Nature and origin of the released prostaglandins following expansion of the blood volume

In a previous study concerning the effects of increasing the blood volume by either saline or blood infusions, in anaesthetized rats, using the blood bathed organ technique (Papanicolaou, 1972), prostaglandin-like substances (PLS) were detected during the subsequent rise of the blood pressure.

In experiments in which blood was cross-circulated in rats, the blood pressure of the recipient dropped while that of the donor rose following the increase of the circulating blood volume, produced by infusion of either saline or blood. The phenomenon was almost imperceptible when the animals were binephrectomized (Papanicolaou, 1972).

We have now investigated the release of PLS in normal and binephrectomized male Wistar rats by using the blood bathed organ technique. In addition, a further identification of the released PLS was made by their extraction from rat blood collected during expansion while the assay organs showed a prostaglandin-like activity (PLA), by chromatographic characterization and by quantitative bioassay estimation.

The rats had free access to food and water until the experiment began. The animals were anaesthetized intraperitoneally with 30 mg kg^{-1} of sodium ethylmethylbutylbarbiturate, their tracheae were catheterized and the animals were allowed to breath spontaneously, then they received 600 U kg^{-1} of heparin dissolved in 1·5 ml kg^{-1} of saline.

Blood was withdrawn from the right femoral artery of rats at the rate of 3·5 ml min^{-1}. The blood was analysed for its hormonal content by cascading over the assay organs. There were three assay organs, rat stomach strip (RSS), chick rectum (CR) and rat colon (RC), continuously superfused. The blood was collected into a reservoir and returned through a catheter to the right femoral vein of the animal at an identical rate by the same roller pump. The extracorporeal circuit was filled with blood collected from other rats before each experiment and thus the animals had not been volume-depleted from the outset. The arterial blood pressure was recorded through a polyethylene catheter (0·03 inch i.d.) inserted into the left femoral artery and connected to a strain gauge manometer (physiograph E & M instruments). The assay organs, loaded with 3 to 5 g, were suspended in polypropylene chambers from frontal writing levers and their contractions were recorded on a Palmer recording drum.

Fig. 1 shows the assay organs demonstrating a PLA following the rise of blood pressure. This was produced by the infusion of 8 ml of blood at the rate of 2 ml min^{-1}, in a normal rat. The contraction of the assay organs corresponded to that produced by standard PGE_2 applied directly to the assay organs by a Braun continuous-infusion pump at the rate of 21 ng min^{-1} (25 μl min^{-1}). The final concentration in the bathing blood was 6 ng ml^{-1}.

Fig. 1 also shows the pattern of response of the assay organs when a binephrectomized rat was used. The contraction of the assay organs corresponded to that produced by standard PGE_2 applied directly to the organs at the rate of 12 ng min^{-1} (25 μl min^{-1}). The final concentration in the bathing blood was 3·4 ng min^{-1}.

The results were the same when antagonists were used, then the blood was not returned to the rat but pooled blood from other rats was used to maintain blood volume.

The mean values of the PLS concentrations detected by the blood bathed organ technique in normal and binephrectomized rats, are also showed in Fig. 1. The released PLS were assayed as ng of PGE_2 equivalent. The difference between the mean values was found to be statistically significant; normal rats: 5·4 $\pm$ 0·3 ng ml^{-1}, n = 7; binephrectomized rats: 3·2 $\pm$ 0·3, n = 5, t = 4·9502, $P < 0{\cdot}025$ ($\pm$ = standard error of the mean).

To further identify the PLS released by this particular mechanism, blood was collected from rats before (control samples) and after expansion as follows: the assay organs were superfused as in Fig. 2. Blood (100 ml) collected from carotid arteries from several rats just before each experiment, was added in the reservoir B (Fig. 2). When both the assay organs and blood pressure showed a base-line level stability 25 ml of blood was collected in a plastic tube (reservoir A) as control sample. Then 8 ml of blood from the reservoir B was transferred in a plastic syringe and the rats were infused by a Braun continuous-infusion pump at the rate of 2 ml min^{-1} through a catheter inserted into the left femoral vein. When the assay organs showed a PLA following the expansion of the intravascular space, 25 ml of blood was collected (in the reservoir A) for extraction and chromatographic characterization of the biologically active substances which provoked the contraction of the assay organs.

The methods used for extraction, purification, chromatographic characterization and quantitative bioassay estimation of PGs were described in detail elsewhere (Papanicolaou, Makrakis & others, 1974).

The AI system used (Gréen & Samuelsson, 1964) separate groups of PGs (PGAs from PGEs and PGFs) but does not separate PGs according to their degree of unsaturation (PGE_1, from PGE_2 etc). Each group of PGs (PGA, PGE and PGF) was assayed as ng of PGA_2, PGE_2 and $PGF_{2\alpha}$, equivalent, respectively.

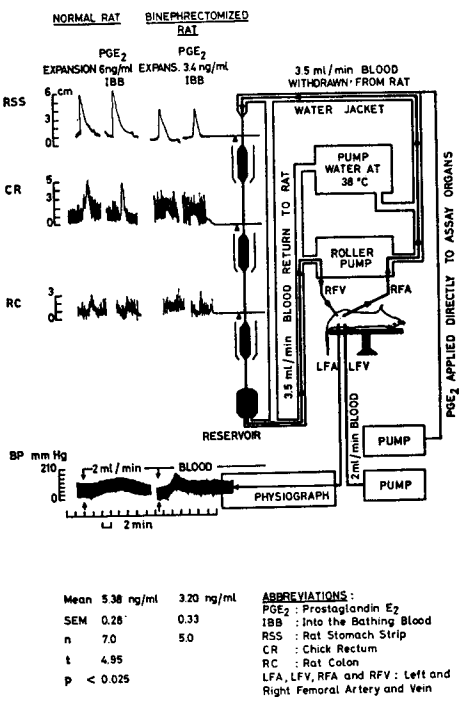

FIG 1

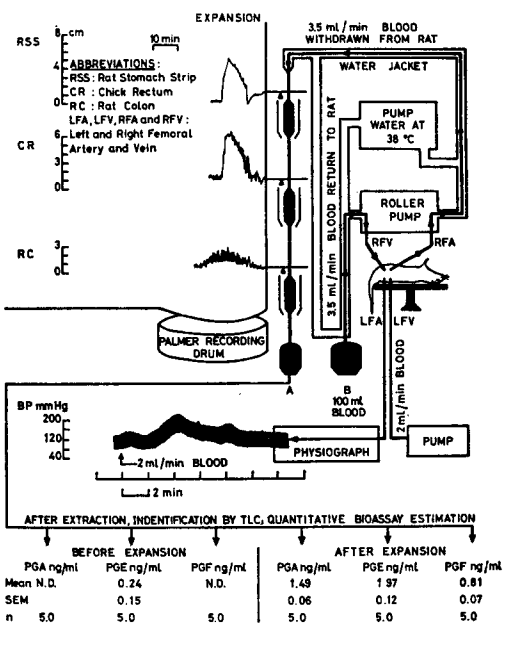

FIG 2

FIG. 1. Release of prostaglandin-like subatances (PLS), following expansion of the rat intravascular space. The difference between the mean values of the released PLS in normal and binephrectomized animals was found to be statistically significant.

FIG. 2. The detected prostaglandins (PGs) in rat blood collected before and after expansion (while the assay organs showed prostaglandin-like activity), after extraction, chromatographic identification and quantitative bioassay estimation.

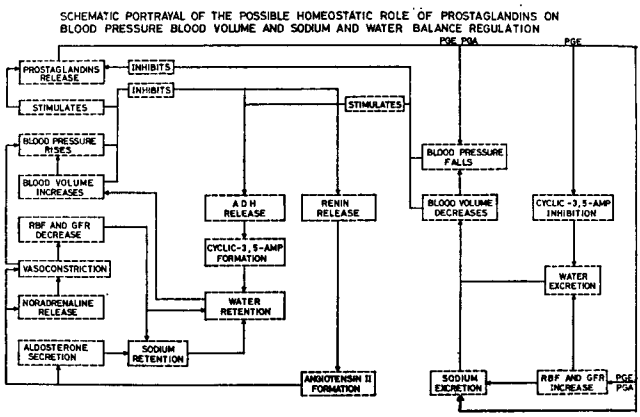

Fig. 2 shows the detected mean values of PGA, PGE and PGF, before (control) and after expansion (in the control samples, PGE was detected only in two cases).

The fact that PGA series was detected must be discussed. Several reports suggest that it may have originated by spontaneous conversion from PGE series (Hamberg, 1969; Hinman, 1972; McGiff, Crowshaw & Itskovitz, 1974), while others support the self-existence of this series (Zusman, Caldwell & others, 1972; Attallah & Lee, 1973; Jaffe, Behrman & Parker, 1973).

This study demonstrated that PLS were released although the animals were binephrectomized, but the detected amounts were less in the operated than in the normal rats. The difference between the mean values was statistically significant. Much of the released PGs could have come from the kidney.

The detection of PLS and mainly PGE-like substances in rat arterial blood is possible because the pulmonary vascular bed of the rat does not remove the PGE series at the same rate as that of the dog and cat (Ferreira & Vane, 1967; Horton & Jones, 1969; McGiff, Terragno & others, 1969; Papanicolaou & Meyer, 1972).

That much of the released PLS did come from the kidney was confirmed: (i) because of the detection of smaller quantities of PLS using the blood bathed organ technique, when the animals were binephrectomized [it is known that the technique used is more sensitive for detecting PGs than the rat blood pressure preparation (Horton & Jones, 1969; Papanicolaou, 1972)] thus it was possible to detect PLS using the blood bathed organ technique, but it was impossible to detect them by using the cross-circulation procedure with binephrectomized rats; (2) because of the detection of a high quantity of PGs in rat renal medulla (Papanicolaou & others, 1974).

The physiological importance of PGs release following expansion is shown in the schematic portrayal.

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Altered 5-HT metabolism with clonazepam, diazepam and diphenylhydantoin

Brain 5-hydroxytryptamine (5-HT) has been implicated in the control of both seizure threshold and action myoclonus, a disorder of movement usually caused by prolonged cerebral anoxia. Thus, in animals with experimental epilepsy, such as the photosensitive baboon or rodents with chemically or electrically induced fits, raising brain 5-HT elevates seizure threshold, while lowering brain 5-HT increases seizure susceptibility (Azzaro, Wenger & others, 1971; Killan & Frey, 1973; Wada, Balzamo & others, 1972). Furthermore, several conventional anticonvulsants, including diphenylhydantoin(DPH) have been shown to raise brain 5-HT (Bonnycastle, Giarman & Paasonen, 1957). In human epileptics "therapeutic" concentrations of phenobarbitone and DPH are associated with a rise in cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) which is particularly striking in clinically intoxicated patients (Chadwick, Jenner & Reynolds, 1975). In subjects with action myoclonus we have found CSF 5-HIAA to be low and treatment with 5-hydroxytryptophan dramatically relieves the abnormal movements (Chadwick, Harris, Reynolds & Marsden, unpublished results), as has been demonstrated by Lhermitte, Marteau & Degos (1972) and Van Woert & Sethy (1975).

Clonazepam, a benzodiazepine derivative with more potent anticonvulsant properties than diazepam in experimental models of epilepsy (Swinyard & Castillion, 1966; Killam, Matsuzaki & Killam, 1972) has recently been introduced into clinical practice for the treatment of epilepsy. In addition to its anticonvulsant action it has been found to be effective in the treatment of post-anoxic action myoclonus (Bourdourques, Roger & others, 1971). We have found it to be markedly more potent than diazepam or other anticonvulsants in this condition although its benefits appear to be transient. We have therefore examined the effect of clonazepam on brain 5-HT metabolism in the mouse and compared it with the effects of diazepam and DPH.

Male Swiss Albino mice (20–25 g; Animal Suppliers Ltd) were treated intraperitoneally with clonazepam (0·5–8·0 mg kg^{-1}; Rivotril, Roche Products Ltd), diazepam (2·0–32·0 mg kg^{-1}; Valium, Roche Products Ltd), diphenylhydantoin (2·5–40·0 mg kg^{-1}; Epanutin, Parke-Davis Ltd) or normal saline (0·1 ml). Animals were housed under conditions of standard laboratory lighting and temperature and were allowed free access to food and water. After 3 h animals were decapitated and the