*Br. J. Pharmac.* (1971), **42**, 569–583.

# Identification of prostaglandins in prevertebral venous blood after preganglionic stimulation of the cat superior cervical ganglion

HEATHER A. DAVIS,* E. W. HORTON,* K. B. JONES† AND J. P. QUILLIAM†

* *Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ*
† *Department of Pharmacology, St. Bartholomew's Hospital Medical College, London EC1*

**Summary**

1. In four out of seven experiments, prostaglandin-like activity was detected in prevertebral venous blood from the superior cervical ganglion, collected during and after preganglionic nerve stimulation.

2. Prostaglandins $E_1$, $E_2$, $F_{2\alpha}$ and a prostaglandin A were identified using solvent partition, column and thin-layer chromatography and bioassay techniques.

3. The release of $PGF_{2\alpha}$ was confirmed by combined gas chromatography-mass spectrometry.

## Introduction

The release of prostaglandins in association with known chemical transmitters has been demonstrated in response to stimulation of nerves to several tissues. These include rat diaphragm (Ramwell, Shaw & Kucharski, 1965 ; Laity, 1969), rat adipose tissue (Shaw, 1966 ; Ramwell & Shaw, 1968), dog spleen (Davies, Horton & Withrington, 1968 ; Ferreira & Vane, 1967), rat stomach (Coceani, Pace-Asciak, Volta & Wolfe, 1967 ; Bennett, Friedmann & Vane, 1967), and the central nervous system (Ramwell, Shaw & Jessup, 1966 ; Ramwell & Shaw, 1966 ; Bradley, Samuels & Shaw, 1969).

This work concerns the release of prostaglandins from the cat superior cervical ganglion as a result of preganglionic nerve stimulation.

## Methods

Seven cats weighing 2·2–4 kg were used. Anaesthesia was induced with ether and maintained with an intravenous dose of chloralose (70 mg/kg).

The isolation of the right superior cervical ganglion followed the classical techniques of Kibjakow (1933), Feldberg & Gaddum (1934) and Perry (1953), but the perfusion was performed by a method similar to that used by Jones & Quilliam (1967).

The superior cervical ganglion, the common carotid, the external carotid and the lingual arteries on the right side, and the right prevertebral vein and left external

jugular vein were cleared. All other blood vessels in the vicinity of the right superior cervical ganglion were tied off. The cat was then given heparin (150 IU/100 g i.v.).

The right lingual artery was connected via a rubber tube and polyethylene cannula to the left external jugular vein. This bridge was then clamped (position (iii), Fig. 1). A cannula, through which modified Locke's solution could be passed, was inserted in a retrograde direction into the right external carotid artery. By clamping at position (ii) (Fig. 1), blood flow was maintained through the superior cervical ganglion and the right prevertebral vein was then cannulated with a polyethylene tube.

When clamps were applied at positions (ii) and (iii) (Fig. 1), blood from the right common carotid artery perfused the ganglion and was collected from the prevertebral vein. With positions (i) and (iii) clamped, and position (ii) unclamped,

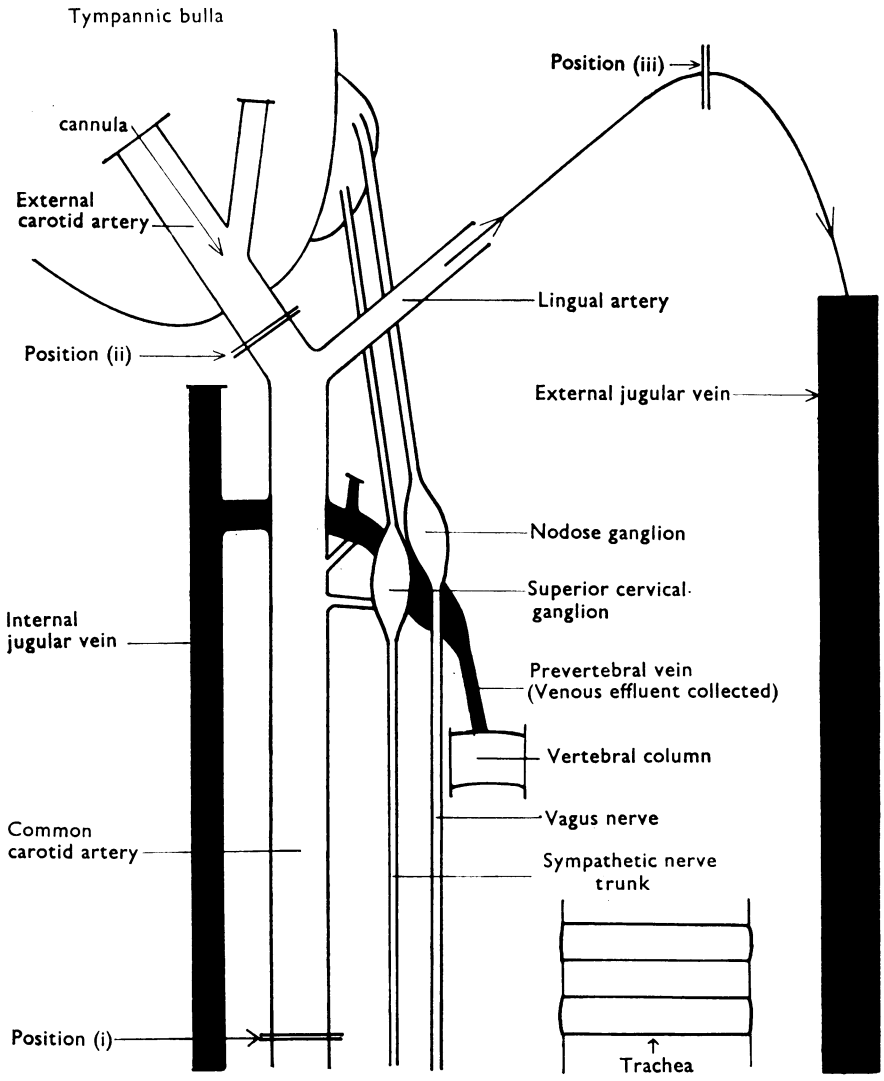


FIG. 1. Diagram of the arrangement for alternate perfusion of the superior cervical ganglion of the cat with Locke's solution and the cat's own blood.

perfusion of the ganglion with modified Locke's solution, under a pressure of 120 mmHg took place (1 mmHg≡1·333 mbar).

The Locke's solution, as modified by Perry (1953), contained double the usual amount of glucose. Choline (1 μg/ml) and eserine (10 μg/ml) were added to it. This solution was filtered, warmed to 37° C and aerated with pure oxygen.

The preganglionic nerve to the superior cervical ganglion was cut proximally to the ganglion and stimulated electrically via platinum electrodes. Liquid paraffin was poured over the electrodes and nerve to provide a localized stimulus. Ganglionic transmission was monitored by recording, on a smoked drum, the retraction of the right nictitating membrane, using an isotonic frontal writing lever. The vagus nerve was ligated both pre- and postganglionically.

When the dissection was completed and the collection of blood samples about to commence, the cat was artificially ventilated with a respiratory pump.

### *Collection of blood samples*

Positions (i) and (iii) (Fig. 1) were clamped and the superior cervical ganglion was perfused initially with modified Locke's solution for 3 minutes. A clear effluent from the ganglion confirmed the absence of blood leaking into the ganglion perfusate. Position (ii) was then clamped and position (iii) followed by position (i) were unclamped. This procedure allowed any cell debris which might have accumulated near clamp (i) to be washed into the lingual artery rather than through the ganglion, and it was always applied when changing from Locke to blood perfusion of the ganglion. After 10 s, clamp (iii) was replaced and the blood flowed through the ganglion to be collected from the prevertebral vein.

The blood was allowed to flow through the ganglion for 3 min and the effluent discarded before an initial control period of a 1 h (three experiments) or a 30 min (four experiments) blood sample collection was taken.

Clamps (i) and (iii) were then applied and clamp (ii) was released. A 5 min 'washover' period with modified Locke's solution followed in order to remove the dead space between the control and subsequent test sample. A similar 'washover' period of 5 min was repeated between each of the collected blood samples.

A 'test' period of 1 h or 30 min blood collection then followed. During this period a supramaximal electrical stimulus of 10–30 V, frequency 10 Hz, and duration 0·5 ms was applied to the preganglionic nerve fibres. After a 5 min 'washover' period, a second control sample of 1 h or 30 min blood collection was taken.

All the blood samples were collected in ice-cooled tubes and stored at −15° C until extraction.

### *Extraction procedure*

The rate of blood flow through the superior cervical ganglion was fairly slow (approximately 1·5 ml/h), and only small volumes of blood were collected. Whole blood was diluted with an equal volume of redistilled water in order to haemolyse it. The pH of this solution was adjusted to 3 with concentrated HCl and it was extracted 3 times with ten volumes of ethyl acetate. A large volume of ethyl acetate was used in order to prevent emulsification of the sample. The bulked ethyl acetate fractions were then subjected to a solvent partition system summarized in Fig. 2 and similar to that described by Horton & Main (1967).

*Silicic acid column chromatography*

Three grammes of silicic acid (Biorad Laboratories, minus 325 mesh) was used for 10 mm i.d. glass columns. It was activated by heating for 1 h at 110° C. After cooling, it was suspended in heavy petroleum, poured into the column and washed with more petroleum to remove impurities. The silicic acid column was then washed with 30% ethyl acetate in benzene.

The dried residue from the solvent partition procedure was dissolved in 1 ml of 30% ethyl acetate in benzene and applied dropwise to the column with a Pasteur pipette. The residue flask was then washed with a further 2 × 1 ml and 2 × 2 ml portions of 30% ethyl acetate in benzene. These separate washings were applied dropwise to the column. Thus, 7 ml of the first 20 ml 30% ethyl acetate in benzene elution fraction was used for transferring activity from the residue flask on to the column. After drying the residue flask, 1 ml of redistilled water was added and the solution was assayed to ensure that no biological activity had been left in the flask.

The columns were eluted under reduced pressure at a rate of approximately 1 ml/min with increasing concentrations of ethyl acetate in benzene, namely 30% (60 ml); 40% (250 ml); 80% (100 ml); followed by ethyl acetate (40 ml) and finally methanol (50 ml) which removed the most polar substances from the column.

The eluates from the column were evaporated to dryness. The 30% ethyl acetate in benzene fractions were dissolved in 1 ml of 0·9% saline and all other fractions in 1 ml of redistilled water. All the samples were bioassayed on the rat fundus and/or the ovariectomized rat uterus. The fractions from the column in which

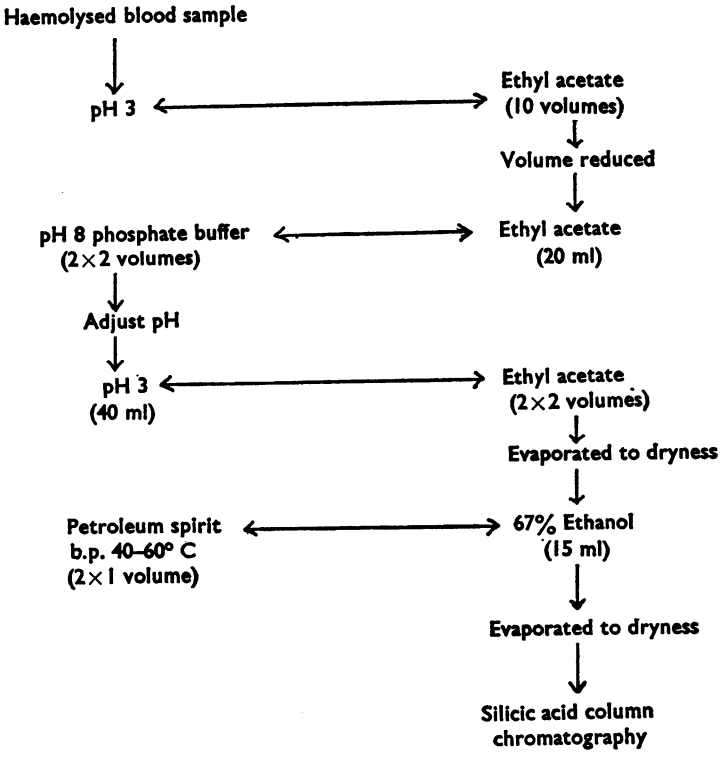


FIG. 2. Outline of extraction procedure.

prostaglandins of the A series could be eluted (30% ethyl acetate in benzene) were also assayed on the cat blood pressure preparation. Similarly, the fractions where prostaglandins of the F series could be eluted (80% ethyl acetate in benzene) were additionally assayed on the rabbit jejunum preparation.

Fractions with biological activity were stored at −15° C for further investigation.

### *Thin-layer chromatography*

The detailed preparation, loading and running of thin-layer chromatography plates and the solvent systems used were described by Horton & Main (1967).

Glass plates of dimensions 20 × 5 cm were used. The plates for use in the AI solvent system (Gréen & Samuelsson, 1964) were coated with a 0·25 mm layer of silica gel G (Merck) whereas those for use in the AII solvent system were coated with a similar layer of silica gel impregnated with 4% silver nitrate. The plates were dried and activated by heating at 110° C for 1 hour. They were then stored in a desiccator over self-indicating silica gel crystals, until required.

Pure prostaglandins and active extract fractions were applied in redistilled methanol to the thin-layer plates with an Agla micrometer syringe. The following authentic prostaglandins were used on marker plates: $E_1$, $E_2$, $F_{1\alpha}$, $F_{2\alpha}$, $A_1$ and $A_2$, but only two such prostaglandins were applied as spots to each plate. Twenty microlitres of a 500 $\mu$g/ml authentic prostaglandin solution were used.

Biologically active extracts were applied in 0·1 ml methanol, as a band, to the preparative plate. The flask containing the extract was washed with 2 × 0·1 ml methanol and these aliquots were also applied to the plate.

The thin-layer chromatography plates were developed in either the AI or AII solvent system of Gréen & Samuelsson (1964) and a 10 cm run was used. Marker plates loaded with pure prostaglandins were always run at the same time as preparative plates. The marker plates were visualized by spraying with 10% phosphomolybdic acid in ethanol, followed by heating to 110° C for 15 minutes.

The whole 10 cm run of each preparative plate was divided into zones and the silica gel from each zone was scraped off. The silica gel from plates run in the AI solvent system was extracted for prostaglandins by shaking it with 3 × 5 ml of methanol. Each methanol suspension was centrifuged and the supernatant removed. The combined methanol supernatants were evaporated to dryness and the residue was dissolved in 1 ml of redistilled water. Each sample was stored at −15° C until ready for bioassay.

The silica gel zones scraped from plates run in the AII solvent system were extracted by initially adding 7 ml of redistilled water, taken to pH 3 with concentrated hydrochloric acid, to them. This procedure precipitated the silver present. This acidified aqueous suspension was then partitioned against 3 × 7 ml of redistilled ethyl acetate. Each of these ethyl acetate/water suspensions was centrifuged and clear supernatant ethyl acetate was collected. The combined ethyl acetate supernatants were evaporated to dryness and the residue was dissolved in 1 ml of redistilled water and stored at −15° C until bioassay.

### *Gas chromatography–mass spectrometry*

The methyl ester/trimethylsilyl ether (Me/TMS) and the methyl ester/trifluoroacetate (Me/TFA) derivatives were prepared using the methods of Thompson, Los & Horton (1970).

The derivatives were prepared on a microscale using 0·5 ml stoppered tubes. Samples were transferred to the tubes in 0·2 ml methanol, then evaporated to dryness *in vacuo*. The samples were methylated at room temperature by reaction for 30 min with a freshly prepared solution of diazomethane in diethyl ether-methanol (9 : 1). After methylation, the samples were dried by the use of a fine jet of air. Trimethylsilyl ether derivatives were formed by the addition of 25 μl of bis (trimethylsilyl)-trifluoroacetamide (BSTFA) to the methyl ester. Ten microlitre samples were injected on to the column after 3 h without removal of the BSTFA. Trifluoroacetates were prepared by reacting the methyl esters for 2 h with 200 μl trifluoroacetic anhydride which was then removed in a vacuum desiccator. The residue was dissolved in 25 μl of hexane and 10 μl samples were injected on to the column.

Analyses were performed on an LKB gas chromatograph-mass spectrometer. The column (1·5 m × 1·5 mm i.d.) was packed with 3% OV 1 on Supasorb AW, 100–120 mesh pretreated with dimethylchlorosilane in carbon tetrachloride. Column temperature was 200° C or 190° C. The carrier gas, helium, flowed at 20 ml/minute. All mass spectra were recorded at an electron voltage of 27·5.

Derivatives were made for 500 ng of authentic $PGF_{2\alpha}$ since only purified extracts with $PGF_{2\alpha}$-like activity were subjected to gas chromatography-mass spectrometry. Ten microlitre samples of the appropriate $PGF_{2\alpha}$ derivative were injected on to the gas chromatograph column, the retention time for the authentic prostaglandin being noted. A mass spectrum was taken at this time. A similar procedure was applied to the samples from the experiments.

### *Biological assays*

*Smooth muscle preparations* in vitro

Segments of various organs were suspended in an 0·5 ml or 4 ml organ bath and longitudinal contractions were recorded isometrically with a force-displacement transducer (Glass FT.03) on a Servoscribe pen recorder. A dose cycle of 4–7 min with a contact time of 1–2 min was used for all preparations. All estimates of content were made by bracketing with pure prostaglandins.

*Rat fundus.* Longitudinal strips from the fundus of rats weighing 100–350 g were prepared, as described by Vane (1957), and suspended in Tyrode's solution at 37° C, vigorously gassed with oxygen.

*Rat uterus.* Single uterine horns from white Wistar strain rats, weighing 200–350 g, which had undergone ovariectomy 14 days previously, were used. The uterine horn was suspended in a 0·5 ml organ bath in de Jalon's solution at 18–22° C gassed with air. These uteri were 5–10 times more sensitive to prostaglandins than uteri from non-ovariectomized rats and they lacked spontaneous activity.

*Rabbit jejunum.* Proximal jejunum from rabbits weighing 1–3 kg was suspended in Tyrode's solution at 37° C gassed with air.

*Guinea-pig ileum.* Terminal ileum from guinea-pigs weighing 200–400 g was suspended in Tyrode's solution at 37° C gassed with air.

*Cat trachea.* Tracheae were obtained from cats which had been used for other experiments and they were stored overnight in Krebs solution at 4° C. Two or three rings of trachea were tied together with the muscle in alignment ; the cartilage was removed. The preparation was suspended in Krebs-Henseleit solution at 37° C

gassed with 5% $CO_2$ in $O_2$. The inhibitory action of a prostaglandin was demonstrated by adding it to the organ bath either 30 s before a dose of acetylcholine or after acetylcholine had produced a sustained contraction (Main, 1964).

*Assay preparations* in vivo

*Kitten blood pressure.* A 500–700 g kitten was anaesthetized with pentobarbitone sodium (40 mg/kg) injected intraperitoneally and anaesthesia was maintained with a slow intermittent intravenous pentobarbitone sodium infusion (5 mg/ml). The blood pressure was recorded from the carotid artery using a Statham transducer attached to a Beckman-Offner Dynograph. Assay samples were administered in 0·9% saline by a rapid intravenous injection into the cannulated external jugular vein and washed in with 0·2 ml of 0·9% saline (Horton & Jones, 1969).

*Spinal cat blood pressure.* Anaesthesia was induced in 0·7–2 kg cats using ethyl chloride followed by ether. The two vagi were cut, the trachea cannulated and the spinal cord cut at the level of the second cervical segment. The cat was artificially ventilated. Blood pressure was recorded from the carotid artery and samples were injected into an external jugular vein.

*Rat blood pressure.* Anaesthesia was induced in 300–400 g rats using 25% urethane (6 ml/kg) injected intraperitoneally. Samples were injected in 0·9% saline via the right femoral vein and blood pressure was monitored from the cannulated left carotid artery using a Statham transducer attached to a Beckman-Offner Dynograph.

*Recovery experiments*

Thirty millilitres of cat blood at 37° C, containing 20 IU/ml of heparin, were divided into six aliquots. Prostaglandins $A_1$, $E_1$ or $F_{2\alpha}$ were added separately to 5 ml of cat blood to give final concentrations of: prostaglandin (PG) $E_1$, 100 ng/ml and 500 ng/ml; $PGF_{2\alpha}$, 100 ng/ml and 500 ng/ml; $PGA_1$, 500 ng/ml and 1 $\mu$g/ml. The samples were extracted as in Fig. 2 and purified using silicic acid column chromatography. $PGA_1$ was eluted from the column with 30% ethyl acetate in benzene and bioassayed on the cat blood pressure preparation. $PGE_1$ was eluted from the column with 40% and $PGF_{2\alpha}$ with 80% ethyl acetate in benzene. $PGE_1$ and $F_{2\alpha}$ were both bioassayed on the rat fundus preparation. The recoveries after column chromatography were: <5% (500 ng/ml $PGA_1$); 14% (1 $\mu$g/ml $PGA_1$); 54% (100 ng/ml $PGE_1$); 67% (500 ng/ml $PGE_1$); 53% (100 ng/ml $PGF_{2\alpha}$) and 60% (500 ng/ml $PGF_{2\alpha}$).

## Results

*Release of prostaglandin-like substances*

In four out of seven experiments prostaglandin-like substances were detected in the venous effluent from the superior cervical ganglion as a result of nerve stimulation (Table 1). In two of these experiments raised concentrations of prostaglandin were detected in the blood sample collected after nerve stimulation as well as in the sample collected during nerve stimulation. Identification of the prostaglandin released from the superior cervical ganglion has been attempted by chromatography and parallel biological assay. The results are presented below.

TABLE 1. *Amounts of prostaglandin E-like material (ng $PGE_1$ equivalent released/min) detected after column chromatography in each of the blood samples taken before, during and after nerve stimulation in seven superior cervical ganglion experiments*

| Experiment number | Before nerve stimulation (control (1)) | During nerve stimulation (test) | After nerve stimulation (control (2)) |
|---|---|---|---|
| 1 | <0·2 | 240 | <2 |
| 2 | <0·25 | 0·5 | 14 |
| 3 | 1·3 | 22 | 27 |
| 4 | 0·7 | <0·3 | <0·3 |
| 5* | <0·3 | 14 | <0·3 |
| 6 | <1 | <1 | <1 |
| 7 | <3 | <2 | <1·5 |

* Prostaglandins F and A released in this experiment. The prostaglandins E were eluted with 40% ethyl acetate in benzene and assayed on rat fundus and/or rat uterus. All values are uncorrected for recovery.

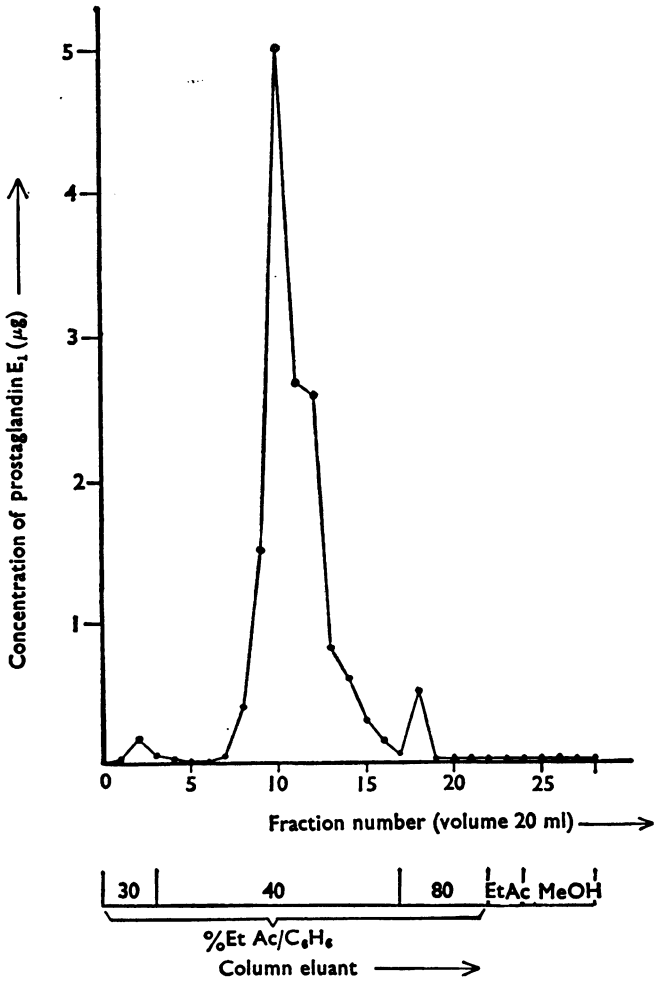


FIG. 3. Silicic acid column chromatography of the sample taken during nerve stimulation in experiment 1 (Table 1). Biological activity assayed on the rat uterus and expressed in terms of μg of $PGE_1$/20 ml elution fraction.

TABLE 2. *Results of thin-layer chromatography in AII solvent of prostaglandin E fractions detected in three experiments*

| Experiment number (Table 1) | Sample | Length of sample collection (min) | Prostaglandin zone eluted | Percentage total biological activity recovered from the plate | Assay preparation | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Rat fundus | Rat uterus | Guinea-pig ileum | Cat trachea | Rabbit jejunum |
| 1 | Test | 60 | $E_1$ | 85 | 167 | 208 | 133 | 233 | |
| | | | $E_2$ | 8·5 | 16·7 | 16·7 | 26·7 | 25 | |
| 3 | Test | 60 | $E_1$ | 13 | 0·42 | | 0·42 | | |
| | | | $E_2$ | 46 | 1·3 | 1·4 | 1·6 | | |
| 3 | Control (2) | 30 | $E_1$ | 17 | 1·3 | | 1·3 | | |
| | | | $E_2$ | 51 | 2·7 | 3·3 | 3 | | |
| 5 | Test | 30 | $E_1$ | <8 | <0·8 | | <0·8 | | |
| | | | $E_2$ | 63·5 | 5 | | 5 | | 6·7 |

Concentrations of prostaglandins $E_1$ or $E_2$, eluted from the appropriate zone of thin-layer preparative plates, are expressed in terms of ng $PGE_1$ or $PGE_2$ released/minute. All values are uncorrected for recovery.

*Identification of prostaglandins*

In the first experiment (Table 1) the venous blood from the right superior cervical ganglion was extracted by the solvent partition procedure outlined in Fig. 2. The material was chromatographed on a silicic acid column and the eluates were assayed on the ovariectomized rat uterus. From the blood sample taken during nerve stimulation, one peak of biological activity was eluted with 40% ethyl acetate in benzene (Fig. 3), corresponding to the chromatographic behaviour of prostaglandins E as found in the recovery experiments and in experiments by Horton & Main (1967). The material from this PGE peak was purified further by thin-layer chromatography on silica gel plates impregnated with $AgNO_3$ using the AII solvent system of Gréen & Samuelsson (1964). About 85% of the biological activity of this peak was recovered from the zone corresponding to the $R_F$ value of $PGE_1$. About 9% was recovered from the $PGE_2$ zone (Table 2).

The material eluted from the $PGE_1$ zone of the thin-layer plate was assayed in parallel on four tissues in terms of $PGE_1$. Like $PGE_1$, the substance extracted from the venous blood contracted the rat fundus, rat uterus and guinea-pig ileum but inhibited contractions of the cat tracheal smooth muscle to acetylcholine. The assay results on the four tissues agreed well quantitatively (Table 2). The highest index of discrimination was 1·7. Similar results were obtained with the material from the $PGE_2$ zone which was assayed in terms of $PGE_2$ (Table 2).

In three subsequent experiments similar evidence was obtained for the release of prostaglandins of the E series as a result of nerve stimulation. On the basis of thin-layer chromatography and parallel biological assay these prostaglandins were further identified as a mixture of $PGE_1$ and $PGE_2$ in experiment 3 and $PGE_2$ alone in experiment 5 (Table 2).

The samples collected in experiment 5 were purified by silicic acid column chromatography. In the sample collected during nerve stimulation, three biologically active fractions were eluted from the column with 30%, 40% and 80% ethyl acetate in benzene. These fractions which contracted the rat fundus, corresponded to the chromatographic behaviour of prostaglandins A, E and F, respectively (Table 3).

The material from the 30% ethyl acetate in benzene fraction was assayed on the cat blood pressure preparation in terms of $PGA_2$. The sample lowered the cat blood pressure, a characteristic of the prostaglandins A (Horton & Jones, 1969). Approximately 200 ng of $PGA_2$ equivalent was identified in the sample, but after bioassay

TABLE 3. *Amounts of prostaglandin (ng PG equivalent released/min) detected after column chromatography in the three 30 min blood samples taken in experiment 5 (Table 1)*

| Sample | Column chromatography eluant (ethyl acetate in benzene) | | | |
|---|---|---|---|---|
| | 30% (PGA) | | 40% (PGE) | 80% (PGF) |
| | Cat blood pressure | Rat fundus | Rat fundus | Rat fundus |
| Before nerve stimulation (control (1)) | <1·5 | <15 | <0·3 | 3 |
| During nerve stimulation (test) | 7 | 37·5 | 14 | 1500 |
| After nerve stimulation (control (2)) | <1·5 | <3 | <0·3 | <0·8 |

The PGA, PGE and PGF-like fractions were assayed in terms of $PGA_2$, $PGE_1$ and $PGF_{2\alpha}$ respectively. All values are uncorrected for recovery.

there was insufficient material left for thin-layer chromatography and further identification.

From recovery experiments using cat blood no $PGA_1$ could be detected when 500 ng/ml was added to the blood and only 14% could be recovered when 1 $\mu$g/ml was added. Therefore, the true concentration of PGA-like material in the sample from experiment 3 could have been very high.

A low concentration of material with the chromatographic behaviour of prostaglandins A was detected in samples from experiments 1 and 3 (Table 1). This material was shown only to contract the rat fundus as no sensitive assay method for the prostaglandins of the A series was known at the time of these experiments.

The material from the 80% ethyl acetate in benzene fraction in experiment 5 (approximately 45 $\mu$g) was purified further by thin-layer chromatography. Half the sample was purified on silica gel plates, using the AI solvent system and the other half was purified on silica gel plates impregnated with silver nitrate using the AII solvent system. In both these systems the active principle behaved like $PGF_{2\alpha}$ (Tables 4 and 5). The material eluted from the $PGF_{2\alpha}$ zone of both thin-layer plates

TABLE 4. *Percentage of biological activity found in the zones of a preparative thin-layer chromatogram of half the prostaglandin F fraction, obtained from column chromatography of the test sample in experiment* 5 (*Table* 3)

| Zone number | Mean $R_F$ | Position of pure prostaglandin | Percentage total biological activity recovered from the plate |
|---|---|---|---|
| 1 | Origin | | 0·3 |
| 2 | | | 0·3 |
| 3 | 0·38 | F | 97·7 ($\equiv$30 $\mu$g $PGF_{2\alpha}$) |
| 4 | 0·56 | E | <1·3 |
| 5 | 0·72 | A | <0·15 |
| 6 | Front | | <0·15 |

The chromatogram was developed in AI solvent system and the zones were assayed on rabbit jejunum in terms of $PGF_{2\alpha}$.

TABLE 5. *Percentage of biological activity found in the zones of a preparative thin-layer chromatogram of half the prostaglandin F fraction obtained from column chromatography of the test sample in experiment* 5 (*Table* 3)

| Zone Number | Mean $R_F$ | Position of pure prostaglandin | Percentage total biological activity recovered from the plate |
|---|---|---|---|
| 1 | Origin | | 1·7 |
| 2 | 0·34 | $F_{2\alpha}$ | 97·6 ($\equiv$22·5 $\mu$g $PGF_{2\alpha}$) |
| 3 | 0·52 | $F_{1\alpha}$ | 0·3 |
| 4 | | | 0·2 |
| 5 | Front | | 0·1 |

The chromatogram was developed in the AII solvent system and the zones were assayed on the rabbit jejunum in terms of $PGF_{2\alpha}$.

TABLE 6. *Parallel biological assay of the material eluted from zone* (3) *of a thin-layer plate developed in the AI solvent system* (*Table* 4) *and from zone* (2) *of a plate developed in the AII solvent system* (*Table* 5)

| Source of assay material | Assay preparations | | |
|---|---|---|---|
| | Rat fundus | Rabbit jejunum | Rat blood pressure |
| Zone (3) (Table 4) | 23·5 | 30 | 11 |
| Zone (2) (Table 5) | 22·5 | 22·5 | 10 |

Biological activity is expressed in terms of $\mu$g prostaglandin $F_{2\alpha}$ detected. The values are uncorrected for recovery.

(approximately 52 $\mu$g) was assayed in parallel on three tissues in terms of $PGF_{2\alpha}$. Like $PGF_{2\alpha}$, it contracted the rat fundus and rabbit jejunum and raised the arterial blood pressure of the rat (Table 6). The highest index of discrimination was 2·7.

Combined gas chromatography-mass spectrometry was performed on two derivatives, the Me/TMS and Me/TFA, of the $PGF_{2\alpha}$-like material. Mass spectra taken at the retention time of authentic $PGF_{2\alpha}$, indicated that the sample contained $PGF_{2\alpha}$. The gas chromatography retention times for the Me/TMS and Me/TFA derivatives were 15·75 min and 5·6 min, respectively. Line diagrams of the mass spectra of the Me/TMS and Me/TFA derivatives of the sample are shown in Figs. 4 and 5 respectively. The spectra are compared with those of authentic $PGF_{2\alpha}$. (Line diagrams are obtained by measuring the heights of prominent m/e peaks, then expressing each peak as a percentage of the highest peak.)

No prostaglandins of the F series were detected in any other experiment.

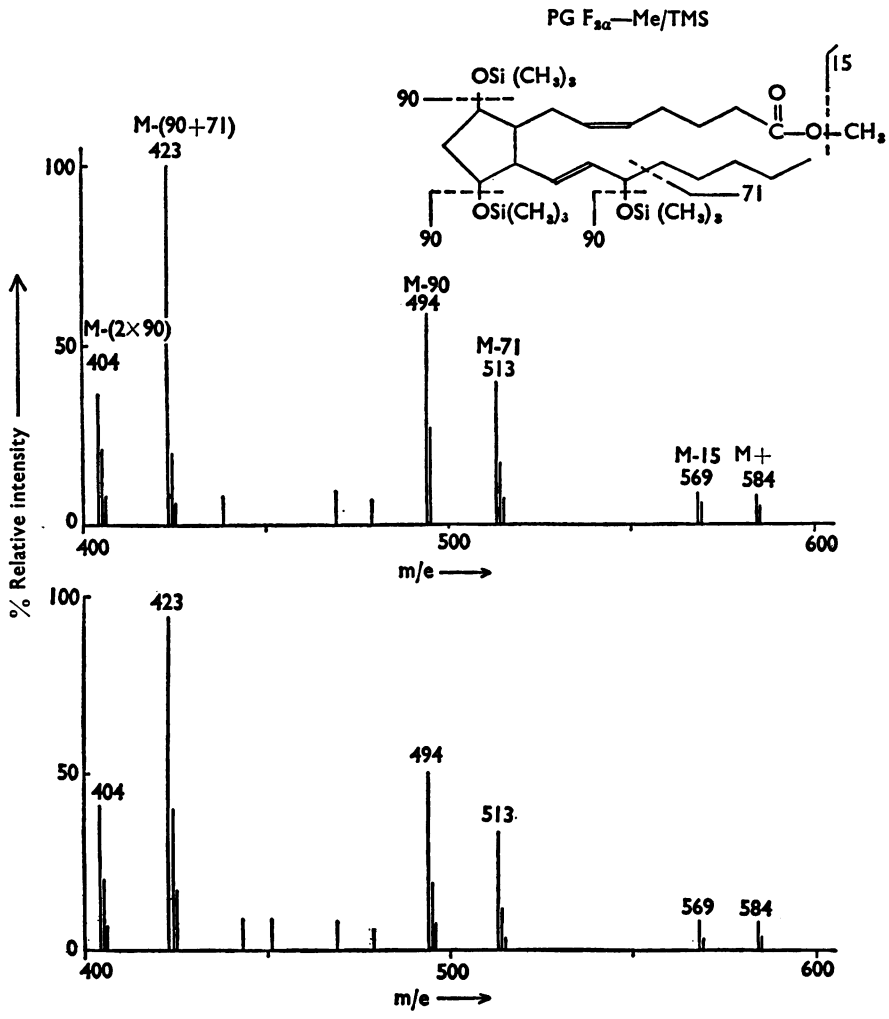


FIG. 4. Mass spectra of the Me/TMS derivatives of authentic $PGF_{2\alpha}$ (upper) and a sample from experiment 5 (lower).

*Occurrence of interfering substances in extracts*

Eight samples were assayed before and after silicic acid column chromatography. In six of these the amount of biological activity recovered from the column was greater than the amount found in the extract before chromatography. This finding suggests that there were smooth muscle inhibitory substances in the extracts which were separated from the prostaglandin by chromatography.

## Discussion

In four out of seven experiments, stimulation of the preganglionic nerve to the superior cervical ganglion resulted in the release of $PGE_1$ and/or $PGE_2$. In one of these four experiments, a prostaglandin of the A series and prostaglandin $F_{2\alpha}$ were also released. The identification of the prostaglandins was based upon their similarity to pure samples of authentic prostaglandins on solvent partition, silicic acid column chromatography, thin-layer chromatography and quantitative parallel biological assay. The identification of the $PGF_{2\alpha}$ was confirmed by combined gas chromatography-mass spectrometry.

At the time these experiments were carried out mass spectrometric methods for the identification of nanogramme quantities of $PGE_1$ and $PGE_2$ were not available.

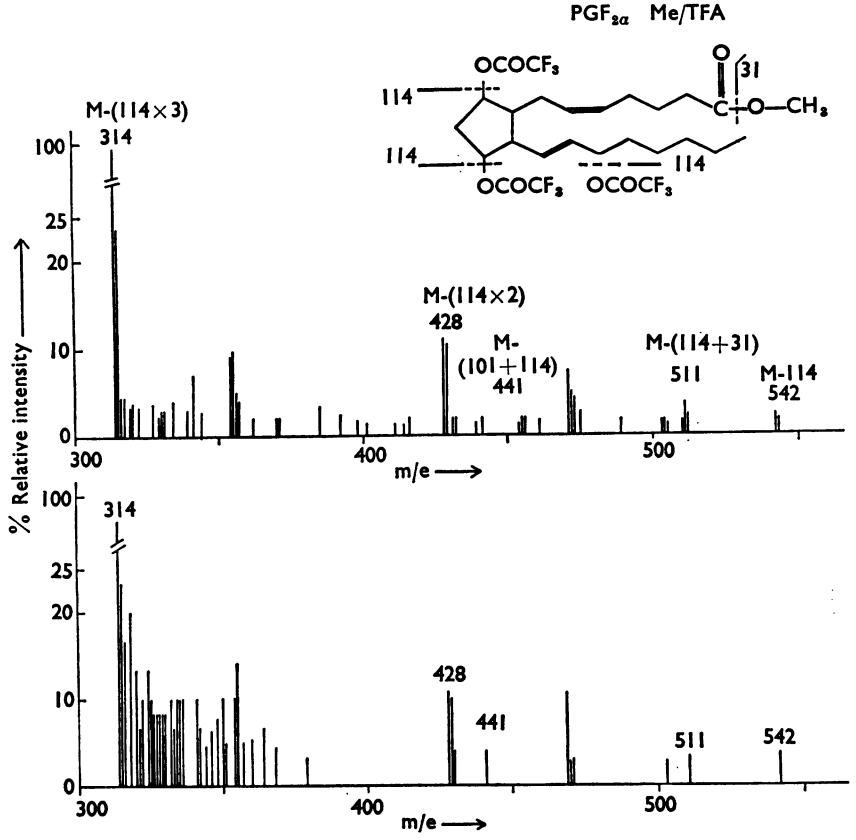


FIG. 5. Mass spectra of the Me/TFA derivatives of authentic $PGF_{2\alpha}$ and a sample from experiment 5.

The identification of these prostaglandins must therefore be regarded as tentative rather than conclusive. The problem of identification of nanogramme quantities of prostaglandins by a combination of chromatography and bioassay has been discussed fully by Horton & Main (1967).

It is concluded that prostaglandins of the E series were released from the cat superior cervical ganglion as a result of preganglionic nerve stimulation in four out of seven experiments. The reason for the three negative results is not known but similar negative results have been obtained in other series of experiments (Davies *et al.*, 1968).

A prostaglandin of the A series and prostaglandin $F_{2\alpha}$ occurred in large amounts in one experiment only; the conditions in this experiment were apparently identical to those used in the other experiments. Factors such as the depth of anaesthesia, blood gas tensions and amount of tissue damage may have been variable. The cats used in these experiments were hyperventilated as the biosynthesis of prostaglandins from precursor polyunsaturated fatty acids requires molecular oxygen (Nugteren & van Dorp, 1965; Samuelsson, 1965) and anoxia inhibits the release rate of prostaglandins (Coceani *et al.*, 1967). The high blood oxygen tension would have inhibited the activity of carotid body chemoreceptors although the possibility that the prostaglandins could have originated from the carotid body can almost certainly be discounted as the venous drainage from that organ was occluded.

Karim, Sandler & Williams (1967) reported the presence of $PGE_2$ in the human cervical sympathetic chain. However, Kayaalp & McIsaac (1968) perfused the cat superior cervical ganglion with $PGE_1$ and $PGE_2$ and could show no change in the responses of the nictitating membrane to preganglionic nerve stimulation. A similar lack of correlation between the $PGE_2$ released on nerve stimulation and any marked pharmacological actions has been demonstrated in the dog spleen (Davies & Withrington, 1968; Davies *et al.*, 1968). It does appear, however, that the release of prostaglandins is a phenomenon associated with nerve stimulation in a variety of tissues. Release of prostaglandins has been demonstrated at post ganglionic nerve endings of both sympathetic and parasympathetic systems, from the adrenal medulla and from the neuromuscular junction. The results in this paper establish that prostaglandins can also be released from an autonomic ganglion on stimulation of preganglionic fibres.

This research was supported by a grant to E.W.H. from the Wellcome Trust.

## REFERENCES

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

BRADLEY, P. B., SAMUELS, G. M. R. & SHAW, J. E. (1969). Correlation of prostaglandin release from the cerebral cortex of cats with the electrocorticogram, following stimulation of the reticular formation. *Br. J. Pharmac.*, **37**, 151–157.

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. (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 response to catecholamines, angiotensin and nerve stimulation. *Br. J. Pharmac. Chemother.*, **32**, 136–144.

FELDBERG, W. & GADDUM, J. H. (1934). The chemical transmitter at synapses in a sympathetic ganglion. *J. Physiol., Lond.*, **81**, 305–319.

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

GRÉEN, K. & SAMUELSSON, B. (1964). Thin-layer chromatography of prostaglandins. *J. Lipid Res.*, **5**, 117–120.

HORTON, E. W. & JONES, R. L. (1969). Prostaglandins $A_1$, $A_2$ and 19-hydroxy $A_1$; their actions on smooth muscle and their inactivation on passage through the pulmonary and hepatic portal vascular beds. *Br. J. Pharmac.*, **37**, 705–722.

HORTON, E. W. & MAIN, I. H. M. (1967). Identification of prostaglandins in central nervous tissues of the cat and chicken. *Br. J. Pharmac. Chemother.*, **30**, 582–602.

JONES, K. B. & QUILLIAM, J. P. (1967). Perfusion of the superior cervical ganglion of the cat alternately with Locke solution and with the cat's own blood. *J. Physiol., Lond.*, **189**, 11–13*P*.

KARIM, S. M. M., SANDLER, M. & WILLIAMS, E. D. (1967). Distribution of prostaglandins in human tissues. *Br. J. Pharmac. Chemother.*, **31**, 340–344.

KAYAALP, S. O. & MCISAAC, R. J. (1968). Absence of effects of prostaglandins $E_1$ and $E_2$ on ganglionic transmission. *Europ. J. Pharmac.*, **4**, 283–288.

KIBJAKOW, A. W. (1933). Über humorale Übertragung der Erregung von einem Neuron auf das andere. *Pflügers Arch. ges. Physiol.*, **232**, 432–443.

LAITY, J. L. H. (1969). The release of prostaglandin $E_1$ from the rat phrenic nerve-diaphragm preparation. *Br. J. Pharmac.*, **37**, 698–704.

MAIN, I. H. M. (1964). The inhibitory actions of prostaglandins on respiratory smooth muscle. *Br. J. Pharmac. Chemother.*, **22**, 511–519.

NUGTEREN, D. H. & VAN DORP, D. A. (1965). The participation of molecular oxygen in the biosynthesis of prostaglandins. *Biochim. biophys. Acta*, **98**, 654–656.

PERRY, W. L. M. (1953). Acetylcholine release in the cat's superior cervical ganglion. *J. Physiol., Lond.*, **119**, 439–454.

RAMWELL, P. W. & SHAW, J. E. (1966). Spontaneous and evoked release of prostaglandins from cerebral cortex of anaesthetised cats. *Am. J. Physiol.*, **211**, 125–133.

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

RAMWELL, P. W., SHAW, J. E. & JESSUP, R. (1966). Spontaneous and evoked release of prostaglandins from frog spinal cord. *Am. J. Physiol.*, **211**, 998–1004.

RAMWELL, P. W., SHAW, J. E. & KUCHARSKI, J. (1965). Prostaglandin: release from the rat phrenic nerve diaphragm preparation. *Science, N.Y.*, **149**, 1390–1391.

SAMUELSSON, B. (1965). On the incorporation of oxygen in the conversion of 8, 11, 14-eicosatrienoic acid to prostaglandin $E_1$. *J. Am. chem. Soc.*, **87**, 3011–3013.

SHAW, J. E. (1966). Prostaglandin release from adipose tissue *in vitro* evoked by nerve stimulation or catecholamines. *Fedn Proc.*, **25**, 770.

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**, 983–988.

VANE, J. R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344–349.

(*Received March* 29, 1971)