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PROSTAGLANDIN RELEASE FROM CAT AND DOG SPLEEN

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1 The output of prostaglandins from the spleens of cats and dogs was studied. Comparison is made between the results found in the two species.

2 The release of prostaglandins was investigated in isolated saline-perfused spleens and in incubates of spleen slices. Release in response to nerve stimulation, and exposure to adrenaline or noradrenaline was compared with resting release.

3 A resting release of prostaglandins was found in the dog but not in the cat spleen.

4 Whereas stimulated dog spleens released microgram quantities of prostaglandins E_2 and $F_{2\alpha}$, prostaglandin output from the cat spleen under similar conditions was undetectable or barely detectable.

5 The identity of the prostaglandins released from the dog spleen (prostaglandins E_2 and $F_{2\alpha}$) was confirmed by mass spectrometry.

6 The species difference in prostaglandin output from the spleen is discussed in relation to the hypothesis that endogenous prostaglandins modify the responses of this organ to nervous stimuli.

Introduction

The dog spleen was among the first of several organs and tissues shown to release prostaglandin-like material in response to autonomic nerve stimulation (Davies, Horton & Withrington, 1967; Ferreira & Vane, 1967). Others include heart (Wennmalm & Stjärne, 1971; Samuelsson & Wennmalm, 1971), kidney (Dunham & Zimmerman, 1970; Davis & Horton, 1972; McGiff, Crowshaw, Terragno, Malik & Lonigro, 1972), adipose tissue (Shaw & Ramwell, 1968), stomach (Bennett, Friedmann & Vane, 1967; Coceani, Pace-Asciak, Volta & Wolfe, 1967), and vas deferens (Hedqvist & von Euler, 1972). Since exogenous prostaglandins modify the responses of various organs to adrenergic nerve stimulation (see review by Brody & Kadowitz, 1974), it is possible that endogenous prostaglandins play a physiological role in the responses of such organs to autonomic control. In testing this hypothesis in any one organ, it is useful to establish whether it releases prostaglandins on stimulation of its autonomic nerve supply.

The cat spleen has been classed with the dog spleen as releasing 'large amounts of prostaglandin' (Hedqvist, 1970a) but there is evidence that the spleens of these two species are not comparable in their ability to release prostaglandins in response to nerve stimulation (Ferreira, Moncada & Vane, 1973; Peskar & Hertting, 1973; Hoszowska & Panczenko, 1974; cf. Davies, Horton & Withrington, 1968; Gilmore, Vane & Wyllie, 1968; Ferreira, Moncada & Vane, 1971). In this investigation, we have measured the quantities of prostaglandins released from cat and dog spleens, at rest and during sympathetic nerve stimulation or exposure to adrenaline or noradrenaline. The stimulated dog spleen released microgram quantities of both prostaglandin E_2 and $F_{2\alpha}$, whereas prostaglandin release from the cat spleen, measured under comparable conditions, was barely detectable.

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Methods

Saline-perfused cat and dog spleens

Eight cats and four dogs of either sex were anaesthetized with pentobarbitone sodium, 40 mg/kg i.v. (dogs) or i.p. (cats). The abdomen was opened, and the spleen was freed of all

vascular connections except for the splenic artery and vein in the splenic pedicle. These were carefully dissected free from surrounding connective tissue. Each animal was then given heparin (1000 i.u./kg i.v.) and polyethylene cannulae were inserted in the splenic artery and vein. Perfusion was started at a rate of 10-15 ml/min (cat) or 15-30 ml/min (dog), with Krebs-Henseleit solution containing 0.4 to 2% dextran (MW 70,000), maintained at 37° C and aerated with 5% CO_2 in O_2.

The spleen was transferred to a perfusion chamber and stimulating electrodes were placed in contact with the splenic nerve plexus surrounding the splenic artery. Changes in spleen volume were detected as changes in weight of the whole perfusion chamber using a Grass force-displacement transducer. Perfusion pressure was monitored by a Statham pressure transducer (P.23 Dc).

Nerve stimulation was carried out at 10-40 V, at frequencies of 10-20 Hz and a pulse duration of 2 ms. Solutions of adrenaline or noradrenaline were infused into the arterial cannula at a rate of 1-2 μg/min, with a Braun infusion pump.

In most experiments, samples of perfusate were collected and centrifuged (500 *g* for 10 min at 4° C) to precipitate red blood cells. The supernatant was extracted for prostaglandins.

Incubated spleen slice experiments

Spleens from one cat and two dogs (killed by an overdose of pentobarbitone) were cut into slices 0.7 mm thick and distributed among incubation flasks containing 5 vol. Krebs-Henseleit solution. Adrenaline or noradrenaline was added to 'test' flasks in a final concentration of 0.2 mM. After incubation at 37° C for 1 h, the tissue was removed by filtration and the supernatant extracted for prostaglandins.

Extraction of prostaglandins

Samples of perfusate and incubation media were titrated to pH 4.5 with 1 N HCl, clarified by centrifugation if necessary, and extracted twice with 2 vol. of ethyl acetate. This extract was evaporated to dryness, and the residue taken up in 30 ml 67% ethanol. The ethanolic solution was washed twice with 15 ml of petroleum spirit (b.p. 40-60° C), then evaporated to dryness. Prostaglandins in this final extract were separated from each other and from accompanying impurities by column or thin layer chromatography.

Separation of prostaglandins by silicic acid column chromatography

The dry residue from the solvent extraction procedure was dissolved in 1 ml of 10% ethyl acetate in benzene and applied to columns of silicic acid (4.5 g Sigma Sil-R activated at 110° C for 1 h) 1 cm in diameter. Columns were eluted at a rate of approximately 1 ml/min with increasing concentrations of ethyl acetate in benzene, namely: 10% (40 ml), 40% (75 ml), 65% (130 ml + 20 ml), 80% (150 ml) and 100% (40 ml). Prostaglandins A (not detected in the present experiments) were eluted by 40% ethyl acetate, E by 65%, and F by 80%.

All eluates from the columns were evaporated to dryness and dissolved in 1 ml distilled water for bioassay.

Separation of prostaglandins and contaminating material by thin layer chromatography

Prostaglandins extracted from the incubation medium in the spleen slice experiments were separated by thin layer chromatography (TLC) on 0.25 mm layers of prepared neutral silica gel, using the 'G.C.M.' solvent system (Millar, 1974). This system separates prostaglandins A, E, and F from each other and from contaminating blood pigment which accompanies the prostaglandins through the extraction procedure. TLC was also used to replace column chromatography in two of the perfused spleen experiments: cat no. 5, and dog no. 2.

Bioassay of prostaglandins

Samples obtained from thin layer or column chromatography were bioassayed on the rat stomach strip. Prostaglandins E_1 and $F_{2\alpha}$ were used as bioassay standards in most experiments (prostaglandin E_1 being more readily available than E_2). However, after mass spectrometry had shown that the dog spleen released prostaglandin E_2 as opposed to E_1, prostaglandin E_2 was used as the assay standard (as indicated in the results from spleen slice experiments).

In some experiments, a portion of the venous effluent was assayed directly by superfusing it over a rat stomach strip. A peristaltic pump was used to deliver the effluent from the spleen (maintained at 37° C) to the tissue at a rate of 10 ml/minute. Calibrating doses of prostaglandin E_1 standards were infused into the effluent as appropriate. In these experiments, a solution of antagonist drugs, which was superfused over the tissue simultaneously, was used to increase the specificity of the assay. This solution contained, per litre of

Krebs solution: 2-bromolysergic acid diethylamide 2 mg, hyoscine hydrobromide 0.88 mg, mepyramine maleate 0.84 mg, phenoxybenzamine hydrochloride 6.7 mg and propranolol hydrochloride 6.8 mg. These concentrations had been found by previous experiment to render the tissue insensitive to 5-hydroxytryptamine, acetylcholine, histamine, adrenaline and noradrenaline, while the tissue's sensitivity to prostaglandins was retained.

Identification of released prostaglandins by combined gas chromatography–mass spectrometry

The techniques for preparing derivatives of prostaglandins and their separation and identification by combined gas chromatography–mass spectrometry have been described by Thompson, Los & Horton (1970). Prostaglandin E's were first converted to the corresponding B's by exposure to 0.1 N KOH in methanol for 1 h at room temperature. The solution was then diluted with water, acidified to pH 5 and extracted twice with ethyl acetate. The extract was evaporated to dryness and transferred (using small volumes of methanol) to Eppendorf tubes prior to methylation and formation of the trimethylsilyl ethers. The prostaglandin $F_{2\alpha}$ derivative used was also the trimethylsilyl ether of the methyl ester. Analyses were performed on an LKB 9000 gas chromatograph–mass spectrometer.

Results

Recoveries

The recoveries of authentic prostaglandins E_1 and $F_{2\alpha}$ after the extraction and column chromatographic procedures used in the majority of the perfusion experiments were 30% and 40% respectively (means of three determinations).

In two of the perfusion experiments, column chromatography was replaced by TLC. In one of these experiments (Table 1, cat no. 5), recoveries of prostaglandins E_1 and $F_{2\alpha}$ were monitored by the addition of trace amounts of tritium labelled compounds to the perfusate. Recoveries of the two prostaglandins were 50% and 53% respectively. In the second such experiment (dog no. 2) recoveries were not determined, but were assumed to be 50% for prostaglandin E and 60% for F, on the basis of recoveries found in other experiments where prostaglandins were separated by TLC.

In the spleen slice experiments, recoveries were determined using tritiated prostaglandins E_1 and $F_{2\alpha}$ which were added to the supernatant from each flask after the incubation. Mean recoveries from the three experiments were 50% for prostaglandin E_1 and 68% for $F_{2\alpha}$.

All the results which follow have been corrected for recovery.

Prostaglandin release from perfused spleens

Resting output The results are shown in Table 1. Perfusate from the resting spleen was collected immediately before the stimulation period (see below) and for the same duration. No prostaglandin was detected in the effluent from unstimulated cat spleens ($\ll 0.26$ ng g^{-1} min^{-1} of either prostaglandin E_1 or $F_{2\alpha}$). However, a small

Table 1 Prostaglandin release from saline-perfused cat and dog spleens, at rest and in response to splenic nerve stimulation

Species and experiment number	Weight of spleen (g)	Prostaglandin released* At rest PGE (as E_1)	At rest PGF (as $F_{2\alpha}$)	Stimulated PGE (as E_1)		Stimulated PGF (as $F_{2\alpha}$)		Nerve† stimulation
Cat No. 1	30	<0.16	<0.25	<0.14	*<0.46*	<0.13	*<0.42*	(a)
2	16	<0.06	<0.10	<0.06	*<0.22*	<0.10	*<0.32*	(a)
3	24	<0.11	<0.13	0.13	*0.43*	<0.13	*<0.43*	(a)
4	20	<0.07	<0.26	<0.07	*<0.30*	<0.26	*<1.20*	(b)
5	25	<0.24	<0.21	<0.20	*<0.68*	*<0.14*	*<0.48*	(c)
Dog No. 1	56	0.52	0.67	4.02	*13.40*	1.45	*4.83*	(d)
2	64	0.39	0.31	6.25	*20.80*	1.56	*5.20*	(e)

* Figures in italic type are output values expressed as nanograms of prostaglandin released per kilogram of spleen per nerve stimulus (ng kg^{-1} $stimulus^{-1}$). All other output values (figures not in italic type) are expressed as nanograms of prostaglandin released per gram of spleen per minute of collection period (ng g^{-1} min^{-1}).

† Nerve stimulation was carried out as follows: (a) 12,000 stimuli in 2 trains of 10 min each, (b) 8700 stimuli, in 8 trains of 1-2 min each, (c) 3000 stimuli in 1 train of 5 min, (d) 12,000 stimuli in 8 trains of 2-3 min each, (e) 6000 stimuli in 2 trains of 5 min each.

resting output (0.31-0.67 ng g^{-1} min^{-1}) of both prostaglandin E and F was detected in effluent from dog spleens.

Stimulated output

(a) *Nerve stimulation* Stimulation of the splenic nerves (carried out between 1 and 3 h after the start of perfusion) produced a rise in perfusion pressure and a contraction of the splenic capsule. The total 'stimulated' collection consisted of effluent collected during the period of stimulation and during an equal time thereafter. The results are shown in Table 1, where prostaglandin output is expressed both as ng g^{-1} min^{-1} (for comparison with control values in the same experiment) and as ng kg^{-1} $stimulus^{-1}$ (for comparison between experiments and species). Various patterns of nerve stimulation were tried as indicated

No prostaglandin F was detected in effluent from the five stimulated cat spleens. In only one cat experiment (cat no. 3) was a release of prostaglandin E detectable. In marked contrast, the stimulated dog spleens released microgram quantities of both prostaglandins E and F.

(b) *Noradrenaline stimulation* The effect of noradrenaline was investigated in two cat spleens and one dog spleen. Perfusate was collected for 40-55 min during which time the spleens were stimulated repeatedly by noradrenaline infusions (1-2 ng/minute). The noradrenaline evoked large contractile and pressor responses. No detectable prostaglandin was recovered from the perfusate in the first cat experiment. The second cat spleen released 0.13 ng g^{-1} min^{-1} prostaglandin E (as E_1) and 0.21 ng g^{-1} min^{-1} prostaglandin F (as $F_{2\alpha}$). A dog spleen similarly treated released 1.2 ng g^{-1} min^{-1} prostaglandin E (as E_1) and 0.65 ng g^{-1} min^{-1} prostaglandin F (as $F_{2\alpha}$).

(c) *Examination of effluent by superfusion over rat stomach strips* This was carried out in experiments with one dog spleen and three cat spleens. The results are shown in Figure 1. The dog spleen released prostaglandins on stimulation of the splenic nerves, evidently in microgram quantities as judged by the response of the assay tissue. Of the three cat spleens (all of which were perfused for at least 3 h) only one showed signs of prostaglandin release in response to stimulation. This occurred after 2-3.5 h perfusion when both adrenaline and nerve stimulation evoked the release of a substance that contracted the assay tissue. Calibrating infusions of prostaglandin E_1 indicated that the concentration of this substance in the effluent was equivalent to approximately 1 ng/ml prostaglandin E_1.

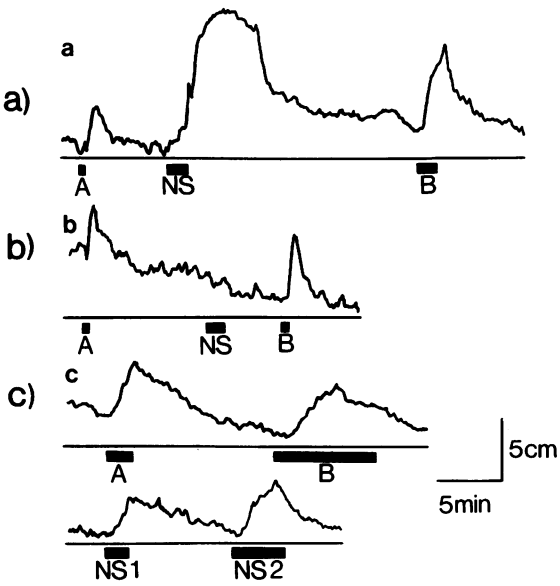

Figure 1 Traces of tension developed in rat stomach strips superfused with effluent from perfused cat and dog spleens. (a) Responses to effluent from a dog spleen, 1 h after the start of perfusion. At A, a calibrating dose of 50 ng prostaglandin E_1 was injected over 15 s into the venous effluent. At NS, the splenic nerves were stimulated at 20 Hz (40 V, 2 ms) for 1.5 minutes. A large contracture of the assay strip resulted. This was not matched by 650 ng prostaglandin E_1 injected (at B) into the venous effluent over 1.5 min (≡43 ng/ml). (b) Responses to effluent from a cat spleen, 1 h after the start of perfusion. The assay tissue responded to 20 ng prostaglandin E_1 infused over 20 s at (A) and (B), but nerve stimulation (NS, 20 Hz, 40 V, 2 ms) for 1.5 min failed to evoke the release of smooth muscle stimulating material. (c) Responses to effluent from another cat spleen. Upper trace—(2.25 h after the start of perfusion). At A, prostaglandin E_1 was infused into the venous effluent for 2 min to give a final concentration of 1 ng/ml. At B, adrenaline was infused into the splenic arterial cannula for 8 min to give a final concentration of 500 ng/ml. This caused the appearance in the venous effluent of a substance that contracted the stomach strip. Lower trace—(3.5 h after the start of perfusion). Nerve stimulation (10 Hz, 40 V, 2 ms) for 2 min (NS1), and 4 min (NS2) caused release of a substance that contracted the stomach strip.

(d) *Identification by GLC-MS of prostaglandins released by the dog spleen* The prostaglandin E- and F-like material isolated chromatographically from the dog spleen perfusate (dog no. 1) collected during the 'stimulated' period was analysed further by gas chromatography–mass spectrometry. Before the preparation of deriva-

tives, the prostaglandin E-like material was converted to prostaglandin B.

Authentic prostaglandin $F_{2\alpha}$ methyl ester/trimethylsilylether (Me/TMS) gave eleven major peaks of m/e values above 300, at 584 (M^+), 569 (M−15), 513 (M−71), 494 (M−90), 423 (M−(90 + 71)), 404 (M−(2 x 90)), 379 (M−(114 + 90 + 1)), 353 (M−141 + 90), 333 (M−(2 x 90 + 71)), 314 (M−(3 x 90)) and 307. The presence of these eleven peaks, in similar ratios in the mass spectrum of the experimental prostaglandin F-like material (Me/TMS) confirmed that this material was prostaglandin $F_{2\alpha}$. There was no indication of the presence of prostaglandin $F_{1\alpha}$.

Authentic prostaglandin B_2 Me/TMS gave 9 prominent peaks of m/e values above 200, at 420 (M^+), 405 (M−15), 389 (M−31), 349 (M−71), 330 (M−90), 321 (M-(71 + 28)), 279 (M−141), 247 (M−173), and 221 (M−199). The presence of these 9 peaks, in similar ratios, in the mass spectrum of the experimental prostaglandin E-like material (after conversion to prostaglandin B Me/TMS) confirmed that this material was prostaglandin E_2. There was no indication of the presence of prostaglandin E_1.

Prostaglandin release from incubated spleen slices

This was investigated in experiments with one cat and two dog spleens. The results are shown in Table 2. Adrenaline had no effect on prostaglandin release from cat spleen slices, but adrenaline and noradrenaline caused a large increase in prostaglandin E output, and a smaller increase in F output from dog spleen slices. Catecholamine-stimulated dog spleen tissue yielded approx. 18 times more prostaglandin E per gram than similarly treated cat spleen tissue. Incubation of dog spleen slices with tritiated prostaglandin E_1 and $F_{2\alpha}$ revealed no detectable metabolism of these compounds under the conditions of the above experiments.

Metabolism of prostaglandin E_1 by the perfused cat spleen

Tritium-labelled prostaglandin E_1 (1 μCi/100 μg) was infused into the inflow cannula of a perfused cat spleen, at a rate of 8 μg/min for 38 minutes. The spleen used was particularly large (110 g) and was thus comparable in mass to a moderately large dog spleen. Chromatographic analysis of the material present in the effluent collected during the infusion and for 1 h thereafter showed that 96.5% of the radioactivity co-chromatographed with standard prostaglandin E_1, and only 3.5% appeared as a less polar metabolite (not identified). Thus there was no evidence for efficient metabolism of this prostaglandin by the cat spleen.

Discussion

We have found that cat and dog spleens, subjected to the same treatment, show marked differences in their ability to release prostaglandins in response to nerve stimulation or catecholamines. This was apparent both in experiments where intact spleens were perfused with a saline solution, and in experiments involving the incubation of spleen slices.

The release of prostaglandins from cat and dog spleens has been investigated by other workers, but no direct comparisons between these species have been made hitherto. Ferreira *et al.* (1973) using the saline-perfused cat spleen detected no basal or stimulated release of prostaglandin during the first hour of perfusion. Thereafter, a basal release of up to 4 ng/ml (prostaglandin E_2 equivalents) was found. During nerve stimulation, prostaglandin release was less than 1 ng/ml in the first few trials, but increased to 10-30 ng/ml after perfusion for 2-3 hours. The authors discuss this increasing release of prostaglandin with time as a possible expression of the gradual deterioration of

Table 2 Prostaglandin release from incubated slices of cat and dog spleen, and the effect of adding adrenaline or noradrenaline

Species and experiment number	*Prostaglandin release (ng g tissue^{-1} h^{-1})*			
	without catecholamine		*with catecholamine*	
	PGE (as E_2)	*PGF (as $F_{2\alpha}$)*	*PGE (as E_2)*	*PGF (as $F_{2\alpha}$)*
			Adrenaline, 0.2 mM	
Cat	15	Traces (<10)	15	Traces (<10)
Dog No. 1	54	27	275	32
			Noradrenaline, 0.2 mM	
Dog No. 2	80	15	280	50

the isolated perfused spleen. We too found, in one experiment, prostaglandin release only after prolonged perfusion of the cat spleen, but we obtained no concentrations as high as those quoted by Ferreira *et al.* Our results agree more closely with those of Peskar & Hertting (1973), who found, using radioimmunoassay, a basal release of prostaglandin E from the cat spleen ranging from undetectable to not more than 0.1 ng/ml, even after 3 h of perfusion. These authors did not investigate the effect of nerve stimulation, adrenaline or noradrenaline on prostaglandin output. However, in a 'representative experiment' angiotensin produced a peak release of only 0.35 ng/ml prostaglandin E and 0.2 ng/ml prostaglandin F during a substantial splenic contraction and rise in perfusion pressure. Hoszowska & Panczenko (1974), in experiments of short duration with saline-perfused cat spleens, found no basal release of prostaglandins, but a release of 1.3 ± 0.1 ng/ml of prostaglandin-like material (measured by superfusion over a rat stomach strip) in response to nerve stimulation.

All the perfused dog spleens examined in the present experiments released microgram quantities of prostaglandins E and F on stimulation, in circumstances where the cat spleen had released undetectable or barely detectable quantities of these compounds. The identity of these prostaglandins, characterized previously only by behaviour on thin layer chromatography and parallel bioassay, has now been confirmed as prostaglandins E_2 and $F_{2\alpha}$ by mass spectrometry. The amounts of prostaglandins we detected in dog spleen perfusates are comparable to those found by other workers using similar techniques (Gilmore, Vane and Wyllie, 1968; Ferreira *et al.*, 1971).

The species difference found in the perfusion experiments was also evident in our incubated spleen slice experiments. Incubation with adrenaline or noradrenaline considerably augmented prostaglandin (particularly prostaglandin E) release from dog spleen slices, but adrenaline had no effect on prostaglandin release from cat spleen slices.

The release of prostaglandins from the spleen may reflect the net result of two processes: synthesis and metabolism. Our experiments do not show definitively which of these processes differs markedly between spleens from cats and dogs. The perfused cat spleen, however, did not appear to be very efficient at metabolizing exogenous prostaglandin E_1. It seems unlikely, therefore, that the large species difference in splenic prostaglandin output is due to a more rapid inactivation of endogenously synthesized prostaglandins in the cat.

The apparent inability of the cat spleen to synthesize prostaglandins in amounts comparable to those produced by the dog spleen might result from a deficiency of prostaglandin synthetase enzymes. Alternatively, it might reflect a paucity of fatty acid prostaglandin precursors, or of the phospholipases necessary for their mobilization prior to prostaglandin synthesis. Experiments with broken cell preparations would help to distinguish between these possibilities.

Cat and dog spleens differ not only in their ability to release endogenous prostaglandin, but also in their susceptibility to exogenous prostaglandins. The responses of the cat spleen to sympathetic nerve stimulation were modified in the presence of exogenous prostaglandins E and prostaglandin synthetase inhibitors (Hedqvist & Brundin, 1969; Hedqvist, 1969, 1970a, 1970b; Hedqvist, Stjärne & Wennmalm, 1971; Ferreira *et al.*, 1973). The responses of the dog spleen, on the other hand, were unaffected by prostaglandins E_1, E_2 or $F_{2\alpha}$ (Davies & Withrington, 1968, 1971). If endogenous prostaglandins play a physiological role in the sympathetic control of spleen function, as proposed by Hedqvist (1970a), it may be that the different degrees of sensitivity of the two species to prostaglandins are correlated with their relative abilities to produce these compounds.

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References

BENNETT, A., FRIEDMANN, C.A. & VANE, J.R. (1967). Release of prostaglandin E_1 from the rat stomach. *Nature, Lond.*, **216**, 873-876,

BRODY, M.J. & KADOWITZ, P.J. (1974). Prostaglandins as modulators of the autonomic nervous system. *Fedn. Proc.*, **33**, No. 1, 48-60.

COCEANI, F., PACE-ASCIAK, C., VOLTA, F. & WOLFE, L.S. (1967). Effect of nerve stimulation on prostaglandin formation and release from the rat stomach. *Am. J. Physiol.*, **213**, 1056-1064.

DAVIES, B.N., HORTON, E.W. & WITHRINGTON, P.G. (1967). The occurrence of prostaglandin E_2 in splenic venous blood of the dog following splenic nerve stimulation. *J. Physiol.*, **188**, 38-39P.

DAVIES, B.N., HORTON, E.W. & WITHRINGTON, P.G. (1968). The occurrence of prostaglandin E_2 in splenic venous blood of the dog following splenic nerve stimulation. *Br. J. Pharmac. Chemother.*, **32**, 127-135.

DAVIES, B.N. & WITHRINGTON, P.G. (1968). The effects of prostaglandins E_1 and E_2 on the smooth muscle of the dog spleen and on its responses to catecholamines, angiotensin and nerve stimulation. *Br. J. Pharmac. Chemother.*, **32**, 136-144.

DAVIES, B.N. & WITHRINGTON, P.G. (1971). Actions of prostaglandin $F_{2\alpha}$ on the splenic vascular and capsular smooth muscle in the dog. *Br. J. Pharmac.*, **41**, 1-7.

DAVIS, H.A. & HORTON, E.W. (1972). Output of prostaglandins from the rabbit kidney, its increase on renal nerve stimulation and its inhibition by indomethacin. *Br. J. Pharmac.*, **46**, 658-675.

DUNHAM, E.W. & ZIMMERMAN, B.G. (1970). Release of prostaglandin-like material from dog kidney during nerve stimulation. *Am. J. Physiol.*, **219**, 1279-1285.

FERREIRA, S.H., MONCADA, S. & VANE, J.R. (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nature, New Biol.*, **231**, 237-239.

FERREIRA, S.H., MONCADA, S. & VANE, J.R. (1973). Some effects of inhibiting endogenous prostaglandin formation on the responses of the cat spleen. *Br. J. Pharmac.*, **47**, 48-58.

FERREIRA, S.H. & VANE, J.R. (1967). Prostaglandins: their disappearance from and release into the circulation. *Nature, Lond.*, **216**, 868-873.

GILMORE, N., VANE, J.R. & WYLLIE, J.H. (1968). Prostaglandins released by the spleen. *Nature, Lond.*, **218**, 1135-1140.

HEDQVIST, P. (1969). Modulating effect of prostaglandin E_2 on noradrenaline release from the isolated cat spleen. *Acta physiol. scand.*, **75**, 511-512.

HEDQVIST, P. (1970a). Control by prostaglandin E_2 of sympathetic neurotransmission in the spleen. *Life Sci.*, **9**, pt. 1, 269-278.

HEDQVIST, P. (1970b). Antagonism by calcium of the inhibitory action of prostaglandin E_2 on sympathetic neurotransmission in the cat spleen. *Acta physiol. scand.*, **80**, 269-275.

HEDQVIST, P. & BRUNDIN, J. (1969). Inhibition by prostaglandin E_1 of noradrenaline release and of effector responses to nerve stimulation in the cat spleen. *Life Sci.*, **8**, pt. 1, 389-395.

HEDQVIST, P. & VON EULER, U.S. (1972). Prostaglandin controls neuromuscular transmission in the guinea-pig vas deferens. *Nature, New Biol.*, **236**, 113-115.

HEDQVIST, P., STJÄRNE, L. & WENNMALM, A. (1971). Facilitation of sympathetic neurotransmission in the cat spleen after inhibition of prostaglandin synthesis. *Acta physiol. scand.*, **83**, 430-432.

HOSZOWSKA, A. & PANCZENKO, B. (1974). Effects of inhibition of prostaglandin biosynthesis on noradrenaline release from isolated perfused spleen of the cat. *Pol. J. Pharmac. Pharm.*, **26**, 137-142.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A., MALIK, K.U. & LONIGRO, A.J. (1972). Differential effect of noradrenaline and renal nerve stimulation on vascular resistance in the dog kidney and the release of a prostaglandin E-like substance. *Clin. Sci.*, **42**, 223-233.

MILLAR, G.C. (1974). A solvent system for thin layer chromatographic separation of prostaglandins and blood pigment. *Prostaglandins*, **3**, 207-208.

PESKAR, B. & HERTTING. G. (1973). Release of prostaglandins from isolated cat spleen by angiotensin and vasopressin. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **279**, 227-234.

SAMUELSSON, B. & WENNMALM, A. (1971). Increased nerve stimulation induced release of noradrenaline from the rabbit heart after inhibition of prostaglandin synthesis. *Acta physiol. scand.*, **83**, 163-168.

SHAW, J.E. & RAMWELL, P.W. (1968). Release of prostaglandin from rat epididymal fat pad on nervous and hormonal stimulation. *J. biol. Chem.*, **243**, 1498-1503.

THOMPSON, C.J., LOS, M. & HORTON, E.W. (1970). The separation, identification and estimation of prostaglandins in nanogram quantities by combined gas chromatography–mass spectrometry. *Life Sci.*, **9**, pt. 1, 983-988.

WENNMALM, A. & STJÄRNE, L. (1971). Inhibition of the release of adrenergic transmitter by a fatty acid in the perfusate from sympathetically stimulated rabbit heart. *Life Sci.*, **10**, pt. 1, 471-479

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