimulation periods, indomethacin was added to the perfusion medium and remained present throughout the rest of the experiment. Twenty-one minutes after the beginning of the perfusion with indomethacin two periods of nerve stimulation at 5 Hz (S'_5) and at 30 Hz (S'_{30}) were repeated with the same time intervals as in the control period. In separate control experiments all four periods of stimulation were performed without the addition of drugs to the perfusion medium.

Similar experiments were carried out in the presence of prostaglandin E_2 (0·28 μM). Prostaglandin E_2 was added to the perfusion medium 11 min before the periods of nerve stimulation at 5 Hz (S'_5) or 30 Hz (S'_{30}) were applied. The experiments with indomethacin or prostaglandin E_2 were carried out in the presence of different calcium concentrations (2·6, 0·65 and 0·26 mM), in the perfusion medium. When either 0·65 or 0·26 mM calcium were employed the Krebs solution with the corresponding calcium concentration was perfused for 1 hr before the first period of nerve stimulation (S_5) and remained present throughout the remainder of the experiment.

In a different group of experiments all four stimulation periods were performed after the spleen was perfused for an additional 2 hr with Krebs solution, with the normal calcium concentration (2·6 mM). In these experiments the first period of nerve stimulation (S_5) was applied 240 min after starting the perfusion of the spleen with Krebs solution. Under these experimental conditions the effects of indomethacin on transmitter overflow elicited by nerve stimulation were determined.

Samples of the splenic effluent were collected at 1 min intervals before, during and after each period of nerve stimulation and until the noradrenaline levels returned to the prestimulation basal values.

In order to study the effect of different concentrations of indomethacin on the overflow of ^{3}H-total and endogenous noradrenaline induced by nerve stimulation, some spleens were labelled with [^{3}H]noradrenaline. In these experiments, 10 min after the supramaximal voltage was determined, an infusion of (−)-7-[^{3}H]noradrenaline (New England Nuclear, Boston, U.S.A., S.A. 4·24 Ci/m-mole) was carried out during 10 min at a rate of 6 μCi/min (total infused: 60 μCi). After the infusion of [^{3}H]noradrenaline was completed, the spleen was perfused for 80 min before the periods of nerve stimulation began.

A total of 300 shocks at a frequency of 5 Hz was delivered during each of the seven periods of stimulation which were applied every 28 min. In the control group all seven periods of stimulation were performed without the addition of

drugs to the perfusion medium. In a second group of experiments, after two control periods of nerve stimulation, increasing concentrations of indomethacin were added to the perfusion medium. Each concentration of indomethacin was perfused for 21 min before the next period of stimulation. Two 1 min samples of the splenic effluent were collected before the period of stimulation. Samples of 1 min were also collected during and after stimulation until the outflow of radioactivity returned to the pre-stimulation basal levels.

In these experiments the overflow induced by nerve stimulation was calculated by subtracting the spontaneous outflow assumed to have occurred in each sample during and after the period of nerve stimulation. The value of the spontaneous outflow subtracted from the stimulation and post-stimulation samples was the basal resting release obtained in the period immediately before stimulation. The total overflow of the labelled transmitter was expressed as a fraction of the total radioactivity remaining in the tissue: total ^3H released per shock divided by total ^3H remaining in the tissue at the onset of stimulation (Langer & Enero, 1974). The total radioactivity remaining in the tissue at the onset of each period of nerve stimulation was determined in each experiment, by adding to the radioactivity remaining in the tissue at the end of the experiment, the radioactivity collected in all the samples from that period of stimulation to the end of the sample collection.

The effects of two alpha-receptor blocking agents, phentolamine (3·1 μM) and phenoxybenzamine (2·9 μM) on ^3H-transmitter overflow induced by nerve stimulation were determined in separate experiments.

Chemical methods. The venous effluent from the perfused spleen was collected and chilled in graduated centrifuge tubes. They were centrifuged to remove the red blood cells at approximately $1000 \times g$ for 10 min and 1 ml. supernatant was assayed for total tritium by liquid scintillation spectrometry, in the experiments in which the spleen was labelled with [^3H]noradrenaline. Six millilitres supernatant were removed and adjusted to pH 2–3 by the addition of 0·25 ml. 1 N-HCl; 0·1 ml. 10 % Na_2 EDTA, and 0·1 ml. 12·5 % sodium sulphite were added and the samples were stored at 0° C until the separation and estimation of noradrenaline was carried out.

For determinations of endogenous noradrenaline and ^3H-total in the tissue at the end of each experiment, the spleen was blotted on filter paper, weighed and homogenized in 10 ml. cold 0·4 N perchloric acid per gram tissue, containing 1 mg Na_2 EDTA and 1·25 mg sodium sulphite per millilitre. The homogenate was kept at 4° C for 60 min and then centrifuged at approximately 1000 *g*.

For the separation of noradrenaline, alumina (Merck A.G.) was prepared according to Crout (1961) and washed with 0·5 M sodium acetate (pH 8·2) before drying. The columns used for chromatography were 0·5 cm diameter and stoppered with glass wool; 200 mg activated aluminium oxide were added to the columns. The samples were brought to pH 8·2 by the addition of tris buffer (0·5 M) pH 8·4 and then put on the alumina columns. The effluent and subsequent washings from the columns (1 ml. followed by 2 ml. bidistilled water) were discarded. After the washings, noradrenaline was eluted by the addition of two portions 1 ml. 0·2 N acetic acid. The fluorometric determination of noradrenaline was carried out using 1 ml. of this eluate according to Laverty & Taylor (1968). The recovery for added internal standards of noradrenaline was $86·7 \pm 1·9$ % ($n = 5$). The results were corrected for recovery.

Radioactivity was measured using a mixture of toluene, 600 ml.; Triton × 100, 300 ml.; absolute alcohol, 100 ml.; 2,5-diphenyloxazole (PPO), 5 g and 1,4-bis 2(5-phenyl-oxazolyl) benzene (POPOP), 0·1 g.

Dose–response curves to (−)-noradrenaline. The isolated cat spleen was set up in a plethysmograph and perfused with Krebs solution as described in the first section of Methods. One hour after the preparation was set up, a dose–response curve to (−)-noradrenaline was determined by injecting the drug through the arterial cannula. This was done by sequential injections of doses of (−)-noradrenaline which increased at each stage by a factor of about three. The time intervals between injections were long enough for full return of the perfusion pressure to pre-injection levels. When prostaglandin E_2 (0·28 μM) was used, it was added to the perfusion medium 10 min before beginning the dose–response curve to noradrenaline and remained present throughout.

The dose–response curves to (−)-noradrenaline in the control group and in the presence of prostaglandin E_2 were determined at two different calcium concentrations, 0·26 and 2·6 mM. In each case the spleen was perfused for 1 hr with the corresponding concentration of calcium before the dose–response curve to noradrenaline was determined.

Detection of prostaglandin-like material in the venous effluent of the spleen. In order to estimate the presence of prostaglandin-like material in the venous effluent from the perfused spleen, bio-assay studies were carried out on strips of fundus from the rat stomach. The strips were dissected as described by Vane (1957) and were fixed to the bottom of a 10 ml. organ bath. The upper end of the strip was connected to a force displacement transducer (Grass FT03) and the tension developed by the muscle was recorded with a Grass polygraph.

The resting tension of the muscle was repeatedly adjusted to 1·5 g and it reached a steady condition after 30–40 min. The rat stomach strip was made insensitive to acetylcholine, 5-hydroxytryptamine and catecholamines by exposure to Krebs containing a mixture of antagonists to give the following final micromolar concentrations: scopolamine hydrobromide, 0·26; methysergide maleate, 0·41; phenoxybenzamine hydrochloride, 2·9 and propranolol hydrochloride 33·0. This solution contained also indomethacin, 28·0 μM, in order to inhibit the release of prostaglandins from the stomach strips. The temperature of this modified Krebs solution was maintained at 37° C and the solution was bubbled with a 95 % O_2 and 5 % CO_2 mixture.

The isolated cat spleen was set up in a plethysmograph and perfused with Krebs solution as described in the first section of Methods. One hour after the preparation was set up and the supramaximal voltage was determined, a contraction of the spleen was induced every 30 min by alternating an injection of 3 μg (−)-noradrenaline through the arterial cannula with a period of nerve stimulation at 5 Hz during 60 sec.

Two minute samples of the splenic venous effluent were collected before, during and after the splenic contraction. These samples were centrifuged to remove the red blood cells at approximately 1000 *g* for 10 min and were then maintained at 37° C until they were assayed on rat stomach strips in order to determine the content in prostaglandin-like material. The tension developed by the fundal strips in response to the prostaglandin-like material was compared with the responses obtained by standard concentrations of prostaglandins E_1 and E_2.

Statistical calculations were performed according to conventional procedures (Snedecor & Cochran, 1967).

The following drugs were used: (−)-noradrenaline bitartrate monohydrate; indomethacin; prostaglandin E_1; prostaglandin E_2; phenoxybenzamine hydrochloride; (±)-propranolol hydrochloride; scopolamine hydrobromide; methysergide maleate and phentolamine hydrochloride. All concentrations and doses refer to the salt except for noradrenaline which is expressed as free base.

RESULTS

Effects of changes in the external calcium concentration on noradrenaline overflow and on responses to nerve stimulation in the perfused cat spleen

As shown in Fig. 1*A* the overflow of noradrenaline elicited by nerve stimulation was dependent on the calcium concentration in the perfusion medium when the lower frequency of stimulation employed, 5 Hz, was considered. When stimulation was applied at 30 Hz the small decrease in transmitter overflow observed as the calcium concentration was reduced was not statistically significant. When vascular responses to nerve stimulation were analysed it was found that in contrast to the

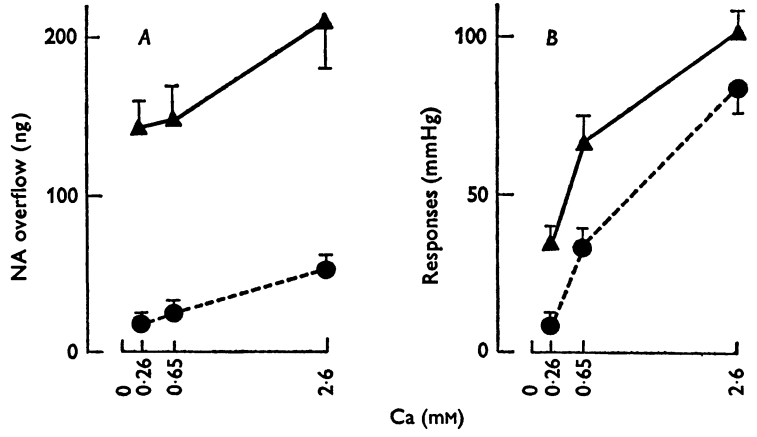

Fig. 1. Effect of the external calcium (Ca) concentration on noradrenaline (NA) overflow and on responses to nerve stimulation in the perfused cat spleen. Abscissa, millimolar calcium concentration in the perfusion medium. Ca, 0·26 mM (n = 15); Ca, 0·65 mM (n = 4); Ca, 2·6 mM (n = 35). Ordinate *A*, transmitter overflow elicited by nerve stimulation expressed in nanograms noradrenaline. Ordinate *B*, responses to nerve stimulation: increase in perfusion pressure, expressed in mmHg. ●——●, stimulation at 5 Hz for 60 sec (0·1 msec, supramaximal voltage). ▲——▲, stimulation at 30 Hz for 10 sec (0·1 msec, supramaximal voltage). n, number of experiments. Shown are mean values ± S.E. of the mean.

results obtained for transmitter overflow there was a significant reduction in responses to nerve stimulation when the external calcium concentration was decreased (Fig. 1*B*). The latter cannot be entirely accounted for by differences in transmitter overflow (Fig. 1*A*) and is most likely related to a decrease in post-junctional sensitivity to noradrenaline at the lower calcium concentration. As shown in Fig. 2, when the concentration of calcium in the perfusion medium was reduced from 2·6 to 0·26 mM

there was a parallel shift to the right in the dose–response curve to (−)-noradrenaline.

Effects of prostaglandin E_2 on responses to nerve stimulation and on noradrenaline overflow at different calcium concentrations in the perfusion medium

Control groups were studied for each of the three calcium concentrations employed. In these experiments, in which two consecutive periods of nerve stimulation were applied at either 5 or 30 Hz, there were no significant differences in the overflow of noradrenaline (Fig. 3) or in the

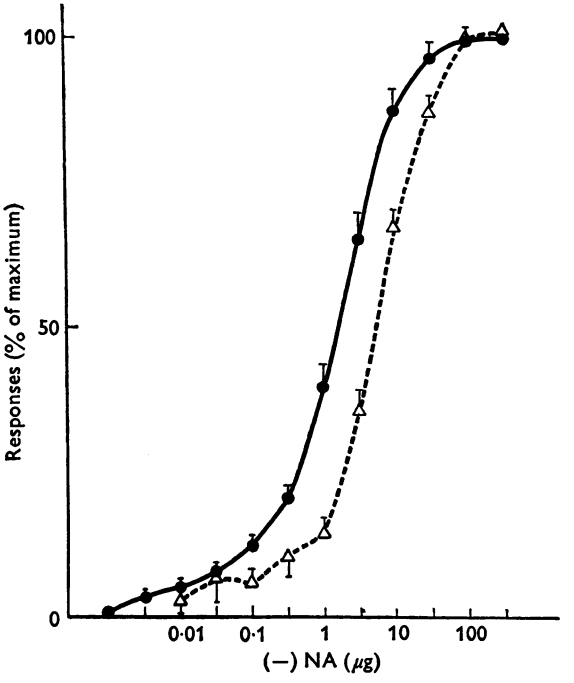

Fig. 2. Effect of the external calcium (Ca) concentration on dose–response curves to noradrenaline (NA) in the perfused cat spleen. Ordinate, increase in perfusion pressure (in mmHg) expressed as percentage of the maximum. Abscissa, dose of (−)-noradrenaline, expressed in μg. ●——● Ca, 2·6 mM (n = 26). △-----△ Ca, 0·26 mM (n = 4). n, number of experiments. Shown are mean values ± S.E. of the mean.

responses (Fig. 4) when the values obtained during the first and the second period of stimulation within the same preparation were compared.

Exposure to prostaglandin E_2 (PGE_2) 0·28 μM, significantly decreased transmitter overflow elicited by nerve stimulation at 5 Hz for the three

concentrations of calcium employed (Fig. 3). Yet, this concentration of PGE_2 did not reduce the overflow of noradrenaline when stimulation was carried out at 30 Hz in the presence of either 2·6 or 0·65 mM calcium. For the lowest calcium concentration employed, 0·26 mM, there was a small but statistically significant reduction in noradrenaline overflow at 30 Hz during exposure to 0·28 μM PGE_2.

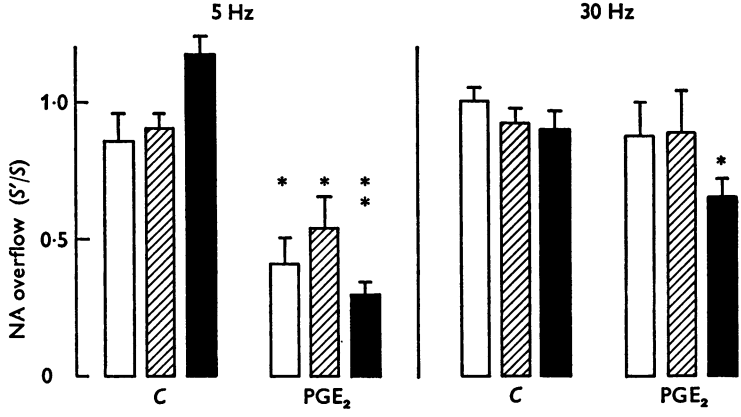

Fig. 3. Effects of prostaglandin E_2 (PGE_2) on noradrenaline (NA) overflow induced by nerve stimulation at different calcium concentrations in the perfusion medium. Ordinate, ratio of transmitter overflow (ng NA) between the second period of stimulation (S') and the corresponding control (S) for each frequency of stimulation. Left, stimulation at 5 Hz for 60 sec (0·1 msec, supramaximal voltage). Right, stimulation at 30 Hz for 10 sec (0·1 msec, supramaximal voltage). C, controls; PGE_2, 0·28 μM; Ca, calcium. □ Ca, 2·6 mM (C, $n = 5$; PGE_2, $n = 4$), ▨ Ca, 0·65 mM (C, $n = 4$; PGE_2, $n = 4$); ■ Ca, 0·26 mM (C, $n = 5$; PGE_2, $n = 6$). n, number of experiments. Shown are mean values ± S.E. of the mean. $*P < 0·05$; $**P < 0·001$ when compared with the corresponding control.

The reduction in vascular responses to nerve stimulation obtained in the presence of PGE_2 tended to be more pronounced as the calcium concentration in the perfusion medium was decreased (Fig. 4). The reduction in vascular responses was observed at both frequencies of nerve stimulation although the inhibitory effects of PGE_2 were more pronounced at 5 than at 30 Hz (Fig. 4).

The absolute values for noradrenaline overflow and for the vascular responses to nerve stimulation at the three concentrations of calcium studied, in the controls and in the presence of PGE_2 are shown in Table 1.

It should be noted that the reduction in vascular responses to nerve stimulation in the presence of 0·28 μM PGE_2 cannot be entirely attributed

to a decrease in transmitter output because as shown in Fig. 5*A* there was a shift to the right in the dose–response curve to exogenous noradrenaline during exposure to this concentration of PGE_2.

For the lowest concentration of calcium employed, 0·26 mM, PGE_2 did not reduce the vascular responses to exogenous noradrenaline (Fig. 5*B*) when compared with the corresponding control.

These results demonstrate that a decrease in responses to nerve stimulation during exposure to PGE_2 does not necessarily indicate a reduction in transmitter output, because it can be due to changes in sensitivity of the effector organ to the released neurotransmitter.

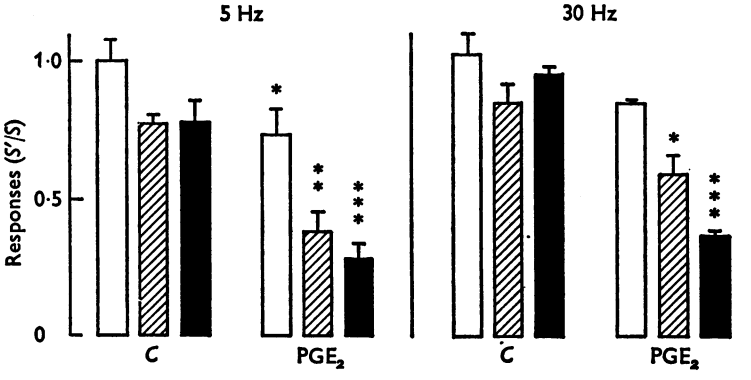

Fig. 4. Effects of prostaglandin E_2 (PGE_2) on responses to nerve stimulation at different calcium concentrations in the perfusion medium. Ordinate, ratio of responses (increase in perfusion pressure in mmHg) between the second period of stimulation (*S'*) and the corresponding control (*S*) for each frequency of stimulation. Left, stimulation at 5 Hz for 60 sec (0·1 msec, supramaximal voltage). Right, stimulation at 30 Hz for 10 sec (0·1 msec, supramaximal voltage). *C*, controls; PGE_2, 0·28 μM; Ca, calcium. □ Ca, 2·6 mM (*C*, $n = 5$; PGE_2, $n = 4$). ▨ Ca, 0·65 mM (*C*, $n = 5$; PGE_2, $n = 4$). ■ Ca, 0·26 mM (*C*, $n = 5$; PGE_2, $n = 6$). *n*, number of experiments. Shown are mean values ± S.E. of the mean. $*P < 0·05$; $**P < 0·01$; $***P < 0·001$ when compared with the corresponding control.

Effects of indomethacin on prostaglandin release elicited by nerve stimulation or by exogenous noradrenaline in the perfused spleen

Inhibition of prostaglandin synthesis by indomethacin has been extensively employed as a tool for the demonstration of the physiological significance played by endogenously formed prostaglandins. The validity of such experiments rests on the demonstration that the concentration of indomethacin employed effectively prevents prostaglandin release under the experimental conditions which are being studied. Therefore, the output of prostaglandins from the perfused spleen was determined

TABLE 1. Effects of prostaglandin E_2 and indomethacin on responses and on transmitter overflow elicited by nerve stimulation at different calcium concentrations in the perfusion medium

Calcium concentration (*a*)	Experimental group	*n*	Responses mmHg (*b*)		Overflow ng NA (*c*)	
			5 Hz	30 Hz	5 Hz	30 Hz
2·6 mM	Controls	35	83·1 ± 7·4	101·4 ± 6·6	53·2 ± 5·5	210·3 ± 29·7
	IND 14 μM	4	84·3 ± 27·8	102·3 ± 31·1	45·4 ± 18·9	173·6 ± 58·3
	PGE_2 0·28 μM	4	83·5 ± 13·9	103·5 ± 4·7	26·5 ± 5·6	207·7 ± 32·6
0·65 mM	Controls	14	33·8 ± 5·4	66·5 ± 7·7	25·5 ± 3·3	148·7 ± 21·1
	IND 14 μM	5	23·4 ± 3·5	57·0 ± 7·8	17·1 ± 5·2	151·7 ± 31·4
	PGE_2 0·28 μM	4	19·0 ± 6·8	52·0 ± 15·5	14·2 ± 5·6	136·1 ± 52·2
0·26 mM	Controls	15	8·1 ± 1·4	34·3 ± 4·2	18·8 ± 1·4	143·7 ± 17·7
	IND 14 μM	4	8·3 ± 2·8	40·0 ± 6·1	9·1 ± 1·5**	157·7 ± 25·1
	PGE_2 0·28 μM	6	2·0 ± 0·5*	15·7 ± 1·3*	5·1 ± 0·7***	77·6 ± 15·5*

(*a*) Calcium concentration in the perfusion medium. The spleens were perfused for 1 hr at the corresponding calcium concentration before nerve stimulation was applied.

(*b*) Increase in perfusion pressure in mmHg elicited by nerve stimulation.

(*c*) Total overflow of noradrenaline elicited by nerve stimulation; a total of 300 stimuli was delivered at each frequency of stimulation.

IND, indomethacin; PGE_2, prostaglandin E_2; NA, noradrenaline. *n*, number of experiments per group. Results are given as mean values ± S.E. of the mean.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ when compared against the corresponding control group.

under conditions of nerve stimulation or after the administration of exogenous noradrenaline. These experiments were carried out before and after the addition of indomethacin to the perfusion medium.

The results obtained in a typical experiment are shown in Fig. 6. The release of PGE-like material induced by exogenous noradrenaline or by nerve stimulation increased as a function of time during the whole experiment (Fig. 6). Usually our bio-assay procedure failed to detect PGE release in the venous effluent during the first periods of nerve stimulation or of exogenous noradrenaline. These negative results obtained at the beginning of the experiment could be due to the fact that PGE release was below the sensitivity of our bio-assay method or may represent a genuine lag period for PGE-induced release under these conditions.

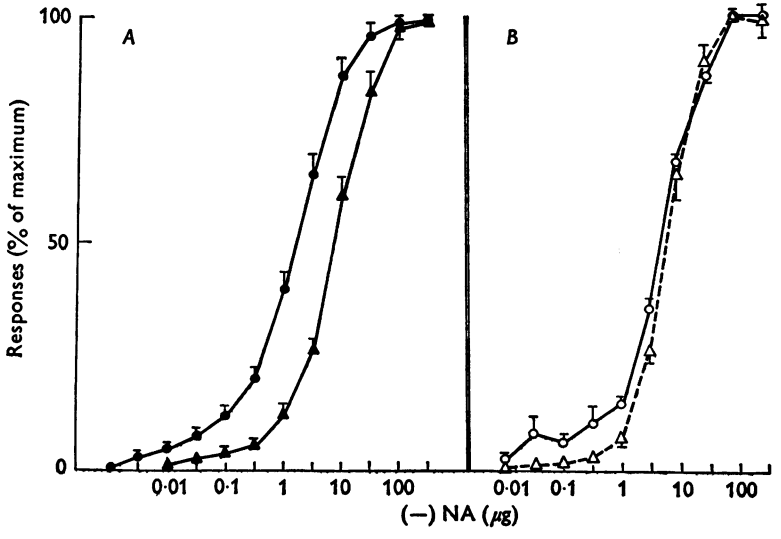

Fig. 5. Effects of prostaglandin E_2 (PGE_2) on dose–response curves to noradrenaline (NA) at different calcium (Ca) concentrations in the perfusion medium. Ordinate, increase in perfusion pressure (in mmHg) expressed as percentage of the maximum. Abscissa, dose of (−)-noradrenaline, expressed in μg. *A*, Ca, 2·6 mM; ●——●, controls (n = 26); ▲——▲, PGE_2 0·28 μM (n = 4). *B*, Ca, 0·26 mM; ○——○, controls (n = 4); △----△, PGE_2 0·28 μM (n = 4), n, number of experiments. Shown are mean values ± S.E. of the mean.

In most experiments PGE release was detected only after 3 hr of perfusion of the spleen. Thereafter a progressive rise in PGE release induced by exogenous noradrenaline or by nerve stimulation was observed as a function of time.

Perfusion with indomethacin 14 μM prevented entirely the release of PGE by either exogenous noradrenaline or by nerve stimulation (Fig. 6).

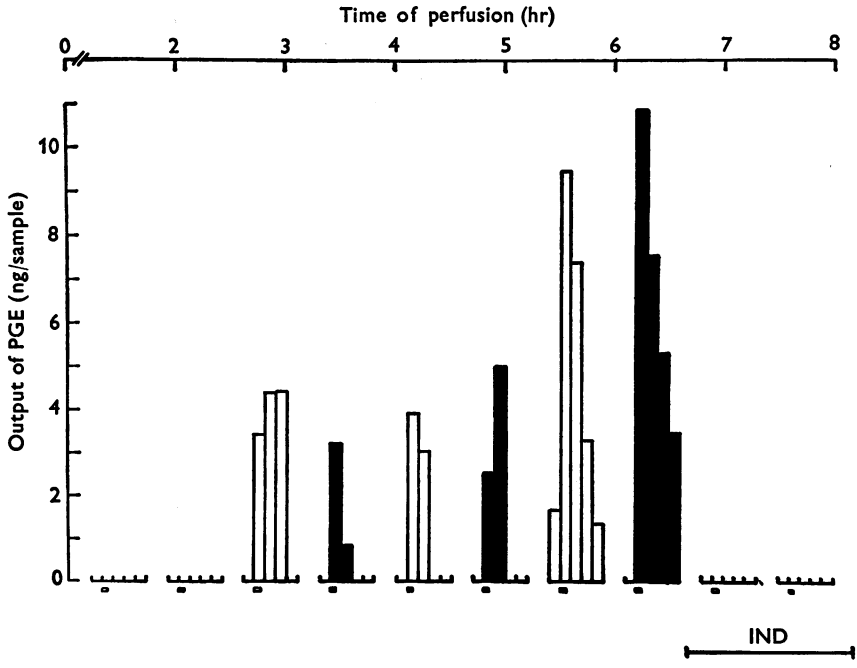

Fig. 6. Effects of indomethacin (IND) on prostaglandin (PGE) release elicited by nerve stimulation or by exogenous noradrenaline (NA) in the perfused spleen. Ordinate, release of PGE-like material expressed in ng PGE in each 2 min sample of the venous effluent. The open bars represent the release of PGE-like material from the perfused spleen elicited by exogenous NA, in each 2 min sample. The open rectangle indicates the injection of 3 μg (−)NA. The filled bars represent the release of PGE-like material from the perfused spleen elicited by nerve stimulation in each 2 min sample. The filled rectangle indicates the period of nerve stimulation at 5 Hz for 60 sec (0·1 msec, supramaximal voltage). The period of perfusion of the spleen with 14 μM indomethacin (IND) is shown by the horizontal line below the abscissa. The time of perfusion of the spleen expressed in hr is shown by the horizontal upper line.

Effects of indomethacin on noradrenaline overflow and on responses to nerve stimulation at different calcium concentrations in the perfusion medium

In order to investigate the possibility that during nerve stimulation endogenously released PGE inhibits the release of the adrenergic transmitter, experiments were carried out before and after exposure to 14 μM indomethacin. This concentration of the drug prevented entirely the

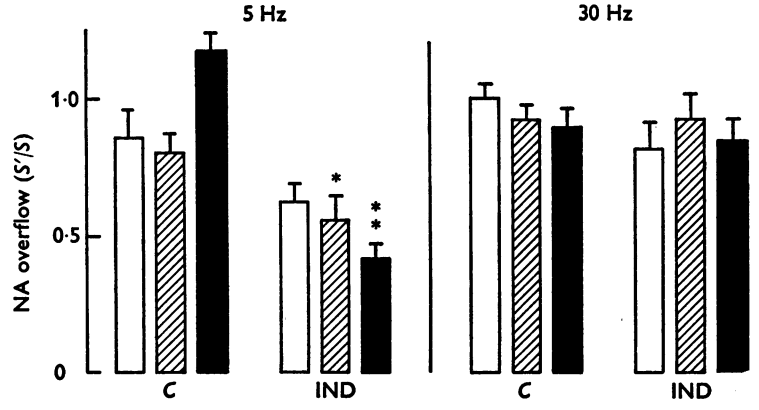

Fig. 7. Effects of indomethacin (IND) on noradrenaline (NA) overflow induced by nerve stimulation at different calcium concentrations in the perfusion medium. Ordinate, ratio of transmitter overflow (ng NA) between the second period of stimulation (S') and the corresponding control (S) for each frequency of stimulation. Left, stimulation at 5 Hz for 60 sec (0·1 msec, supramaximal voltage). Right, stimulation at 30 Hz for 10 sec (0·1 msec, supramaximal voltage), C, controls; IND, 14 μM; Ca, calcium. □ Ca, 2·6 mM (C, $n = 5$; IND, $n = 4$). ▨ Ca, 0·65 mM (C, $n = 4$; IND, $n = 5$). ■ Ca, 0·26 mM (C, $n = 5$; IND, $n = 4$). n, number of experiments. Shown are mean values ± S.E. of the mean. $*P < 0{\cdot}05$; $**P < 0{\cdot}001$ when compared with the corresponding control.

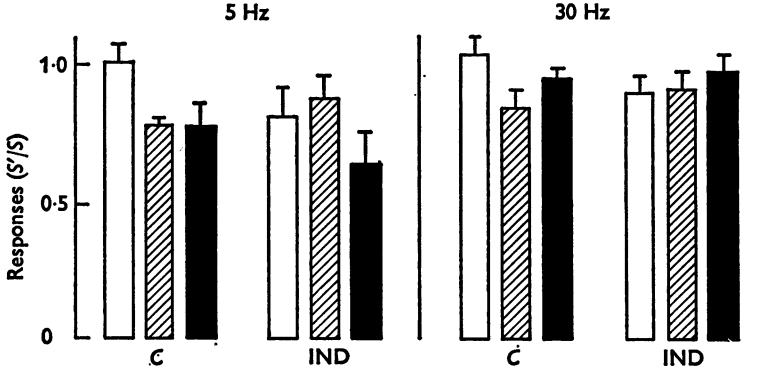

Fig. 8. Effects of indomethacin (IND) on responses to nerve stimulation at different calcium concentrations in the perfusion medium. Ordinate, ratio of responses (increase in perfusion pressure in mm Hg) between the second period of stimulation (S') and the corresponding control (S) for each frequency of stimulation. Left, stimulation at 5 Hz for 60 sec (0·1 msec, supramaximal voltage). Right, stimulation at 30 Hz for 10 sec (0·1 msec, supramaximal voltage). C, controls; IND, 14 μM; Ca, calcium. □ Ca, 2·6 mM ($C, n = 5$; IND, $n = 4$). ▨ Ca, 0·65 mM ($C, n = 4$; IND, $n = 5$). ■ Ca, 0·26 mM (C, $n = 5$; IND, $n = 4$). n, number of experiments. Shown are mean values ± S.E. of the mean.

release of PGE during nerve stimulation under our experimental conditions (Fig. 6).

As shown in Fig. 7, exposure to 14 μM indomethacin did not affect significantly the overflow of noradrenaline in response to nerve stimulation

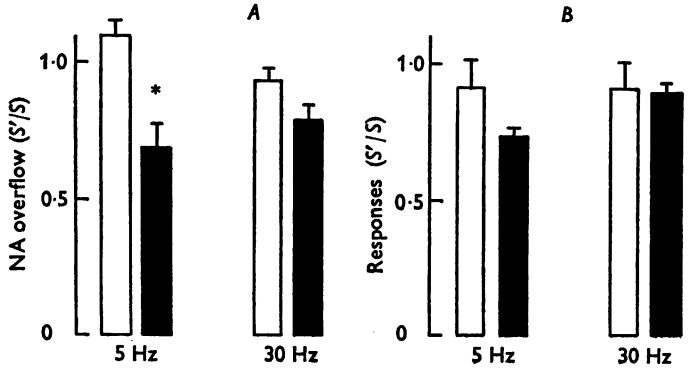

Fig. 9. Effects of indomethacin (IND) on noradrenaline (NA) overflow and on responses to nerve stimulation after 4 hr of perfusion of the spleen. Ordinates, *A*, ratio of transmitter overflow (ng NA) between the second period of stimulation (*S'*) and the corresponding control (*S*) for each frequency of stimulation. The nerves were stimulated at 5 Hz for 60 sec or at 30 Hz for 10 sec (0·1 msec, supramaximal voltage). □ *C*, controls (n = 4), ■ IND, indomethacin (14 μM) (n = 5). The calcium concentration was 2·6 mM. n, number of experiments. Shown are mean values ± S.E. of the mean. *P < 0·01 when compared with the corresponding control.

TABLE 2. Effects of indomethacin on responses and on transmitter overflow elicited by nerve stimulation after 4 hr perfusion

Experimental group (*a*)	n	Responses mmHg (*b*) 5 Hz	Responses mmHg (*b*) 30 Hz	Overflow ng NA (*c*) 5 Hz	Overflow ng NA (*c*) 30 Hz
Controls	9	61·6 ± 17·8	80·4 ± 17·7	77·9 ± 17·3	251·4 ± 45·1
IND 14 μM	5	67·0 ± 22·0	96·6 ± 23·5	66·0 ± 19·9	231·6 ± 36·9

(*a*) The spleens were perfused for 4 hr with Krebs solution (calcium concentration 2·6 mM) before beginning with the stimulation periods.

(*b*) Increase in perfusion pressure in mmHg elicited by nerve stimulation.

(*c*) Total overflow of noradrenaline elicited by nerve stimulation; a total of 300 stimuli was delivered at each frequency of stimulation.

IND, indomethacin; NA, noradrenaline; n, number of experiments per group. Results are given as mean values ± S.E. of the mean.

at either 5 or 30 Hz when the calcium concentration in the perfusion medium was 2·6 mM. For lower external calcium concentrations (0·65 or 0·26 mM) indomethacin induced a significant reduction in noradrenaline

overflow during nerve stimulation at 5 Hz (Fig. 7). There were no changes in the overflow of noradrenaline elicited by stimulation at 30 Hz during perfusion with indomethacin at external calcium concentrations of either 0·65 or 0·26 mM.

As shown in Fig. 8 the vascular responses to nerve stimulation at either 5 or 30 Hz were unaffected by exposure to indomethacin for the three concentrations of calcium employed.

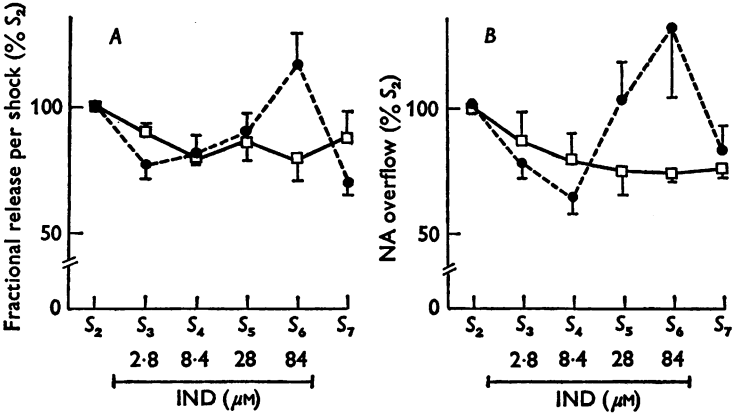

Fig. 10. Effects of different indomethacin (IND) concentrations on noradrenaline (NA) and on ^{3}H-total overflow induced by nerve stimulation in the perfused cat spleen. Ordinate *A*, fraction of the total radioactivity released per shock expressed in percentage of the second period of nerve stimulation within the same experiment. Ordinate *B*, transmitter overflow (ng NA) expressed in percentage of the second period of nerve stimulation within the same experiment. The nerves were stimulated at 5 Hz for 60 sec (0·1 msec, supramaximal voltage). Abscissa, periods of nerve stimulation (S_2 to S_7). □—□, controls (n = 4) S_2–S_7, ●--● IND, Indomethacin (n = 4) S_3, 2·8 μM; S_4, 8·4 μM; S_5, 28 μM; S_6. 84 μM, S_7, after wash-out of IND. n, number of experiments. Shown are mean values ± S.E. of the mean.

The absolute values for noradrenaline overflow and for the vascular responses to nerve stimulation obtained during exposure to 14 μM indomethacin are shown in Table 1.

The failure of inhibition of PGE synthesis by indomethacin to enhance noradrenaline overflow during nerve stimulation (Fig. 7) could be due to the fact that PGE release induced by sympathetic nerve stimulation reaches levels of significance only after the spleen has been perfused for approximately 3 hr (Fig. 6). In order to test this possibility experiments were carried out in which the spleen was perfused for 4 hr before the periods of nerve stimulation were started. In these experiments the

TABLE 3. Effects of repeated periods of nerve stimulation on the responses and on the overflow of ^{3}H-total and of endogenous noradrenaline (NA) in the perfused cat spleen

	n	S_1	S_2	S_3	S_4	S_5	S_6	S_7
^{3}H-total $F.R. \times 10^{-5}$ (*a*)	4	8·06 ± 2·11	7·27 ± 1·97	6·40 ± 1·64	5·76 ± 1·54	5·92 ± 1·44	5·37 ± 1·32	5·82 ± 1·33
Noradrenaline (ng) (*b*)	3	89·6 ± 7·5	82·1 ± 6·0	70·8 ± 7·6	65·1 ± 10·4	61·0 ± 6·9	61·2 ± 7·2	62·6 ± 6·2
Responses (mmHg) (*c*)	4	59·8 ± 19·6	60·5 ± 19·9	57·5 ± 19·5	56·5 ± 18·5	56·5 ± 15·4	54·8 ± 15·1	53·8 ± 13·9

(*a*) Total radioactivity released by nerve stimulation expressed as fractional release per shock ($\times 10^{-5}$). For details see Methods.
(*b*) Total overflow of NA elicited by nerve stimulation expressed in nanograms.
(*c*) Increase in perfusion pressure in mmHg elicited by nerve stimulation.
The nerves were stimulated at 5 Hz for 60 sec (0·1 msec, supramaximal voltage) every 28 min (S_1 to S_7). Results are given as mean values ± S.E. of the mean. *F.R.*, fractional release per shock. *n*, number of experiments.

TABLE 4. Effects of several concentrations of indomethacin on the responses and on the overflow of ^{3}H-total and of endogenous noradrenaline (NA) in the perfused cat spleen

		S_1	S_2	S_3	S_4	S_5	S_6	S_7
	n	Control	Control	IND, 2·8 μM	IND, 8·4 μM	IND, 28 μM	IND, 84 μM	Control
^{3}H-total $F.R. \times 10^{-5}$ (*a*)	4	7·13 ± 1·29	6·18 ± 1·28	4·82 ± 1·03	4·99 ± 1·23	5·90 ± 1·49	7·52 ± 2·09	4·29 ± 0·87
Noradrenaline (ng) (*b*)	4	62·2 ± 10·0	53·6 ± 9·6	38·3 ± 3·9	32·3 ± 3·7	49·4 ± 6·3	62·1 ± 10·5	40·5 ± 5·5
Responses (mmHg)	4	96·7 ± 21·2	105·0 ± 24·5	101·5 ± 28·1	99·5 ± 28·2	83·3 ± 22·9	53·3 ± 14·5	94·0 ± 33·0

(*a*) Total radioactivity released by nerve stimulation expressed in fractional release per shock ($\times 10^{-5}$). For details see Methods.
(*b*) Total overflow of NA elicited by nerve stimulation, expressed in nanograms.
(*c*) Increase in perfusion pressure in mmHg elicited by nerve stimulation.
The nerves were stimulated at 5 Hz for 60 sec (0·1 msec, supramaximal voltage) every 28 min (S_1 to S_7). IND, indomethacin. *F.R.*, fractional release per shock. *n*, number of experiments per group. Results are given as mean values ± S.E. of the mean.

effects of indomethacin on neurotransmission were tested. As shown in Fig. 9*A*, *B* exposure to 14 μM indomethacin failed to increase the overflow of noradrenaline or the responses to nerve stimulation even when the spleen was perfused for 4 hr before the periods of stimulation were started. In fact, the overflow of noradrenaline elicited by stimulation at 5 Hz in the presence of indomethacin was significantly reduced when compared with the corresponding controls (Fig. 9*A*).

TABLE 5. Effects of indomethacin, phentolamine and phenoxybenzamine, on ^{3}H-transmitter overflow elicited by nerve stimulation in the perfused cat spleen

Experimental group	n	Fractional release per shock: S_1 ($\times 10^{-5}$) (*a*)	S_2 ($\times 10^{-5}$) (*b*)	S_2/S_1 (*c*)
Controls	4	7·27 ± 1·97	5·92 ± 1·44	0·86 ± 0·07
IND, 28·0 μM	5	6·71 ± 1·12	6·05 ± 1·17	0·90 ± 0·07
PHENT, 3·1 μM	3	9·03 ± 0·65	73·67 ± 7·31**	8·28 ± 1·09**
PBA, 2·9 μM	4	8·80 ± 1·14	55·89 ± 6·75**	6·53 ± 0·96*

(*a*) Fractional release per shock during the first control period of nerve stimulation (S_1) at 5 Hz for 60 sec (0·1 msec, supramaximal voltage).

(*b*) Fractional release per shock during the second period of nerve stimulation (S_2) obtained in the presence of the drug.

(*c*) Ratio of fractional release per shock obtained between the second period of nerve stimulation (S_2) and the corresponding control (S_1).

IND, indomethacin; PHENT, phentolamine; PBA, phenoxybenzamine IND, PHENT or PBA were added 21 min before S_2. Shown are mean values ± S.E. of the mean n, number of experiments per group.

* $P < 0{\cdot}005$; ** $P < 0{\cdot}001$ when compared with the corresponding controls.

Table 2 shows the absolute values of noradrenaline overflow and of responses to nerve stimulation obtained in the group in which the effects of indomethacin were tested after the spleen had been perfused for 4 hr.

Effects of different concentrations of indomethacin on the overflow of noradrenaline, total tritium and on responses elicited by nerve stimulation

The failure of indomethacin to increase noradrenaline overflow elicited by stimulation could be ascribed to a direct effect of the drug whereby a reduction in transmitter output is obtained independently of the ability of indomethacin to inhibit PGE synthesis. In order to explore this possibility, several concentrations of indomethacin were studied to determine whether the drug induces inhibition of transmitter output.

In these experiments the spleens were labelled with [^{3}H]noradrenaline and the overflow of endogenous noradrenaline was determined together with that of total tritium. In the control group the decline in overflow

of total ^3H and of endogenous noradrenaline as a result of seven consecutive periods of nerve stimulation was rather small when these values were expressed with reference to the second period of nerve stimulation (Fig. 10*A*, *B*).

Perfusion with indomethacin in the range of concentrations from 2·8 to 84·0 μM did not affect significantly the overflow of either total ^3H (Fig. 10*A*) or of endogenous noradrenaline (Fig. 10*B*). During exposure to 84 μM indomethacin there was a small increase in transmitter overflow which was readily reversed by washing (Fig. 10*A* and *B*). Yet this increase in overflow did not reach levels of significance as compared to the controls at S_6 and furthermore, it was obtained with a concentration of indomethacin six-times higher than that required to inhibit release of PGE-like material from the spleen during nerve stimulation.

The absolute values for the overflow of total ^3H, endogenous noradrenaline and for the vascular responses to nerve stimulation are shown in Table 3 for the controls and in Table 4 for the group exposed to the different concentrations of indomethacin. In the presence of indomethacin the responses to nerve stimulation were not significantly different from the controls.

Changes in transmitter overflow elicited by blockade of prejunctional α-adrenoceptors or by inhibition of prostaglandin synthesis

In order to compare the effectiveness of the α-prejunctional receptors and of endogenously formed prostaglandins in the regulation of noradrenaline release during nerve stimulation, experiments were carried out in the presence of phentolamine or phenoxybenzamine to block the prejunctional α-adrenoceptors and during exposure to indomethacin to inhibit prostaglandin synthesis.

As shown in Table 5 there was a pronounced increase in ^3H-transmitter overflow in the presence of either phentolamine or phenoxybenzamine. In contrast to these results, during exposure to indomethacin 28 μM there were no significant changes in transmitter overflow.

DISCUSSION

The reduction in noradrenaline overflow elicited by nerve stimulation obtained in the presence of exogenous PGE_2 was only observed at low frequencies of nerve stimulation (5 Hz). This inhibitory effect was not obtained when the higher frequency of stimulation (30 Hz) was employed. The higher effectiveness of exogenous PGE_2 to inhibit transmitter release at low frequencies of nerve stimulation has already been reported (Hedqvist *et al.* 1970; Hedqvist, 1973).

In addition to the frequency-dependence of the inhibition of transmitter release by exogenous PGE_2 several authors have reported that the reduction in noradrenaline release during exposure to PGE_2 is more pronounced when the calcium concentration in the medium is decreased (Hedqvist, 1970*c*, 1973; Stjärne, 1973*d*, *e*). Yet, in our experiments at 5 Hz, inhibition of noradrenaline release by nerve stimulation in the presence of PGE_2 was not modified by decreasing the external calcium concentration. In contrast to these results, the reduction in responses to nerve stimulation during exposure to PGE_2 was more pronounced the lower the external calcium concentration. These results were observed at both 5 and 30 Hz.

This apparent dissociation between transmitter overflow and responses to nerve stimulation can be explained on the basis of post-junctional changes in the sensitivity to noradrenaline. Under our experimental conditions, perfusion with PGE_2 decreased the responses to exogenous noradrenaline. A similar decrease in responses to exogenous noradrenaline was obtained when the calcium concentration in the perfusing medium was reduced from 2·6 to 0·26 mM. Consequently, the reduction in responses to nerve stimulation as a result of the combined effects of exogenous PGE_2 and low external calcium concentration represented a mixture of prejunctional effects (i.e. decrease in transmitter output) and post-junctional effects (i.e. reduction in sensitivity of the effector organ to the neurotransmitter). Therefore, a reduction in responses to nerve stimulation does not necessarily imply inhibition of transmitter output. When studies on the effects of exogenous PGE on neurotransmission are based on responses to nerve stimulation the contribution of post-junctional changes in sensitivity to the neurotransmitter has to be taken into account (von Euler & Hedqvist, 1969; Hedqvist, 1972; Taylor & Einhorn, 1972; Illés, Hadházy, Torma, Vizi & Knoll, 1973).

The absence of relationship between the external calcium concentration and the inhibitory effects of PGE_2 on neurotransmission under our experimental conditions does not necessary reflect a conflict of evidence with other authors and it may be due to species or organ differences.

The greater effectiveness of PGE_2 in reducing transmitter output at 5 Hz when compared with 30 Hz was strikingly similar to the results obtained in the controls with low calcium concentrations. Under both experimental conditions the reduction in transmitter output was most pronounced for the lower frequency of stimulation. It is of interest that for this high frequency of nerve stimulation (30 Hz) blockade of presynaptic receptors by phenoxybenzamine or by phentolamine results in very small increases in transmitter overflow (Dubocovich & Langer, 1974; Dubocovich, M. L. & Langer, S. Z., unpublished observations)

when compared with the effect of alpha-receptor blockade at 5 Hz. Therefore, the frequency of stimulation at which the presence of a regulatory mechanism for transmitter release is investigated appears to be a very important parameter.

The association between the inhibition of noradrenaline release by stimulation induced by PGE and the possible role of these substances in an endogenously mediated feed-back mechanism is based on the finding that PGE is released into the venous effluent of the perfused spleen during periods of nerve stimulation (Davies *et al.* 1968; Gilmore *et al.* 1968; Ferreira *et al.* 1973; Hoszowska & Panczenko, 1974). Under our experimental conditions both nerve stimulation and exogenous noradrenaline elicited PGE release from the perfused spleen. Yet, there were characteristics to this phenomenon that we consider of special interest: (*a*) we did not detect PGE release by either nerve stimulation or exogenous noradrenaline at the beginning of the experiment (i.e. during the first 2 hr of perfusion of the spleen) and (*b*) when both treatments elicited PGE release (i.e. after at least 3 hr of perfusion) there was a progressive increase in the release of PGE as a function of time. These two special features about the time course of PGE release from the perfused spleen raise the question as to whether the release of PGE elicited by nerve stimulation or exogenous noradrenaline represents a physiologically significant phenomenon. The increase in PGE production as a function of time was also observed in the perfused cat spleen by Ferreira *et al.* (1973). It is possible that the time lag observed indicates that an additional, time-dependent phenomenon is involved in the release of PGE from the spleen. The possibility of a gradual deterioration of the perfused spleen as a result of oedematous changes was suggested by Ferreira *et al.* (1973). It is known that tissue damage of various kinds leads to prostaglandin formation (Piper & Vane, 1971). If the latter were true, the physiological significance of PGE release elicited by nerve stimulation or by exogenous noradrenaline would require an extensive revision.

The concentration of indomethacin employed in our experiments effectively prevented the release of PGE which was observed coinciding with the periods of nerve stimulation or the administration of noradrenaline after the perfusion of the spleen was carried out for at least 2 hr. Consequently, the crucial test as to whether or not this concentration of indomethacin enhances transmitter overflow elicited by nerve stimulation should be considered as a valid one. The failure of this concentration of indomethacin to enhance noradrenaline overflow by nerve stimulation at the three concentrations of calcium which were employed does not support the view that endogenous PGE released by nerve stimulation is involved in an endogenous feed-back inhibitory mechanism that

regulates the release of the transmitter by nerve stimulation. It is of interest that the failure of indomethacin to enhance transmitter release by nerve stimulation was also observed when the experiments were carried out after 4 hr of perfusion of the spleen (i.e. at a time when PGE release was detected as a result of nerve stimulation under our experimental conditions).

In the presence of low external calcium concentrations exposure to indomethacin resulted in a significant reduction in transmitter overflow during nerve stimulation. This effect of indomethacin could be related to a direct depressant action of the drug on transmitter output which might be independent of the ability of indomethacin to inhibit PGE synthesis. However, when the effects of different concentrations of indomethacin were determined on transmitter overflow elicited by nerve stimulation it was found that this drug did not reduce transmitter overflow in a wide range of concentrations, when the calcium concentration in the perfusion medium was 2·6 mM.

It could be argued that the failure of indomethacin to enhance transmitter overflow under our experimental conditions was due to the fact that in most of our experiments we measured endogenous noradrenaline. The small increase in transmitter output which was observed when testing inhibitors of PGE synthesis has generally been determined for the labelled transmitter (Stjärne, 1972, 1973*a*, *b*, *c*; Fredholm & Hedqvist, 1973). Yet, when the overflow of the tritiated transmitter was determined in the presence of indomethacin under our experimental conditions the results obtained were essentially identical with those observed for endogenous noradrenaline.

It is of interest to note that when a different drug was employed to inhibit prostaglandin synthesis in the perfused cat spleen (ETA, 5, 8, 11, 14-eicosatetraenoic acid, 30 μM) essentially the same results were obtained: the overflow of noradrenaline during nerve stimulation was not increased (Dubocovich, M. L. & Langer, S. Z., unpublished observations). In addition, inhibition of PGE synthesis by meclophenamate in the perfused cat spleen does not enhance transmitter overflow during nerve stimulation (Hoszowska & Panczenko, 1974).

In the perfused kidney of the rabbit, indomethacin prevents the release of PGE-like material by electrical stimulation of the renal nerve fibres. Nevertheless, in the presence of indomethacin the release of catecholamines and the renal constriction in response to nerve stimulation was not affected (Needleman, Douglas, Jakschik, Stoecklein & Johnson, 1974).

In contrast to the failure of inhibition of PGE synthesis to enhance transmitter release during nerve stimulation, blockade of prejunctional α-adrenoceptors by phentolamine or phenoxybenzamine resulted in a

marked increase in transmitter overflow (Table 5). These results support the view that the negative feed-back regulatory mechanism for noradrenaline released by nerve stimulation which is mediated by prejunctional α-adrenoceptors plays a major physiological role in adrenergic neurotransmission (Langer, Adler, Enero & Stefano, 1971; Farnebo & Hamberger, 1971; Kirpekar & Puig, 1971; Enero, Langer, Rothlin & Stefano, 1972; Starke, 1972*a*; Enero & Langer, 1973; Langer, 1973, 1974; Rand, Story, Allen, Glover & McCulloch, 1973; Cubeddu, Barnes, Langer & Weiner, 1974; Dubocovich & Langer, 1974; Farah & Langer, 1974). According to this hypothesis the neurotransmitter released by nerve stimulation inhibits its own release through the activation of prejunctional α-adrenoceptors by triggering a negative feed-back mechanism which leads to a decrease in transmitter output.

In support of this regulatory mechanism it was reported that stimulation of these prejunctional sites by α-adrenoceptor agonists reduces transmitter release (Farnebo & Hamberger, 1971; Starke, 1971, 1972*b*; Langer, Enero, Adler-Graschinsky & Stefano, 1972; Kirpekar, Furchgott, Wakade & Prat, 1973; Langer, 1973; Starke, Montel, Gayle & Merker, 1974). On the other hand, blockade of these prejunctional α-adrenoceptors markedly increases the release of noradrenaline by nerve stimulation (Brown & Gillespie, 1957; Langer, 1970; Langer, Enero, Stefano & Rothlin, 1970; Farnebo & Hamberger, 1971; Kirpekar & Puig, 1971; Langer *et al.* 1971; Starke, Montel & Schumann, 1971; Enero *et al.* 1972; Dubocovich & Langer, 1974; Farah & Langer, 1974).

It is of interest to note that stimulation or block of prejunctional α-adrenoceptors by drugs lead to the expected effects on transmitter release in every tissue in which it is tested. Yet, the same does not seem to apply for the reduction in noradrenaline overflow obtained with exogenous PGE: in the cat nictitating membrane, neither PGE_1 nor PGE_2 in concentrations up to 84 μM reduced ^{3}H-transmitter release during nerve stimulation (Langer, Enero, Adler-Graschinsky, Dubocovich & Celuch, 1975). In addition, in the perfused cat spleen a reduction in transmitter overflow by PGE_1 was observed only in four out of ten experiments (Hedqvist & Brundin, 1969).

It is of interest to note that a possible link between prostaglandins of the E series and the prejunctional negative feed-back mechanism mediated by α-adrenoceptors has been excluded in experiments in which inhibition of the synthesis of prostaglandins by indomethacin or other drugs did not affect the increase in noradrenaline release induced by alpha-receptor blockade nor the inhibition of transmitter release obtained during exposure to alpha-receptor agonists (Starke & Montel, 1973, Stjärne, 1973*a*, *c*).

The present results do not support the view that prostaglandins of the E series are involved in an endogenous regulatory mechanism for noradrenaline released by nerve stimulation in the perfused cat spleen. In addition, it appears that even in the tissues in which exogenous PGE_1 or PGE_2 reduce transmitter release during nerve stimulation, this effect might be of a pharmacological nature instead of reflecting the presence of an endogenously mediated negative feed-back mechanism.

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