J. Pharm. Pharmac., 1970, **22**, 302

LETTERS TO THE EDITOR

Prostaglandins in dog renal medulla

During an investigation into the effects of acute experimental renal ischaemia in dogs (McGiff, Crowshaw & others, 1969), we detected prostaglandin-like substances in the venous blood of both the ischaemic and contralateral kidneys. Prostaglandins (PGE_2, $PGF_2\alpha$ and PGA_2) have been unequivocally identified in rabbit renal medulla (Lee, Crowshaw & others, 1967; Daniels, Hinman & others, 1967). This report concerns the isolation of three biologically-active lipids from dog renal medulla which exhibited the typical chromatographic and pharmacological properties of PGE_2, $PGF_2\alpha$ and PGA_2.

Kidneys from 20 dogs were sectioned and the medulla was separated from the cortex and stored at −10°. The pooled medullary tissue (453 g) was homogenized for 2 min in 400 ml water at 4°. Five volumes of ethanol were added and the mixture was vacuum filtered. The residue was washed with a further 500 ml ethanol and the combined filtrates were evaporated *in vacuo* to a small volume, then diluted to 800 ml with water and extracted (3 × 800 ml) with ethyl acetate. The extracts were combined and evaporated to 100 ml. The concentrate was extracted (4 × 50 ml) with 0·1M phosphate buffer (pH 8). The pH of the buffer phase was adjusted to 3 with 2N HCl and the acidic solution extracted (6 × 200 ml) with chloroform. The combined chloroform extracts were evaporated to dryness, the residue dissolved in ethanol (10 ml) and 0·9 ml of the solution was taken for preliminary chromatographic and biological testing. The remainder was evaporated and dissolved in 10 ml of benzene–ethyl acetate (9 : 1, v/v) and applied to a 20 g silicic acid column as described by Lee & others (1967).

To minimize losses of prostaglandins during silicic acid chromatography, small volumes of solvents (200 ml) were used in the elution sequence (benzene-ethyl acetate 7 : 3; 2 : 3; 1 : 9; methanol) so that the time during which prostaglandins were on the silicic acid was relatively short and the eluates were quickly evaporated. Fractions containing prostaglandins were eluted with more polar solvent mixtures than usually used (Samuelsson, 1963), but we consistently obtained a reproducible sequence of column separations; PGA compounds were eluted with benzene–ethyl acetate (2 : 3), PGE compounds with benzene–ethyl acetate (1 : 9) and PGF compounds with methanol. The eluates were evaporated to dryness, dissolved in ethanol (1·0 ml) and portions taken for bioassay, those with biological activity being stored at 4° for further assay and thin-layer chromatographic (TLC) characterization as described previously (Lee & others, 1967). For separation of those prostaglandins differing only in their degree of unsaturation, e.g., PGE_1 and PGE_2, $AgNO_3$/silica gel G (1 : 30) was used (Gréen & Samuelsson, 1964). Solvent systems were "AI", benzene–dioxan–acetic acid (20 : 20 : 1, v/v); B, chloroform– methanol–acetic acid (18 : 1 : 1, v/v); C, chloroform–methanol–acetic acid (18 : 2 : 1, v/v). After separation, 1 cm wide zones were scraped off the plates and eluted with organic solvents.

Purified samples were sonified in saline for testing. Smooth-muscle stimulating activity was determined using rat stomach strip, rat colon and chick rectum continuously superfused in series by Krebs solution at 37·5° (Ferreira & Vane, 1967). Both PGE_2 and $PGF_2\alpha$ contract all three tissues. The effects of PGE_2 were usually distinguishable from $PGF_2\alpha$ because of the differential magnitude of their effects on the assay organs. The rat blood pressure bioassay was used to detect PGA-like material and to characterize further the PGE and PGF fractions obtained from column and thin-layer chromatography (McGiff, Terragno & others, 1969).

The biologically active material was isolated and estimated after each stage of the purification procedures as shown in Fig. 1. The extraction of 453 g of dog renal

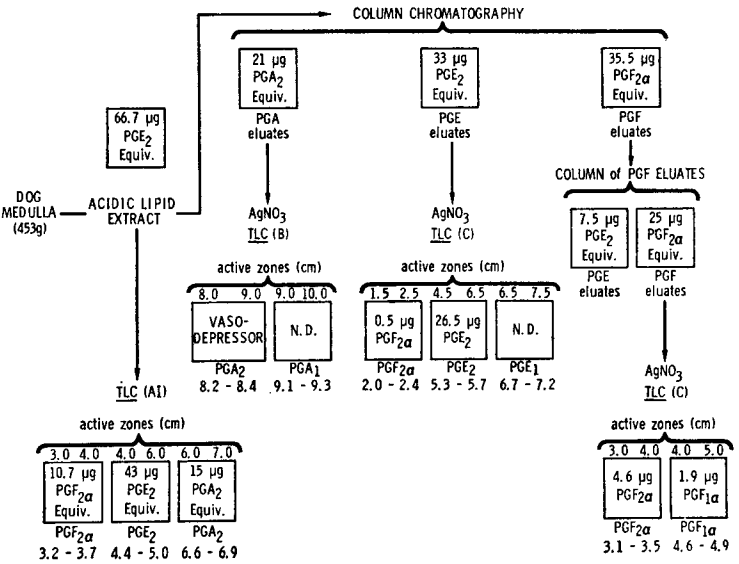

FIG. 1. Extraction and chromatographic separation of prostaglandins in 453 g of dog renal medulla. The prostaglandin-like activity isolated after each step of these procedures is shown in boxes. Silica gel TLC of a portion of the acidic lipid extract using solvent system AI (not equilibrated) yielded 3 biologically active zones. Silicic acid chromatography of the bulk of the extract also yielded a comparable separation, although re-chromatography of the PGF eluates was necessary to remove some PGE-contamination. Final TLC separation of the PGA, PGE and PGF eluates was effected on layers of silica gel containing $AgNO_3$ and using solvent systems B, C and C respectively (see text for solvent composition; solvent front 15 cm). Prostaglandin-like substances were extracted from certain zones characterized by their distance from the origin. Prostaglandin standards, run on the edge of each plate, were visualized after the unknowns were removed. Comparison of these zones with the position of prostaglandin standards (the mobilities are shown under each box in the figure) provides a tentative identification of these compounds. Important zones in which activity was not detected are indicated N.D.

medulla and the subsequent purification of the extract by column chromatography resulted in the recovery of 21 μg PGA_2 (0·033 μg/g), 40·5 μg PGE_2 (0·090 μg/g) and 25 μg $PGF_{2\alpha}$ (0·055 μg/g). These three prostaglandins have also been identified in rabbit renal medulla (Lee & others, 1967) although much higher prostaglandin concentrations were reported in the rabbit. The demonstration that prostaglandins are present in the kidney medulla of two species suggests that these biologically active compounds may be important renal hormones. The demonstration of PGE-like material in the renal venous blood of hypertensive humans (Edwards, Strong & Hunt, 1969) and in renal venous blood of dogs with experimentally induced ischaemia (McGiff & others, 1969) supports this possibility. Furthermore, in both cases, evidence indicated that the major prostaglandin constituent was PGE_2.

This work was supported by American Heart Association Grant 65 G 54, the Missouri Heart Association, U.S. Public Health Service Grants HE 11041, HE 0347, 1RO1 AM 13036 and General Research Support Grant 203.

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January 5, 1970

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Response of female mice to anticonvulsants after pretreatment with sex steroids

We have examined in female mice the effects of lynestrenol (progestin) and mestranol (oestrogen) on the intensity and duration of activity of a series of anticonvulsant drugs.

Rumke & Noordhoek (1969) had shown previously that pretreatment of mice with large doses of lynestrenol (20 and 200 mg/kg) 48 h before receiving either phenytoin or phenobarbitone resulted in decreased protection against bemegride convulsions, and increased metabolism of the drugs. Banziger (1965), and Swinyard & Castellion (1966) have demonstrated that the minor tranquillizers chlordiazepoxide (Librium) and diazepam (Valium) in high doses protect mice from tonic extensor seizure produced by maximal electroshock. Chlordiazepoxide is rapidly and extensively metabolized in mice (Coutinho, Cheripko & Carbone, 1968) and its anticonvulsant activity pattern follows closely the disappearance rate of the parent drug and its major metabolites from the brain and plasma. Since both lynestrenol and mestranol have marked and opposite effects upon the metabolism of certain barbiturates (Blackham & Spencer, 1969) it occurred to us that these effects may also occur with phenobarbitone, phenytoin, chlordiazepoxide, and diazepam.

For each anticonvulsant studied 5 groups of 10 female TO mice, weighing 20–25 g, were pretreated with progestin (lynestrenol, 10 mg/kg), or oestrogen (mestranol, 1·0 mg/kg), or their oily vehicle, daily, for four days. On the fifth day, each of the 5 groups received an intraperitoneal injection of one of the following anticonvulsant drugs; phenytoin sodium, 20 mg/kg; phenobarbitone sodium, 40 mg/kg; chlordiazepoxide hydrochloride, 80 mg/kg; or diazepam, 20 mg/kg. At various times afterwards, they were subjected to maximum electroshock, using a shock of 70 V at 100 pulses/s (pulse-width 0·2 ms), for a duration of 0·3 s. This was delivered through silver ear electrodes, according to the method of Cashin & Jackson (1962). The current was supplied by a Scientific Research Instruments' square-wave stimulator. This shock produced tonic extensor convulsions in 100% of all animals not receiving anticonvulsant drugs; each mouse was tested once only.

Our results are summarized in Fig. 1. Lynestrenol produced a reduction in intensity and duration of activity with all of the anticonvulsant drugs examined, whilst mestranol had the reverse effect. Administration of the microsomal enzyme inhibitor SKF 525A mimicked the effect of mestranol, increasing the protection of mice receiving these anticonvulsant drugs. The observed changes in activity of the above anticonvulsant drugs may be due therefore to alterations in their rate of metabolism.

There may be however an alternative explanation. We have already shown that marginal but opposite changes in brain 5-hydroxytryptamine levels occur in female