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Prostaglandin E_2 and the bovine sphincter pupillae

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Summary

1. The bovine isolated sphincter pupillae incubated in Krebs solution releases a biologically active substance tentatively identified as prostaglandin E_2.

2. The prostaglandin did not appear to be of neural origin or to result merely from tissue degeneration.

3. The spontaneous release of prostaglandin E_2-like material was related to the tone of the sphincter. Output increased as tone was acquired after setting up the tissue and fell when various procedures were used to reduce the tone.

4. Low concentrations of E and F-type prostaglandins produced slow, well-sustained contractions of the atonic sphincter, prostaglandin E_2 being the most potent of those tested. The responses to prostaglandin E_2 were antagonized selectively by a prostaglandin antagonist SC-19220 (a dibenzoxazepine derivative) which in higher concentrations caused dose-dependent relaxations of the preparation.

5. Prostaglandins did not appear to modulate transmission from nerve to muscle in the sphincter.

6. The hypothesis that prostaglandin E_2 might be produced to act as a local hormone causing tonic contraction of the sphincter pupillae is discussed.

Introduction

Ambache (1955, 1957, 1959) extracted a pharmacologically active material from the rabbit iris which he called ' irin '. This was found to be a hydroxy unsaturated fatty acid (Ambache, 1957). Thin layer chromatography of irin from cat and rabbit indicated the presence of prostaglandins E_2 and $F_{2\alpha}$ (Ambache, Brummer, Rose & Whiting, 1966). Waitzman and co-workers also concluded that prostaglandins are present in the rabbit iris (Waitzman, Bailey & Kirby, 1967). Prostaglandin $F_{2\alpha}$ has been isolated from the sheep iris (Anggard & Samuelsson, 1964) and Van Dorp, Jouvenaz & Struijk (1967) demonstrated that pig iris contains the prostaglandin precursor arachidonic acid and can synthesize prostaglandin.

Some prostaglandins have potent biological activities in the eye. Ambache (1963) considered that irin mediated the atropine-resistant miosis and vasodilatation produced by antidromic stimulation of the trigeminal nerve (Bernard, 1858). Prostaglandins have been shown to produce miosis in the eyes of the cat, monkey and rabbit, and to elevate intraocular pressure in various species (Beitch & Eakins, 1969 ; Eakins, 1970 ; Waitzman & King, 1967). Vasodilator effects are also reported (Starr, 1971 ; Whitelocke & Eakins, 1973). Prostaglandins have been implicated in inflammatory conditions of the eye such as uveitis (Eakins, Whitelocke, Bennett & Martenet, 1972 ; Eakins, Whitelocke, Perkins, Bennett & Unger, 1972) and it is

possible they are involved in glaucoma (Wyllie & Wyllie, 1971). However, the possibility of a physiological role in the eye has not been given due consideration. The present work was designed to test the hypothesis that prostaglandins play a physiological role in the bovine iris. Preliminary reports of this work have already been published (Posner, 1970, 1971a).

Methods

Eyes were obtained from Friesian cows at a local abattoir immediately after killing and were taken to the laboratory in cold Krebs solution. They were stored in Krebs solution at 3 to 4° C for up to 48 h from the time of slaughter. The irides were removed immediately before use and a loop of tissue was dissected by a circular cut 3 to 4 mm from the pupillary margin. Each preparation thus consisted of the sphincter pupillae and some dilator muscle fibres

The sphincter loop was bathed in 10 ml Krebs solution at $37 \pm 0{\cdot}5$° C and gassed with a mixture of 95% O_2 and 5% CO_2. The preparation was suspended between two vertical platinum wires and, by means of a timing device, could be stimulated with trains of pulses from a square wave stimulator at timed intervals. Movements of the circular muscle were recorded on a kymograph by a frontal-writing lever (×7 to 8 magnification) with a load of 500 mg. The parameters of electrical stimulation for routine monitoring of the state of the tissue were 75V/cm, 0·3 ms pulse width, 5–80 Hz for 15 s at intervals of 5·5 minutes.

In experiments to investigate the release of biologically active material from the sphincter loop, recordings were made under isometric conditions. The preparation was attached to an Ether dynanometer UF1 under a tension of 450 to 500 mg and recordings were made on a Grass polygraph using a low level DC pre-amplifier.

The rat stomach strip (Vane, 1957) was used for the bioassay of material released from the sphincter loop. The preparation was suspended in 5 ml Krebs solution at 37° C and gassed with 5% CO_2 in O_2. Longitudinal muscles of the rat colon, chick rectum, rabbit duodenum and guinea-pig ileum, (segments approximately 20 mm in length suspended in 10 ml Krebs solution, gassed with 5% CO_2 in O_2, 37° C), were also used for identification of the active material.

Preparative thin-layer chromatography

The methods used were almost identical to those of Gréen & Samuelsson (1964). Clean glass plates were coated with silica gel G (0·3 mm thickness); when the AII solvent system was to be used the gel was prepared in a solution of 3% silver nitrate. Plates were dried and stored in a dessicator in the dark, and activated before use by heating in the dark for 30 min at 110° C.

Extracts and reference markers of prostaglandin E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ were applied with a Hamilton syringe. After chromatography one set of markers was visualized by development with 10% phosphomolybdic acid in ethanol and subsequent heating to 120° C for 10 minutes. The remainder of the gel containing chromatographed extracts and markers was divided into 1·5 cm squares. Each section was scraped into a test tube and eluted with methanol which was then evaporated to dryness *in vacuo*. The residue was dissolved in chloroform, transferred to another container, and the residue left after further evaporation was dissolved in 2·0 ml of water.

With silver nitrate impregnated plates, the silver was precipitated as the chloride from the aqueous solution by addition of solid sodium chloride. Following centrifugation the supernatant was decanted and assayed.

Paper chromatography of 5-hydroxytryptamine and related compounds

5-Hydroxytryptamine, tryptamine, 5-hydroxytryptophan and tryptophan (1 μg each) were applied in aqueous solution to Whatman No. 1 paper in volumes of 1 μl. Unextracted bath fluid collected from several preparations over a few hours was pooled and the volume reduced to less than 0·1 ml by freeze drying. Ascending chromatograms were run in butanol, acetic acid, water, (60:15:25) for 12 h at 21° C and developed with Ehrlich's reagent (1 vol. 10% w/v dimethylaminobenzaldehyde in concentrated HCl and 4 vol. acetone) or were cut into sections and eluted with 2·0 ml Krebs solution for subsequent bioassay.

Extraction of prostaglandins and other acidic lipids

The irides were dissected, dried on Whatman No. 1 paper, and weighed in groups of four. Analar acetone (2 ml/100 mg) was added to each group, which was placed on ice after 15 min and homogenized for 45 s with an Ultraturrax homogenizer. After a further 45 min the contents of each tube were centrifuged at 0° C for 60 min at 38,000 *g*. The supernatants were decanted and the acetone evaporated in a stream of O_2-free N_2 at 35° C. The residues were re-extracted similarly and the combined acetone extracts were evaporated to dryness. The residue was dissolved in water, acidified to pH 2 to 3 by addition to 0·1 N HCl, and partitioned three times against peroxide-free diethyl ether. The total volume of ether used was at least three times greater than that of the aqueous phase. The ether was evaporated to dryness in a stream of O_2-free N_2 and the residue was dissolved in 2 ml Krebs solution. Extracts were assayed in terms of prostaglandin E_2 on rat stomach strips by means of a matching assay correct to within 0·2 ng/ml bath fluid. Prostaglandins and other acidic lipids released into the bath fluid were extracted and assayed similarly.

Drugs, solvents and physiological solutions

The following drugs were used: acetylcholine chloride, atropine sulphate, bethanidine sulphate, bromolysergic acid diethylamide, carbachol, dimethyl-phenylpiperazinium iodide (DMPP), histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate, hyoscine hydrobromide, (−)-isoprenaline sulphate, lignocaine hydrochloride, mepyramine maleate, piperoxan hydrochloride, procaine hydrochloride, propranolol hydrochloride, prostaglandins E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, SC-19220 (1-acetyl-2-(8-chloro-10, 11 dihydrodibenz (b,f) (1,4) oxazepine-10-carbonyl hydrazine) and tetrodotoxin. The concentration of drugs, stated in terms of the base where applicable, are g/ml or molar, M.

Stock solutions of SC-19220 in ethanol (10^{-2}M) or propylene glycol (3×10^{-3}M) were diluted in Krebs solution before use. Stock solutions of prostaglandins were prepared by dissolving in ethanol (0·1 ml/mg prostaglandin) and diluting with sodium carbonate solution (0·2 mg/ml) to give a final concentration of 1 mg/ml. The pH was adjusted to about 7 with anhydrous sodium carbonate.

In all experiments Krebs solution of the following composition was used (mM): sodium chloride 118, potassium chloride 5, magnesium sulphate 1·2, potassium dihydrogen phosphate 1·2, sodium bicarbonate 25, calcium chloride 3·5, dextrose 11.

Results

Identification of a prostaglandin E_2-like substance in the fluid bathing the iris

The sphincter loop preparation set up in the organ bath under a load of 450 to 500 mg relaxed over the course of 5 to 10 min and then remained atonic for about 1 hour. It then began to contract slowly and after a further 45 to 60 min a steady level of tone was maintained. Longitudinal muscles of the rat stomach fundus and colon, chick rectum, rabbit duodenum, and guinea-pig ileum were contracted by fluid that had bathed the tonically contracted sphincter loop for a period of 1 hour.

Hyoscine, mepyramine or bromolysergic acid diethylamide (all 0·5 μg/ml) sometimes slightly reduced responses of the rat fundus or chick rectum to the bath fluid but a similar reduction of contractions to pure prostaglandin E_2 was also apparent. These concentrations virtually abolished responses to large doses of acetylcholine, histamine and 5-hydroxytryptamine respectively. The prostaglandin antagonist SC-19220 (1 to 10 μg/ml) (Sanner, 1969; Bennett & Posner, 1971) selectively inhibited responses of the rat fundus and guinea-pig ileum to prostaglandin E_2, $F_{2\alpha}$ and to the bath fluid (Figure 1).

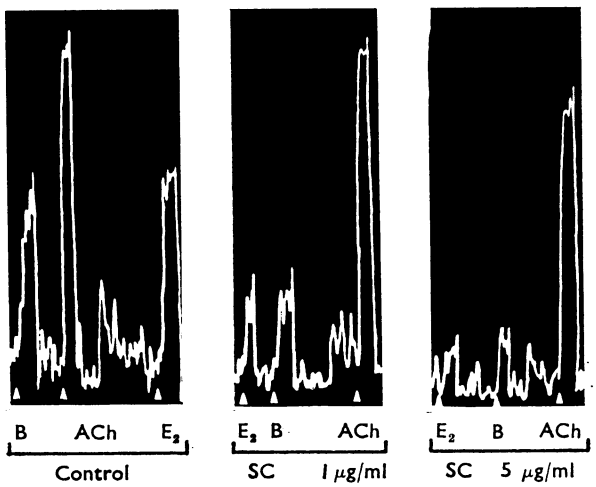

FIG. 1. SC-19220 inhibited responses of the rat stomach strip to prostaglandin E_2 (E_2) and an ethereal extract of the bath fluid (B) but not to acetylcholine (ACh).

On partition of the acidified bath fluid with ether the active material entered the organic phase and there was no detectable activity in the aqueous phase (limit of assay sensitivity=0·2 ng prostaglandin E_2/ml) after adjusting the pH to 7·4. Recovery estimated by similar partition of an acidified standard solution of prostaglandin E_2 was greater than 80%.

Preparative thin-layer chromatography on silica gel G in the AI ascending solvent system of Gréen & Samuelsson (1964) with the bioassay of eluates on rat stomach

strips indicated the presence of an E-type prostaglandin (R_F values of prostaglandin E and F=0·74 and 0·56 respectively, mean of 4 experiments). This finding was supported by the inhibitory effect of the eluates, the ethereal extracts of bath fluid and the unextracted fluid itself on the circular muscle of the guinea-pig colon; E-type prostaglandins relax this muscle whereas F-type compounds cause contractions (Fleshler & Bennett, 1969) (Figure 2).

FIG. 2. E-type prostaglandins and the fluid bathing the sphincter pupillae (B) caused relaxation of the circular muscle of guinea-pig colon; F-type compounds caused contraction.

Incubation with 0·1 N NaOH for 1 h at 38° C virtually abolished (90–100%) the effect on the rat fundus of standard solutions of prostaglandin E_1 or E_2 and caused a 65–70% reduction in the activity of extracted bath fluid. Incubation with 0·1 N HCl or 0·1 N NaCl caused no loss of activity and prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ were unaffected by any of these treatments. These results indicated the presence of an E-type prostaglandin and preparative thin-layer chromatography on silica gel G prepared with 3% w/v silver nitrate in the AII solvent system of Gréen & Samuelsson (1964) supported the conclusion that the predominant prostaglandin present was E_2 (R_F values of prostaglandin E_1=0·87, E_2=0·70, $F_{1\alpha}$=0·71, $F_{2\alpha}$=0·50; mean of 6 experiments). The possibility that some prostaglandin $F_{1\alpha}$ was present was not excluded.

It seemed unlikely that 5-hydroxytryptamine or related compounds contributed to the activity of the unextracted bath fluid or the ethereal extract. Paper chromatography supported this view since no 5-hydroxytryptamine, tryptamine, 5-hydroxy-

tryptophan or tryptophan were detected, in chromatograms of the bath fluid on development with Ehrlich's reagent or after elution and subsequent bioassay (limit of assay=1 ng 5-hydroxytryptamine).

The biological activity was unaltered by chymotrypsin 100 μg/ml at 38° C for 30 min indicating that a polypeptide was not contributing to the activity of the bath fluid.

The tissue content of the acidic lipids

Irides were extracted with acetone and the residue left after evaporation was dissolved in HCl and partitioned against ether. The final residue after evaporation of the ether was dissolved in Krebs solution and the acidic lipid content assayed in terms of prostaglandin E_2 was found to be 0·25 μg/g wet tissue after correction for recovery (about 45% of prostaglandin E_2). In view of the comparatively small quantities of biologically active material extracted a more precise identification was not undertaken.

The release of prostaglandin E_2-like material from the isolated sphincter

Resting release

Bath fluid collected during the first hour after setting up the atonic iris sphincter in Krebs solution did not affect the rat fundus preparation. The output of active material increased during the second hour when the preparation was acquiring tone. After the third hour it remained approximately constant for the following four to five hours although there was considerable variation in the rate of release between individual preparations. Periods of 60 min incubation were used and the release during the fourth hour was taken as the control value. There were a minimum of five irides in each experimental group.

The mean resting release of 29 tonically contracted preparations assayed in terms of prostaglandin E_2 on rat stomach strips was $0{\cdot}91 \pm 0{\cdot}09$ (μg/g)/h (mean $\pm$ S.E.). The output during a 30 min incubation period was almost exactly half of that in 1 h (30 min: 60 ± 7 ng; 60 min: 111 ± 7 ng; $n=6$) indicating that the accumulation of active material did not inhibit further release for at least one hour. Incubation of added prostaglandin E_2 (20 ng/ml) with the tissue indicated no significant uptake or metabolism of prostaglandin in the bath fluid over a period of one hour.

Tension and stretch. When the tissue was stretched by an increase in the applied load from 0·5 g during the control hour to 2 g for a further hour the output of active material was not affected (Figure 3).

Isoprenaline. The release of biologically active material in 1 h from the sphincter relaxed maximally with isoprenaline (2×10^{-8}M) was the same as that from the tonically contracted preparation.

Duration of Storage. There was no significant difference in the quantities of active material released in 1 h from irides stored for 2, 24 or 48 h (23 irides/group; Figure 3). Longer durations of storage were not tried.

A greatly diminished response of irides to transmural stimulation after storage of eyes for 48 h indicated that considerable nerve degeneration had occurred but the output of prostaglandin-like material was not significantly altered. It therefore

seemed unlikely that the material was neurogenic in origin. The effects of blockade and stimulation of the neuronal elements on the release of active material support this conclusion.

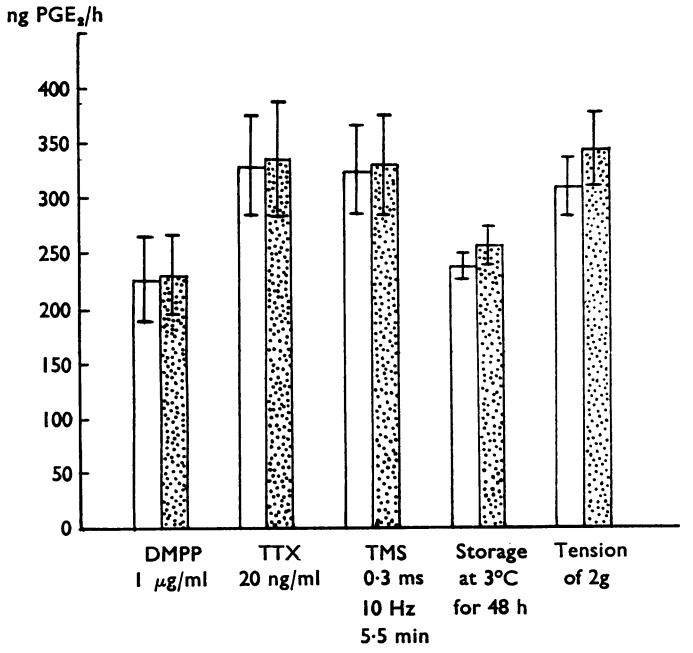

FIG. 3. A histogram to show conditions that did not significantly affect the output of active material (in terms of prostaglandin E_2) from the sphincter pupillae. TMS=transmural stimulation. Open columns=control output (500 mg load, stored up to 24 h). Stippled columns=output under treatment.

Tetrodotoxin and local anaesthetics. Tetrodotoxin 20 ng/ml abolished the response to transmural stimulation wihin 5 min but did not alter the output of active material (Figure 3). Similar results were obtained with procaine 200 μg/ml. Lignocaine caused the rat fundus to contract and could not be used.

Adrenergic neurone blockade. Bethanidine (1 μg/ml) selectively inhibited the adrenergically mediated relaxant component of the biphasic response to transmural electrical stimulation (Posner, 1969) but it did not affect the output of active material. Similar results were obtained with DMPP (1 μg/ml) which also possesses adrenergic neurone blocking activity (Birmingham & Wilson, 1965) (Figure 3).

Transmural stimulation 0·3 ms pulse width. At this pulse width only nervous elements appeared to be stimulated. Stimulation for periods of 15 s to 15 min did not affect the output of material from the sphincter (Figure 3).

The following experiments demonstrated dependence of release on metabolic factors, and an association between the rate of release and the muscle tone.

Temperature. Stepwise reductions in temperature caused corresponding reversible depressions of output (Table 1). The reduction to 20° C also caused a gradual increase in tone but the additional shortening of the muscle was less than 5% of the tonic shortening at 37° C. It therefore seems unlikely that a mechanical

TABLE 1. *Various procedures that reduce the release of active material from the iris sphincter.*

Procedure	% Control output	*n*	*P*
Temperature (°C)			
37	100		
31	45	6	0·1
25	27	6	0·05
20	19	6	0·001
DNP			
10^{-5}M	52	5	0·1
5×10^{-5}M	35	5	0·02
Anoxia	19	12	0·001
Calcium-free Krebs solution	68	8	0·05
Stimulation at 3*ms* 10 Hz			
Duration of stimulation in 1 hour			
1 min×10	65	6	0·1
2 min×5	33	6	0·001
15 min×3	5	6	0·001

Incubation = 1h; *n* = number of experiments; *P* = probability.

response of the muscle caused the reduction in output especially since the length of the tissue was shown previously to be unrelated to the release of active material.

Metabolic inhibition. 2,4-Dinitrophenol (DNP), one of several substituted phenols capable of uncoupling phosphorylation from electron transport, produced a dose-dependent relaxation of the sphincter and a corresponding depression of the output of active material (Figure 4). Both effects proved irreversible on repeated washing of the preparation.

Anoxia. Anoxia would also be expected to inhibit an active synthetic or secretory process. When the O_2/CO_2 mixture was switched off, relaxation of the muscle began immediately and was maximal after 30 minutes. The bath fluid was then replaced with pre-gassed Krebs solution, but during the subsequent hour without oxygenation the output was reduced by more than 80% (Figure 4). The gas supply was then re-established and the tissue regained tone rapidly and the output during the following hour was of the same magnitude as control values.

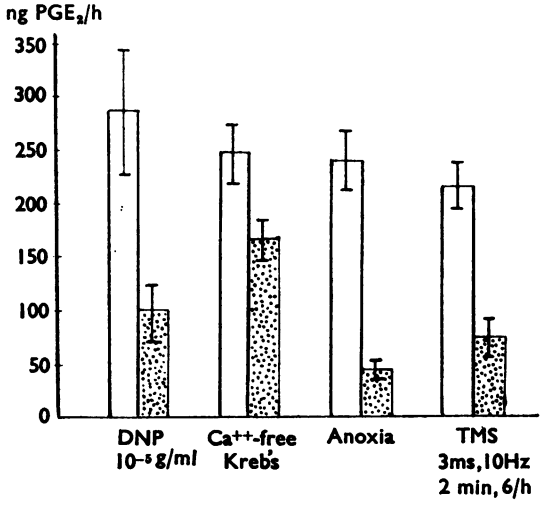

FIG. 4. A histogram to show conditions which significantly reduced the output of active material (in terms of prostaglandin E_2) from the sphincter pupillae. Open columns = control output. Stippled columns = output under treatment.

Calcium-free Krebs solution. Repeated washing of the preparation with calcium-free Krebs solution produced a gradual relaxation of the sphincter. During 1 h of incubation under these conditions the iris relaxed to 55% maximum and output of active material fell to less than 70% of control values (Figure 4). The effect of a chelating agent such as disodium ethylene diamine tetraacetate was not tried.

Electrical stimulation at 3 ms pulse width. Transmural stimulation of the iris with wide pulses (3 ms at 10 Hz) produced a transient contraction (cholinergic), followed by a relaxation which was little affected by α- and β-adrenoceptor or adrenergic neurone blocking agents. The extent of relaxation increased for periods of stimulation up to about 2 min and recovery was prolonged for several minutes after this duration of stimulation. A reduced output of active material accompanied the non-adrenergically mediated relaxation (Figure 4). For periods of stimulation of 2 min or more, the reduction was statistically highly significant.

The actions of prostaglandins and their antagonism on the sphincter pupillae

Concentrations of prostaglandin E_2 greater than 10^{-6}M were required to elicit contraction of the tonically shortened muscle. The maximum contraction of the tissue was only about 10% greater than the degree of spontaneous tonic shortening. Prostaglandin E_1, $F_{1\alpha}$, and $F_{2\alpha}$ produced similar responses to prostaglandin E_2.

Much lower concentrations of prostaglandin (10^{-9}M prostaglandin E_2) caused the atonic sphincter to contract shortly after it had been set up. Responses were therefore studied only within 1 h of setting up the preparation when tone was absent. Cumulative dose-response curves to prostaglandins E_2, E_1, $F_{1\alpha}$ and $F_{2\alpha}$ (contact time 3–5 min) were begun 15 min after setting up the preparation and in general the maxium response was reached before the control iris from the other eye of the same animal had begun to acquire tone spontaneously. The curves for E_2, E_1 and $F_{1\alpha}$ and $F_{2\alpha}$ appeared to be parallel and their potency ratios at the 50% maximal response were 100:13:3:1 respectively (Figure 5).

It proved impossible to construct a dose-response curve 30 to 40 min after setting up the preparation, since prostaglandin E_2 then produced a slow contraction which did not plateau for about 20 minutes. The total degree of prostaglandin-induced shortening was similar to the spontaneous tonic shortening of control preparations.

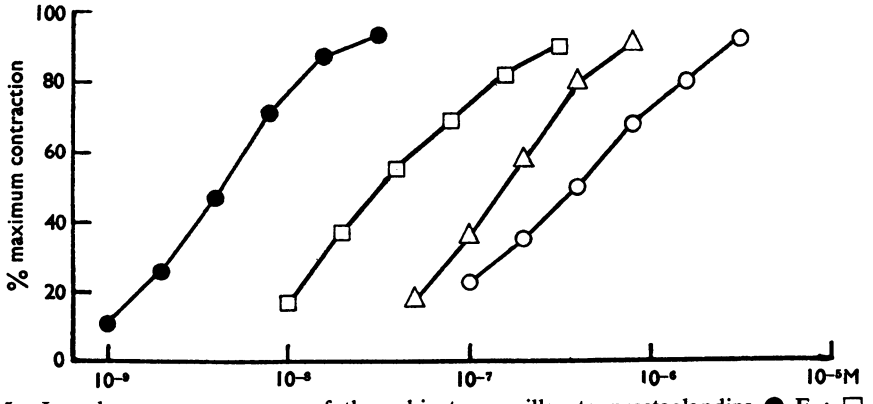

FIG. 5. Log dose-response curves of the sphincter pupillae to prostaglandins ● E_2; □ E_1; △ $F_{1\alpha}$; ○ $F_{2\alpha}$; (n=6).

A threshold concentration of 10^{-9}M prostaglandin E_2 was sufficient to produce this contraction and the response was not reversible on washing. The muscle subsequently behaved in an identical manner to control preparations.

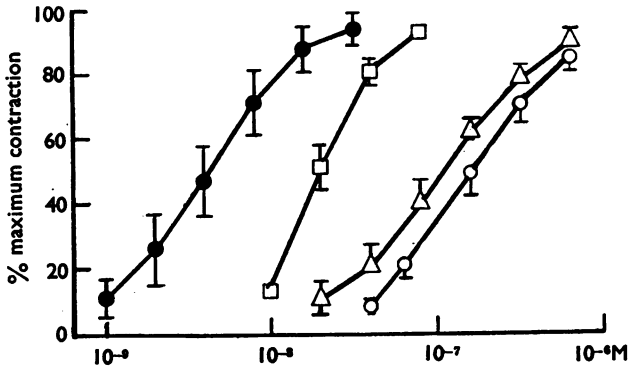

FIG. 6. The effect of SC-19220 on the log dose-response curve of the sphincter pupillae to prostaglandin E_2. ● control; □ 5×10^{-7}M; △ 10^{-6}M; ○ 10^{-5}M ($n=6$).

The prostaglandin antagonist SC-19220 (5×10^{-7}M) selectively inhibited responses of the sphincter to prostaglandin E_2 (Figure 6). A virtually maximal inhibition was obtained with 5×10^{-6}M SC-19220 which was somewhat surprising since data obtained on the guinea-pig ileum (Sanner, 1969; Bennett & Posner, 1971) supported a simple, competitive mode of action of this compound. The diluted alcohol solvent had no effect on the responses of the sphincter. SC-19220 did not alter the dose-response curve to histamine nor did it inhibit responses to carbachol or transmural electrical stimulation in concentrations up to 3×10^{-5}M. Higher concentrations of the antagonist inhibited the responses to transmural stimulation at 0·3 ms pulse width probably indicating a non-specific depression of contractility and also caused a dose-dependent relaxation of the preparation (Figure 7).

The possibility that prostaglandins may interact with neurohumoral transmission in the sphincter was investigated. Responses to transmural stimulation at 20 Hz, 0·3 ms and 75 V/cm for 15 s were not significantly affected by prostaglandin E_2 10^{-9}M to 10^{-6}M, by prostaglandin E_1 up to 2×10^{-6}M or by prostaglandin $F_{2\alpha}$ 10^{-6}M. Hyoscine, in concentrations sufficient to abolish contractile responses to carbachol and transmural stimulation did not affect the responses to prostaglandin E_2. Alpha- and β-adrenoceptor blocking agents were also ineffective.

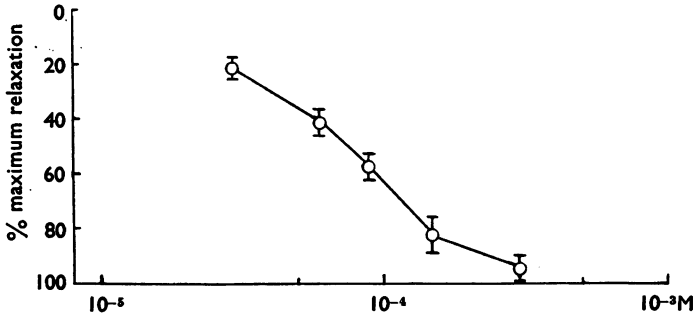

FIG. 7. Log dose-response curve of the sphincter pupillae to SC-19220 ($n=6$).

Discussion

These experiments show that a prostaglandin E_2-like substance is released into the fluid bathing the bovine isolated sphincter pupillae, and suggest that it is formed enzymically just prior to release. Thus the output was dependent on temperature and the presence of oxygen and calcium in the bathing medium, and it was reduced by the metabolic inhibitor dinitrophenol. Furthermore, considerably more active material was released in 1 h than could be extracted from other irides. The formation and release of material did not seem to involve neural elements since its release was not affected by nerve degeneration on storage, electrical stimulation of cholinergic or adrenergic nerves or block of conduction with tetrodotoxin, local anaesthetics or selective adrenergic neurone blockade.

The idea that muscle cells release prostaglandin as the result of contraction seems unlikely. The output from the tonic tissue did not fall during mechanical stretching or on maximal relaxation with isoprenaline. On the other hand, the release of material did appear to be related to the level of tonic contraction ; certain procedures reduced both the tone of the preparation and the output of the material. Moreover, soon after the preparation was set up it had no tone and there was no detectable release of active material at this time ; the output increased later as tone was acquired. The question therefore arises whether there is a causal relationship between endogenous prostaglandin production and the acquisition and maintenance of tone.

The actions of prostaglandins on the sphincter lend support to such a relationship. Of several substances tested only prostaglandins and histamine were found to produce sustained contractions of the atonic sphincter (Posner, 1971b) and it is interesting that prostaglandin E_2, the most potent prostaglandin tested, was the substance tentatively identified in the fluid bathing the tonically contracted sphincter. In addition, the threshold concentration of prostaglandin E_2 (10^{-9}M) was capable of producing irreversible contraction of the atonic sphincter with a similar time course and magnitude to the spontaneous tonic contraction of control preparations. This response to prostaglandin E_2 could only be produced 30 to 40 min after setting up the sphincter, although transmural stimulation and carbachol could induce maximal contractions immediately. It therefore seems that some change in the tissue other than improvement of muscle contractility had occurred in this period. One possibility is that although no prostaglandin had yet been released, synthesis had begun and only a small amount of exogenous prostaglandin was now required to initiate shortening.

As would be expected if prostaglandins are responsible for tone, the prostaglandin antagonist SC-19220 prevented the spontaneous acquisition of tone and produced a dose-dependent relaxation of the tonic preparation. It is noteworthy that both SC-19220 and another prostaglandin antagonist, polyphloretin phosphate (Eakins, Karim & Miller, 1970), have been shown to cause relaxations of various smooth muscles (Bennett & Posner, 1971). Furthermore, indomethacin, an inhibitor of prostaglandin synthesis, caused a reduction in tone of the rabbit isolated jejunum which was accompanied by an inhibition of prostaglandin release (Ferreira, Herman & Vane, 1972). These workers concluded that the tone of this muscle might be maintained by continuous generation of prostaglandins. Eckenfels & Vane (1972) have suggested a similar role for prostaglandins in some blood vessels.

A role for prostaglandins as modulators of neuromuscular transmission has been proposed by Hedqvist (1970) but the results of the present experiments do not support any direct interaction of prostaglandins with adrenergic or cholinergic nerves or their receptors. However, a possible role of prostaglandins could be one complementary to that of the cholinergic and adrenergic nerves which respectively cause rapid contraction and relaxations of the bovine sphincter (Posner, 1969). The hypothesis that prostaglandin E_2 might be continually synthesized by the smooth muscle cells in order to contribute to the acquisition and maintenance of tone in the bovine sphincter pupillae merits further examination.

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