The concept that the kidney may possess antihypertensive endocrine activity in various forms of experimental hypertension\(^1\)\(^-\)\(^3\) has stimulated investigations into the presence and localization of renal antihypertensive substances. Although vasodepressor substances have been found in extracts of whole kidney and kidney cortex,\(^4\)\(^-\)\(^5\) little evidence is available to suggest a physiological role in blood pressure regulation. On the other hand, Muirhead et al.\(^6\) have shown that extracts of renal medulla prevent the development of renoprival hypertension which otherwise occurs in the bilaterally nephrectomized animal.\(^7\)

Although the principle active in the prevention of renoprival hypertension possessed antihypertensive activity in established experimental hypertension, no vasodepressor effect was observed in intact normotensive animals.\(^8\)

Recently, crude homogenates of renal medulla have been shown to produce sustained blood pressure depression in the normotensive, vagotomized, pentolinium-treated rat.\(^9\) Although the material responsible for the vasodepressor effect is dialyzable, ethanol soluble, and of relatively low molecular weight, little is known of its chemical and physiological properties. Moreover, although blood pressure depression has been observed with rabbit, rat, pig, and human renomedullary extracts,\(^5\) the mechanism of the sustained depressor effect is obscure. Thus the present studies were undertaken to (1) isolate and characterize renomedullary vasodepressor substance(s), (2) determine the mechanism of their hypotensive action, (3) investigate their activity on nonvascular smooth muscle, and (4) compare the chemical and physiological properties of renomedullary vasodepressor substance(s) and prostaglandin E-1 (PGE-1). The results suggest that sustained lowering of blood pressure by extracts of renal medulla is the result of at least two unsaturated acidic lipids which are closely related derivatives of prostanoic acid. One substance is a hitherto unknown, highly unsaturated compound with potent vasodepressor effects, but with relatively weak activity in stimulating nonvascular smooth muscle. This material has been called medullin in contrast to the second renomedullary compound which has been identified tentatively as prostaglandin E-1 (PGE-1). Medullin appears to exert its hypotensive action by a direct effect on peripheral arterioles resulting in peripheral vasodilation and lowered peripheral resistance with a
compensatory increase of heart rate and cardiac output.

Methods

CHEMICAL STUDIES

Crude Homogenates

Specific chemical methods relating to the purification, isolation, and chemical characteristics of renomedullary depressor substance are described under the appropriate heading in the section on Results. Either fresh or quick-frozen rabbit medulla was the source material for all chemical and physiological experiments. For studies with fresh medulla, male albino rabbits of the New Zealand/Flemish strain, weighing between 3 and 4 kg, were killed by neck stroke followed by carotid exsanguination. The kidneys were rapidly removed, decapsulated, and placed on ice-cold petri dishes for dissection. Each kidney was quartered, and the medulla separated from the cortex by dissection through the cortical-medullary junction. The medulla represented about 80% inner medulla (papilla) and 20% outer medulla. Pooled medullary tissue was homogenized in ice-cold 0.005 M Na$_2$HPO$_4$ (1 g medulla/2 ml Na$_2$HPO$_4$), the homogenate centrifuged to remove cellular debris, and the supernate (containing vasodepressor activity) utilized for further procedures. For experiments with quick-frozen tissue, commercially available predissected medulla* was thawed and homogenized in 0.005 M Na$_2$HPO$_4$ by a glass hand-type homogenizer or, with large amounts of tissue, by means of a Waring blender. Homogenates of fresh or quick-frozen rabbit medulla were assayed for depressor activity by injection into the jugular vein of the pentobarbital-anaesthetized, vagotomized, pentolinium-treated rat as previously described. Mean carotid blood pressure was recorded kymographically.

Figure 1A shows that sustained renomedullary vasodepressor activity was present in the crude homogenate of both fresh and quick-frozen rabbit medulla. In order to determine its ethanol solubility, absolute alcohol was added to the crude homogenate to a final concentration of 80%. After centrifugation, the ethanol supernate was evaporated to dryness and the residue restored to the original homogenate volume with 0.005 M Na$_2$HPO$_4$. Figure 1B illustrates that the depressor material of both fresh and quick-frozen medulla was ethanol-soluble. In addition, the quick-frozen depressor substance(s) were dialyzable, of low molecular weight, and did not possess the chemical and rapid-acting depressor characteristics of nucleotides, findings previously observed with sustained vasodepressor material from fresh rabbit medulla. Therefore, it appeared that renomedullary depressor substance(s) present in the fresh medulla, were well preserved in commercially available frozen tissue. Except as noted, experiments were carried out with frozen medulla because of the greater amount of readily available material.

Column Chromatography

Column chromatography of crude ethanol extracts of medulla was performed with diethylaminoethyl ether-cellulose (DEAE-cellulose). For preparation of the column, 60 g DEAE-cellulose were sprinkled on 0.1 N NaOH, allowed to settle, and the supernate decanted. The resin was washed with distilled water, the pH adjusted to 4.5 with 10 N HCl, and allowed to settle. The supernate was decanted and the DEAE-cellulose washed with water, resuspended in 0.005 M Na$_2$HPO$_4$ buffer and poured into a 60 x 5 cm glass column under 100 cm water pressure (height of DEAE-cellulose approximately 30 cm). The column was washed repeatedly with 0.005 M Na$_2$HPO$_4$ until the eluate pH was 7.8 to 8.2.

Approximately 25 to 35 ml of medullary extract were placed on the column and eluted at 20°C by gradient elution: (A) 0.005 M Na$_2$HPO$_4$, (B) 0.12 M Na$_2$HPO$_4$/0.08 M NaH$_2$PO$_4$, and (C) 1.0 M NaH$_2$PO$_4$. Gradient elution was accomplished by a tandem arrangement of bottles containing solutions A, B, and C placed about

*Pel-Freeze Biologicals Inc., Rogers, Arkansas.

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four feet above the column head in order to insure adequate eluate flow. Ten-ml eluate fractions were collected in an automatic fraction collector with a total eluate volume of 800 to 1000 ml. After passage of approximately 300 ml of 0.005 M Na$_2$HPO$_4$ buffer (pH 8), the initial eluate (100 to 150 ml, possessing no depressor activity) appeared as a deep yellow band associated with a rise in pH from 8 to 10. Depressor material was recovered during passage of the next 250 to 350 ml. Cessation of elution of depressor activity was associated with a decrease in eluate pH from 10 to 6; no subsequent elution of depressor substance was observed. Table 1 illustrates the recovery of organic solids in a representative small scale experiment utilizing only 0.005 M Na$_2$HPO$_4$ elution; it is evident that sustained depressor material was recovered in fractions 40 to 52 with the exclusion of about 90% of the organic solids initially placed on the column. A similar tenfold purification was achieved in larger scale experiments utilizing gradient elution.

Thin-Layer Chromatography

Thin-layer chromatography of purified depressor substance was employed utilizing a Desaga-Brinkman apparatus. Glass plates, 20 x 5 cm (analytical) or 20 x 20 cm (preparative), were coated with 250 µ or 500 µ, respectively, of a slurry of 30 g silica gel G in 65 ml distilled water. In certain experiments, 2% silver nitrate was incorporated into the silica gel. The plates were allowed to dry in room air for fifteen minutes, activated at 100°F for one hour, and stored in a dessicator. Material was spotted with Hamilton syringes (50 µliters), dried in a cold air stream, and placed in sealed, filter paper-lined glass tanks containing the appropriate solvents. All organic solvents for extraction and partition as well as thin-layer chromatography were spectro-grade and redistilled before use.

The solvent systems used were:

I. Trimethylpentane 120
   Isopropyl alcohol 40
   Acetic acid 1
II. Benzene 80
    Dioxane 80
    Acetic acid 4
III. Ethyl acetate 110
     Acetic acid 30
     Methanol 35
     2, 2, 4 trimethylpentane 10
     Water 100
IV. Petroleum ether 90
    Diethyl ether 10
    Acetic acid 1
V. Chloroform 65
   Methanol 25
   Acetic acid 8
   Water 4
VI. Ethyl acetate 160
    Methanol 3
    Water 100

System I was adopted from Eneroth, systems II and III from Grén and Samuelsson, system IV from Vogel et al., and system V from Skipski et al. Systems III and VI were allowed to equilibrate for one hour and the less polar phase used. The plates were allowed to develop by the ascending technique to 10 cm at 20°C with a total development time of 20 to 40 minutes. After development, the plates were allowed to dry and visualized by ultraviolet light.

<table>
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<tr>
<th>Eluate number</th>
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<th>Pooled volume</th>
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<th>Ash weight</th>
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<tr>
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<td>34</td>
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<tr>
<td>Total off column</td>
<td>—</td>
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<td>395</td>
<td>204</td>
<td>191</td>
</tr>
</tbody>
</table>

*Column: 17 g DEAE-cellulose packed under 100 cm water pressure. Column height, 25 cm; width, 3 cm. 80% ethanol extract of 7 g wet wt medulla dried under nitrogen and reconstituted to 6.0 ml with .005 M Na$_2$HPO$_4$, 5.0 ml on column. Elution: .005 M Na$_2$HPO$_4$; ℃65: 10 ml eluate fractions pooled to 130 ml as indicated.
iodine vapors, charring following 50% H₂SO₄ spray, or by spraying with 10% phosphomolybdic acid in 95% ethanol and heating at 125°C for 20 minutes.

The spots were removed with a spatula into an ultratine sintered glass funnel, and the material eluted with 3 ml x 3 of methanol. Since the biologically active substances could not be completely extracted with less polar organic solvents from a dried methanol residue, the methanol was evaporated to about 1 ml, diluted to 10 ml with distilled water, acidified with hydrochloric acid, and extracted five times with chloroform, benzene, or diethyl ether (v:v). The organic phases were washed with distilled water until neutral and evaporated to a small volume. This extract was used for rechromatography or, in the case of the pure material, for determination of chemical and physiological properties.

CARDIOVASCULAR STUDIES

Intact Animals

In the first series of experiments, the direct effect of renomedullary depressor substance and prostaglandin E-1 (PGE-1)* on peripheral resistance was determined in ten dogs by measuring the change in femoral blood flow and arterial pressure following intra-arterial injection of these compounds. Adult mongrel dogs were anaesthetized with pentobarbital sodium (30 mg/kg iv) and the left femoral artery was cannulated for the measurement of blood pressure by means of a pressure transducer. The right carotid and right femoral arteries were then exposed and cannulated, so that blood flow was diverted by means of polyethylene tubing from the carotid artery through a Shipley-Wilson rotameter into the distal end of the femoral artery. This provided a continuous measurement of blood flow to one hind limb of the dog. Simultaneous recordings of the blood pressure and blood flow were made on a Sanborn two-channel recorder. From these measurements, peripheral resistance was calculated:

\[ \text{PRU} = \frac{\text{mean blood pressure, mm Hg}}{\text{blood flow, ml/min}} \]

After control observations, either renomedullary depressor substance or PGE-1 was administered through a catheter in the jugular vein. At the time of maximal hypotensive effect, indocyanine green was administered intravenously for repeat determination of cardiac output.

Isolated Heart and Aortic Strips

The direct effect of renomedullary depressor substance on the isolated rabbit heart was studied in a group of seven rabbits. Rabbits were stunned by neck stroke, and the heart removed and placed in a beaker of aerated Krebs-Ringer bicarbonate buffer. The aorta was cannulated and attached to a Carver-Anderson coronary perfusion apparatus. The hearts were perfused with Krebs-Ringer bicarbonate buffer (glucose 10 mM), aerated with 95% O₂ to 5% CO₂, and maintained at a constant temperature of 37°C. In order to measure cardiac contractility, a ligature was passed through the apex of the left ventricle and attached, in turn, to a Grass force displacement transducer; contractile force was then recorded by means of a Sanborn recorder. Injections of depressor substance were made into the perfusion fluid through the aortic cannula in such a fashion that dilution of depressor activity by the perfusion fluid was minimal. The effect of 0.5 ml (50 μg) of renomedullary depressor substance on heart rate and myocardial contractility was compared with the effect of 0.5 ml of 1% potassium chloride and 0.5 ml of 1% calcium chloride.

For studies on isolated aortic strips, rabbits were killed by neck stroke and sections of thoracic aorta were removed, cut into helical strips, and suspended in a 10-ml bath containing oxygenated Tyrode solution.

NONVASCULAR SMOOTH MUSCLE STUDIES

Nonvascular smooth muscle studies were performed in a group of ten dogs. A catheter was passed into the aorta by way of the left femoral artery, and aortic blood pressure measured by means of a pressure transducer. The right carotid artery was cannulated for the purpose of obtaining blood samples for measurements of cardiac output according to the dye dilution technique. Indocyanine green* was injected intravenously via the jugular vein, and blood concentration curves obtained by means of a Gilson densitometer. Total peripheral resistance (TPR) was calculated:

\[ \text{TPR (total peripheral resistance)} = \frac{\text{mean aortic blood pressure, mm Hg}}{\text{cardiac output, ml/min}} \]

After control measurements of cardiac output and blood pressure, either renomedullary depressor substance or PGE-1 was administered through a catheter in the jugular vein. At the time of maximal hypotensive effect, indocyanine green was administered intravenously for repeat determination of cardiac output.

*For these studies, crystalline PGE-1 was generously supplied by Professor Sune Bergström of the Karolinska Institute, Stockholm, Sweden.
formed utilizing isolated segments of rabbit jejunum; rat duodenum, stomach, uterus, bladder, vas deferens, seminal vesicle; and terminal guinea pig ileum. With the exception of isolated uteri, the tissues were suspended in 10 ml of oxygenated Tyrode solution at either 30 or 37°C. Uteri were removed from rats which received 500 μg/kg of stilbesterol, sc, 18 hours before use and were suspended in 10 ml of Jalon's solution at 37°C. All tissues were allowed to equilibrate with the media for one hour before use; isotonic contractions were recorded at one gram resting tension and eightfold magnification.

Results

CHEMICAL STUDIES

Acidic Lipid Properties

Previous observations had established that renomedullary vasodepressor substance(s) were dialyzable, ethanol soluble, of relatively low molecular weight (< 4500 mol wt), and resistant to a mixture of peptide hydrolases. In addition, they possessed chemical and physiological characteristics distinct from those of renomedullary nucleotides which produce short-acting vasodepressor effects.

In order to determine whether the active material was lipid soluble, fresh medullary slices were prepared with a Stadie-Riggs microtome and 1 g of slices placed in each of two 40-ml capped test tubes containing 20 ml chloroform-methanol (2:1, v:v). They were allowed to stand overnight and the tissue was then removed and discarded. Four ml 0.018 N H₂SO₄ were added to one chloroform extract and four ml 0.01 N NaOH to the second extract. The tubes were inverted slowly and allowed to stand according to the method of Bragdon. They were then centrifuged and the acid and alkaline aqueous phases (pH 3 and 9 respectively) aspirated, dried under nitrogen to a volume of 2 ml, and neutralized for assay in the pentolinium-treated rat. The chloroform phase was also dried and the residue redissolved in 2 ml 0.2 M sodium phosphate buffer (pH 8) for assay. Figure 2A illustrates that the vasodepressor substance(s) partitioned almost completely into chloroform from an acid-methanol phase. However, in an alkalinized chloroform-methanol extract, most of the vasodepressor activity was recovered in the alkaline-methanol phase (fig. 2B).

In order to compare the lipid solubility of vasodepressor substance(s) extracted directly from fresh medullary tissue with that of purified depressor substance(s), 1 ml of eluate, after DEAE-cellulose chromatography, was injected forcefully into each of two tubes containing chloroform-methanol (2:1, v:v). The chloroform-methanol extracts were acidified and alkalinized as described with fresh medulla. Figure 3A reveals purified depressor substance(s) dialyzable, ethanol soluble, of relatively low molecular weight (< 4500 mol wt), and resistant to a mixture of peptide hydrolases. In addition, they possessed chemical and physiological characteristics distinct from those of renomedullary nucleotides which produce short-acting vasodepressor effects.
activity was recovered in the organic phase from acidified chloroform-methanol, and in the alkaline-methanol phase from alkalinized chloroform-methanol (fig. 3B), findings similar to a direct lipid extract of fresh medulla.

Figure 4 illustrates the effect of pH on the partition of purified vasodepressor substance(s) between aqueous and certain organic phases. It is evident that partition into petroleum ether is relatively minimal at all hydrogen ion concentrations, while partition into benzene, chloroform, and ethyl acetate is increased by a progressive reduction in pH. Although partition into benzene, chloroform, and ethyl acetate is almost complete at pH 1 to 2, recovery of depressor activity in the organic phase at any given higher pH appears to be greater as the polarity of the organic phase increases (ethyl acetate > chloroform > benzene). These studies indicate that the substance(s) responsible for sustained lowering of blood pressure are relatively polar acidic lipids. The acidic lipid soluble residue which remained after evaporation of benzene, chloroform, and ethyl acetate appeared as a yellow oil. Depressor activity could be recovered from this material after extraction with saturated NaHCO₃, 5% Na₂CO₃, as well as 1 n NaOH.

**Purification**

Figure 5 illustrates schematically the preliminary purification procedures which were based on the known ethanol soluble, strongly acidic lipid characteristics of renomedullary depressor substance. An 80% ethanol extract of 3 to 5 kg of wet weight medulla was distilled in vacuo to 2 to 3 liters. After adjustment of the pH to 7.5, the ethanol extract was partitioned 3X against petroleum ether (v:v) and the petroleum ether discarded. The aqueous-ethanol phase was then distilled in vacuo to 300 to 400 ml (phase I extract) and column chromatographed on DEAE-cellulose by gradient elution as described in the section under Methods. The pooled DEAE eluate was acidified and extracted with benzene (chloroform or ethyl acetate) and reduced to a small volume. From 3 to 4 kg wet weight frozen medulla, 100 to 150 mg of oily material were recovered, which was extremely active when assayed for depressor effects. This material was redissolved in buffer.
MEDULLIN: CHEMICAL AND PHYSIOLOGICAL PROPERTIES

PREPARATION OF MEDULLIN FROM FROZEN RABBIT MEDULLA

1. Weigh 4 kg wet weight medulla
   Homogenized in .005 M Na\textsubscript{2}HPO\textsubscript{4} (100 ml/200 g medulla)
   Centrifuged 10,000 x g. Sediment discarded
   Supernate + absolute ethanol (final concentration 80%)
   Filtered, & precipitate discarded
   Supernate distilled in vacuo to 2-3 l. at 35 °C
   pH adjusted to 7.5
   Extracted 3 x with petroleum ether
   Pet. ether phase discarded
   (no depressor activity)

2. Aqueous-ethanol phase distilled in vacuo to 300-400 ml
   Column chromatographed: DEAE-cellulose, gradient elution
   Eluate containing depressor activity pooled
   Acidified to pH 1-2
   Extracted 3 x with benzene, chloroform or diethyl ether
   Organic phase discarded
   (no depressor activity)

3. Aqueous eluate distilled in vacuo to 10 ml
   Yellow oil (100-150 mg)
   Organized phase dried under nitrogen
   Redissolved in phosphate buffer (100 μg/ml)
   Maximum rat depressor effect: 5 μg

**FIGURE 5**
Schematic representation of sequential steps utilized in the purification and isolation of renomedullary depressor substance (medullin).

for physiological studies (phase II extract) or was utilized directly as the free acid for isolation of the pure material (medullin) by thin-layer chromatography (phase III).

**Thin-Layer Chromatographic Isolation**

Initial observations revealed that vasodepressor activity was recovered only at the origin in a neutral lipid solvent system (system IV). No activity was observed in areas corresponding to cholesterol, cholesterol esters, free fatty acids, diglycerides, or triglycerides. In a solvent system utilized for phospholipid separation (system V), vasodepressor material was recovered only at the solvent front. These experiments suggested that renomedullary depressor substance(s) did not appear to be an unsubstituted fatty acid or phospholipid with thin-layer mobilities which resemble known serum neutral lipids or phospholipids.

For the isolation of depressor substances, a combination of two successive thin-layer chromatographic systems were employed. Figure 6 illustrates the results of chromatography in system I of phase III extract, prepared by extraction of acidified, pooled DEAE-eluate with benzene. Depressor activity was recovered only in spot 3 which was an intensely iodine-staining, nonfluorescent spot with an Rf of 0.48. In system II, the Rf of spot 3 was 0.57 to 0.60, which is represented as spot 4 in figure 7. In the initial chromatogram, no depressor activity was observed in other spots, unless phase III extract was prepared by extraction of pooled DEAE-eluate with chloroform or ethyl acetate rather than benzene. Figure 7 illustrates the results of chromatography in system II under such conditions. As noted, spot 4 (Rf = 0.57 to 0.60) was an intensely iodine-staining spot.
with marked vasodepressor activity which corresponded to spot 3 in system I (fig. 6). In addition, marked depressor activity was also observed in spot 2, with very weak activity present in spot 1; these were less mobile, nonfluorescing spots with weak iodine-staining properties. The ability to stimulate isolated rabbit jejunum was an additional biological activity present in spots 1 and 2, and to a lesser extent in spot 4. It appeared therefore, that rabbit medulla possessed at least two acidic lipids capable of lowering of blood pressure and stimulating nonvascular smooth muscle, while a third compound displayed only nonvascular smooth muscle stimulating properties. In the present study, the material of primary interest was the relatively mobile, intensely iodine-staining substance

![Figure 6](https://circres.ahajournals.org/content/17/3/64.f6)

**FIGURE 6**
Thin-layer chromatography of phase III renomedullary extract prepared by extraction of acidified pooled DEAE-eluate with benzene. System I.

<table>
<thead>
<tr>
<th>Spot</th>
<th>Rf</th>
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<th>U-V Fluorescence</th>
<th>Depressor Activity</th>
<th>Spot Number</th>
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</tbody>
</table>

![Figure 7](https://circres.ahajournals.org/content/17/3/64.f7)

**FIGURE 7**
Thin-layer chromatography of phase III renomedullary extract prepared by extraction of acidified pooled DEAE-eluate with chloroform. System II. PMA: phosphomolybdic acid.
(spot 3, system I; spot 4, system II) with marked depressor, but relatively weak intestinal smooth muscle stimulating properties. This material has been designated "medullin." For comparative purposes, the more polar substances are referred to as compounds 1 and 2, utilizing spots 1 and 2, respectively, of the chromatogram in figure 7 as reference. The Rf values of these compounds are summarized in table 2.

Ultraviolet and Infrared Spectra
The ultraviolet (UV) absorption of pure medullin (57 µg/ml methanol) is shown as the solid line in figure 8A. A very weak maximum is evident at 2780 to 2800Å. The dashed line (fig. 8A) illustrates the UV spectrum of a solution of 51 µg medullin in 1 ml 96% H₂SO₄, which was allowed to stand at 22°C for four hours. Two strong maxima were observed at 2580 to 2600Å and at 3200 to 3220Å, with an inflexion point at approximately 3600Å. Although not shown, a third maximum was present in the visible range associated with the development of yellow color during the period of acid treatment. The solid line in figure 8B shows the UV spectrum of 55 µg medullin following treatment with 1 ml of 0.01 N NaOH for one hour at 100°C; a strong absorption at 2800 to 2810Å was evident. After treatment of this solution with excess 3 N HCl, the previously observed maximum at 2800Å was no longer evident, and a weak maximum appeared at 3280 to 3310Å (dashed line, fig. 8B).

Figure 9 illustrates an infrared spectrum obtained on a solution of medullin in chloroform (3.5 mg/ml). The following characteristic peaks were present: 3470(w), 2890(s), 2820(m), 1700(s), 1410(m), and 972(m) cm⁻¹. In addition to the well-defined peak at 1700, an inflexion point was present at approximately 1725 cm⁻¹.

Comparison with Prostaglandin E₁
The chemical, chromatographic, and spectral properties of medullin closely resembled those of a recently isolated series of biologically active acidic lipids called prostaglandins E₁ (PGE). Although medullin appears closely related to these substances, figure 10 illustrates important chromatographic differences between these compounds. It is evident that medullin has a significantly greater mobility than PGE-1, a characteristic which has been found in a variety of solvent systems (table 2). In certain experiments, 2% silver nitrate was incorporated into the silica gel, conditions

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4000 3000 2000 1500 1000 900 800 700 600 500 4000 3000 2000 1500 1000 900 800 700

**FIGURE 8**
Ultraviolet spectra of (A) medullin (solid line) and chromophores derived after treatment with 96% H₂SO₄ (dashed line); (B) following heat treatment with 0.01 N NaOH before (solid line), and after addition of 3 N HCl (dashed line). Perkin-Elmer spectrophotometer, model 202.
which separate derivatives of PGE and prostaglandins F (PGF) according to the degree of unsaturation. Medullin consistently ran as a single spot in a more mobile position than PGE, confirming the homogeneity of the isolated material. Since all known unsaturated compounds of PGE and PGF are less mobile than PGE-1, it is evident that the more mobile unsaturated medullin represents an unknown although closely related compound. In addition to its characteristic mobility, medullin was much more intensely iodine-staining than PGE-1. In contrast to medullin, medullary compound-2 displayed weak iodine-staining characteristics and greater smooth muscle-stimulating ability (fig. 7). In addition, compound-2 revealed identical chromatographic mobilities as PGE-1 in several solvent systems, with and without added silver nitrate (table 2).

**CIRCULATORY STUDIES**

**Intact Animals**

Peripheral blood flow studies were carried out in eight dogs with partially purified medullin (phase II extract)* and in two dogs with chromatographically pure medullin and PGE-1. Additional studies were not possible with pure medullin and PGE-1 because of the limited amount of available material.

Figure 11 shows a typical response following the intra-arterial injection of 50 µg of partially purified medullin. Femoral blood flow rose promptly, followed by a slight reduction of arterial blood pressure. The average changes in mean arterial blood pressure,

*"Partially purified" medullin contained several substances in addition to medullin. Individually, however, none of these substances possessed biologic activity when isolated by thin-layer chromatography (fig. 6). Observations of biological activity with partially purified medullin, therefore, represent only the activity associated with chromatographically pure medullin.
blood flow, and peripheral resistance following the intra-arterial injection of partially purified medullin, pure medullin, and PGE-1 are presented in table 3. The predominant effect was a significant increase of femoral arterial blood flow accompanied by a slight reduction of arterial blood pressure. The relationship between these changes of blood flow and blood pressure was such that the calculated resistance of the peripheral vasculature decreased significantly. The only difference between partially purified medullin, pure medullin, and PGE-1 was one of potency, i.e., 0.5 μg of pure medullin or PGE-1 was equivalent to 50 μg of partially purified medullin.

An attempt was made to obtain a dose-effect relationship between intra-arterial injections of medullin and arterial blood flow. This was not possible in all cases since, in some dogs, the arterial pressure failed to return to the pre-injection control level after repeated administration of medullin. However, in several animals, the blood pressure remained sufficiently constant throughout the experimental procedure to permit a dose response study. Figure 12 shows the relationship between varying doses of partially purified medullin and femoral arterial blood flow in a representative experiment. It is evident that the increase of blood flow was directly proportional to the dose injected until maximum flow occurred.

The systemic hemodynamic effects of partially purified medullin were evaluated in an
additional series of experiments on eight dogs. The mean values for aortic pressure, cardiac output, and total peripheral resistance, before and after administration of partially purified medullin, are shown in figure 13. In all eight animals, there was a significant decrease in mean aortic pressure which persisted for 10 to 17 minutes before returning to control levels. The decrease in mean aortic pressure was almost exclusively the result of a fall in diastolic pressure which decreased an average of 40 ± 6 mm Hg. The reduction of systolic pressure was statistically insignificant and the net result was a marked increase of pulse pressure. At the time of maximum decrease in mean aortic pressure, a 30% increase in cardiac output was observed. Thus, the total peripheral resistance decreased significantly in all eight experiments. An average increase in heart rate of 18.5 ± 5.0 beats/min was also observed after treatment with partially purified medullin. However, the acceleration of heart rate occurred after the establishment of maximal blood pressure depression suggesting a tachycardia of reflex origin. From the heart rate and cardiac output data, stroke volume was calculated and found to increase an average of 2.2 ± 0.6 ml/beat following injection of partially purified medullin.

A comparison of the systemic hemodynamic effects of partially purified medullin with pure medullin and PGE-1 is shown in table 4. In these studies, the initial mean, systolic, and diastolic pressures were considerably higher than in experiments with partially purified medullin. Injection of pure medullin or PGE-1 under these conditions reduced systolic pressure from 176 to 151 mm Hg. Again, however, the decrease in diastolic blood pressure was greater and pulse pressure increased. As in the experiments with partially purified medullin, there was an increase in cardiac output with a resultant 26 to 30% decrease in total peripheral resistances. No differences were observed between pure medullin and PGE-1. The only difference between the pure compounds and partially purified medullin was one of potency: 10 μg of pure medullin elicited responses similar to that produced by 300 μg of partially purified medullin. Figure 14 illustrates the dose response relationship between pure medullin and PGE-1 on blood pressure reduction. Although the number of experiments with pure medullin or PGE-1 were limited by the amount of material available, it would appear that maximum hypotensive effect with both compounds.

Hemodynamic effects of medullin in the dog. Phase II extract. n = 8. See text for experimental details.
### TABLE 4

**Effect of Partially Purified Medullin, Pure Medullin, and PGE-1 on Aortic Pressure, Cardiac Output, and Total Peripheral Resistance in the Intact Dog**

<table>
<thead>
<tr>
<th>Partially purified medullin (300 µg iv) n = 8</th>
<th>Control</th>
<th>Postinjection</th>
<th>Mean difference</th>
<th>Per cent change</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean aortic pressure, mm Hg</td>
<td>143 ± 7</td>
<td>120 ± 6</td>
<td>-23 ± 4</td>
<td>- 16</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>161 ± 6</td>
<td>155 ± 6</td>
<td>-6 ± 6</td>
<td>- 4</td>
<td>&gt;0.3</td>
</tr>
<tr>
<td>Diastolic pressure</td>
<td>126 ± 5</td>
<td>86 ± 7</td>
<td>-40 ± 6</td>
<td>- 32</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>34 ± 2</td>
<td>69 ± 9</td>
<td>+35 ± 8</td>
<td>+106</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cardiac output, liters/min</td>
<td>2.20 ± 0.27</td>
<td>2.85 ± 0.27</td>
<td>+0.65 ± 0.1</td>
<td>+ 30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total peripheral resistance, PRU</td>
<td>0.074 ± 0.01</td>
<td>0.048 ± 0.003</td>
<td>-0.026 ± 0.006</td>
<td>- 35</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

| Pure medullin (10 µg iv) n = 2              |         |              |                |                |    |
| Mean aortic pressure, mm Hg                | 158     | 129          | -29            | - 18           |    |
| Systolic pressure                          | 176     | 151          | -25            | - 14           |    |
| Diastolic pressure                         | 140     | 106          | -34            | - 24           |    |
| Pulse pressure                             | 36      | 46           | +10            | + 28           |    |
| Cardiac output, liters/min                 | 2.9     | 3.1          | +0.2           | + 7            |    |
| Total peripheral resistance, PRU            | .058    | .043         | -0.015         | - 26           |    |

| PGE-1 (10 µg iv) n = 2                      |         |              |                |                |    |
| Mean aortic pressure, mm Hg                | 156     | 126          | -30            | - 19           |    |
| Systolic pressure                          | 175     | 150          | -25            | - 14           |    |
| Diastolic pressure                         | 139     | 103          | -36            | - 26           |    |
| Pulse pressure                             | 36      | 46           | +10            | + 25           |    |
| Cardiac output, liters/min                 | 2.6     | 2.9          | +0.3           | + 10           |    |
| Total peripheral resistance, PRU            | .066    | .046         | -0.020         | - 30           |    |

*P: significance determined by Student's t test utilizing paired data analysis.
occurred at a dose between 10 and 50 \( \mu g \) (1.0 to 5.0 \( \mu g/kg \)).

**Isolated Hearts and Aortic Strips**

Studies were made on the isolated rabbit heart and isolated aortic strips. Table 5 illustrates that no change in either heart rate or contractile force was observed following the direct injection of 0.5 ml of partially purified medullin into the aortic cannula of the isolated rabbit heart. On the other hand, 0.5 ml of 1\% calcium chloride produced a mean increase in amplitude of contraction of 28.3 ± 3.4 mm, while 0.5 ml of 1\% potassium chloride resulted in a mean decrease of 18.4 ± 2.3 mm. Typical responses of the isolated rabbit heart are shown in figure 15.

High concentrations of crude medullary extract produced very weak contractions of isolated aortic strips. However, pure medullin at concentrations of 0.1-0.3 \( \mu g/ml \) produced no stimulation of such strips, nor did it result in relaxation of strips partially contracted with either norepinephrine or angiotensin.

**NONVASCULAR SMOOTH MUSCLE STUDIES**

Because of the direct effect of these renomedullary substances on vascular smooth muscle, their effect on nonvascular smooth muscle was examined. Crude ethanol extracts of rabbit medulla, possessing depressor activity, produced strong contractions of isolated segments of rabbit jejunum, and rat duodenum, stomach, and uterus; absent or weak contractions were observed with terminal guinea pig ileum, rat bladder, vas deferens, and seminal vesicle. It appeared that the isolated rabbit jejunum was particularly responsive to medullary extracts and this preparation was subsequently employed for assaying the nonvascular smooth muscle stimulating activity.

Figure 16 shows the results when crude medullary extracts were column-chromatographed on DEAE-cellulose as previously described and the eluate fractions assayed for both depressor and intestinal smooth muscle-stimulating activity. Although both hypotensive and nonvascular smooth muscle-stimulating activities were observed in fractions 27 to 60, it is apparent that considerable intestinal smooth muscle-stimulating activity without associated depressor activity was initially eluted in fractions 19 to 25. Fractions 27 to

<table>
<thead>
<tr>
<th>TABLE 5</th>
<th>Effect of Medullin on the Isolated Perfused Rabbit Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>Control: 98 ± 6</td>
</tr>
<tr>
<td>Amplitude of contraction, mm</td>
<td>Control: 30.8 ± 3.7</td>
</tr>
</tbody>
</table>

Perfusion media: Krebs-Ringer bicarbonate buffer, glucose 10 \( \mu M \), 37°C aerated with 95\% \( O_2 \)-5\% \( CO_2 \). \( n = 7 \), with SEM.

*50 \( \mu g \) medullin injected into the aortic cannula.
MEDULLIN: CHEMICAL AND PHYSIOLOGICAL PROPERTIES

Effect of partially purified medullin (phase II extract) and potassium chloride on contractile force and heart rate in the isolated perfused rabbit heart. Perfusion media: Krebs-Ringer bicarbonate buffer, glucose 10 mM. Gas phase: 95% O₂-5% CO₂. Typical experiment.

60 were pooled, acidified, extracted with chloroform, and the organic phase subjected to thin-layer chromatography. As previously indicated (fig. 7), nonvascular smooth muscle-stimulating activity was present in three chromatographically distinct substances: compound 1, compound 2, and, to a much lesser extent, medullin.

Concentration-effect curves for a crude ethanol extract of rabbit medulla, medullin, PGE-1, and acetylcholine are shown in figure 17. The typical response of rabbit jejunum to these substances is shown to consist of an initial phase of rapid contraction followed by progressive relaxation. A comparison of the potency of medullin, PGE-1, and acetylcholine reveals that the activity of PGE-1 was equal to acetylcholine, as evidenced by superimposable concentration-effect curves. On the other hand, medullin, a compound equal to PGE-1 in vasodepressor activity, was approximately 1/50th as potent as PGE-1 in producing stimulation of nonvascular smooth muscle. Analysis of the relative potencies of pure medullin and relatively crude renomedullary extract (phase I) indicates a purification of approximately one-hundredfold.

Discussion

Original studies in 1963 revealed that crude extracts of rabbit, rat, pig, and human renal medulla contained substance(s) which resulted in a sustained lowering of blood pressure when injected into the vagotomized, pentobarbital-anaesthetized, pentolinium-treated rat. The present study demonstrates that the depressor activity of such crude homogenates is soluble in the lipid phase after direct lipid extraction of medullary tissue or acidified renomedullary extract, an observation recently reported by Hickler et al. In addition, such fractions possessed nonvascular smooth muscle-stimulating properties. Assay of the eluate fractions after column chromatography revealed differing eluate recovery of vasodepressor and nonvascular smooth muscle-stimulating activities suggesting the presence of more than one active substance, each with differential effects upon blood pressure and nonvascular smooth muscle. After final isolation by thin-layer chromatography, three substances were found to be principally responsible for the vasodepressor and nonvascular smooth muscle-stimulating properties of the crude homogenate. These substances have been designated, “medullin,” “compound 1,” and “compound

![Figure 15](image1)

![Figure 16](image2)

*Figure 15* Effect of partially purified medullin (phase II extract) and potassium chloride on contractile force and heart rate in the isolated perfused rabbit heart. Perfusion media: Krebs-Ringer bicarbonate buffer, glucose 10 mM. Gas phase: 95% O₂-5% CO₂. Typical experiment.

*Figure 16* Eluate recovery of intestinal smooth muscle-stimulating and vasodepressor activity after gradient elution of crude medullary extract on DEAE-cellulose. 10 ml eluate fractions.
All three substances stimulate intestinal smooth muscle, but only medullin and compound 2 possess significant activity in lowering blood pressure.

At the present time, there are several classes of naturally occurring acidic lipids which are known to stimulate either nonvascular smooth muscle and/or lower blood pressure. "Darmstoff," extracted from the intestine is probably acetalphosphatidic acid; this compound stimulates intestinal smooth muscle, but possesses little depressor activity. G-acid, and hemolytic acid, from human plasma, are straight chain unsaturated monocarboxylic acids without hydroxyl or keto groups and are probably Δ-3-octadecenoic and cis-Δ-11-octadecenoic acid (cis-vaccenic acid) respectively. Slow reacting substance A from guinea pig lung produces only slow contraction of isolated nonvascular smooth muscle.

The most well-defined group of lipid soluble acids which possess vasodepressor and nonvascular smooth muscle activity is a recently isolated series of compounds referred to as prostaglandins. Of the various known biologically active acidic lipids, medullin and compounds 1 and 2 appear to belong to this general class of compounds. The vasodepressor and smooth muscle-stimulating activities associated with prostaglandins were first independently described in human seminal fluid and sheep vesicular glands by Goldblatt and von Euler. In further investigations, the activity was found to depend on an acidic lipid that was named prostaglandin by von Euler. More recently, Bergström et al. have isolated and identified a series of such compounds including PGE-1, PGE-2, and PGE-3, which structurally are unsaturated, hydroxylated, ketonic derivatives of the parent 20 carbon, five-membered ring compound, prostanoic acid. Figure 18 illustrates the structure of PGE-1 which is a carboxylic acid containing a cyclopentanone ring (C8-12), two hydroxyl groups (C11, 15), one keto group (C9), and one trans double bond (C13-14). Reduction of the keto group in the PGE compounds results in the formation of PGF-1, PGF-2, and PGF-3 each of which may exist in two stereo-isomeric forms. The widespread occurrence of such compounds is evidenced by their isolation from such sources as sheep seminal vesicles, sheep and pig lung, human and sheep seminal plasma, bovine brain, calf thymus, human menstrual fluid, and sheep iris. The PGE compounds possess both vasodepressor and nonvascular smooth muscle-stimulating activity while the PGF substances produce only nonvascular smooth muscle stimulation.

**CHEMICAL PROPERTIES OF MEDULLIN**

Of the three biologically-active compounds isolated from rabbit renal medulla in the present study, the compound of major interest was medullin. Medullin was observed to be an ethanol soluble compound which was recovered in the organic phase by direct lipid extraction from an acid aqueous phase, but only incompletely from an alkaline aqueous phase. In addition, medullin was extracted by saturated NaHCO₃ and 5% Na₂CO₃, as
well as 1 N NaOH, suggesting that it is a relatively strong acidic lipid. Medullin was isolated by thin-layer chromatography in a variety of solvent systems, with and without silver nitrate, verifying the homogeneity of the isolated material. An infrared spectrum of pure medullin shows the presence of carbonyl (C = O) and methylene (-CH₂-) groups, with evidence for the existence of hydroxyl groups (-OH) and trans ethylene bonds (> C = C <), features in common with the structural composition of prostaglandins. Comparing the UV spectrum of medullin in sulfuric acid with similar spectra of prostaglandins E₂⁹ and prostaglandins F²⁸ reveals a striking spectrophotometric similarity between medullin and prostaglandins E, both of which have maximal absorption at 2600Å and 3200Å with an inflexion point at 3600Å this is in contrast to prostaglandins F which display a single maximal absorption at approximately 3000Å. Furthermore, a comparison between the spectra obtained from heat-treated alkaline solutions of medullin and prostaglandins E-1³⁴ show similar absorption maxima at approximately 2800Å. The chromophore formed after treatment with alkali is believed to be the result of a dienione formed by dehydration and isomerization of a double bond introduced between C₈ and C₁₂ (fig. 18).³⁷ In addition, acid treatment of these solutions results in disappearance of the maxima at 2800Å and appearance of a new maximum at 3280Å for both medullin and prostaglandin E-1. These studies obtained on derivatives of medullin and PGE-1 suggest identity of major structural components in the two molecules.

Although the chemical properties and composition of medullin and PGE-1 reveal close structural relationship, important differences exist which prove clearly that the two compounds are not identical: (1) Medullin reveals a consistently greater mobility on thin-layer chromatography in comparison to the less mobile PGE-1, PGE-2, and PGE-3. (2) Medullin stains much more intensely with iodine vapors than equal amounts of PGE-1. (3) PGE-1 in ethanol possesses no characteristic absorption at higher wavelengths, while a methanolic solution of medullin displays a weak but definite absorption maximum at 2780-2800Å. (4) Medullin is approximately fifty times weaker than PGE-1 in stimulating nonvascular smooth muscle. Thus, the available evidence indicates that medullin is a derivative of prostanoic acid which possesses a basic structural similarity to PGE-1 but appears to be a more unsaturated compound with fewer polar (i.e., hydroxyl) components.

In addition to medullin, two additional biologically active acidic lipids were isolated from renomedullary extracts. The first substance (compound 2) displayed weak iodine-staining characteristics and produced vasodepression and nonvascular smooth muscle...
stimulation comparable in grade to PGE-1. Since the thin-layer chromatographic mobilities of compound 2 and PGE-1 were identical in a variety of solvent systems, with and without added silver nitrate, this substance has been tentatively identified as PGE-1. The third isolate (compound 1) was less mobile than PGE on thin-layer chromatographic separation, and revealed strong nonvascular smooth muscle-stimulating activity, but absent to weak vasodepressor properties, characteristics common to PGF.

**Physiological Properties of Medullin**

Although the ability of medullin to reduce blood pressure in the pentolinium-treated rat suggests that its action is independent of ganglionic blockade, little information has been available on the mechanism of its vasodepression. The present study was done in order to determine whether the hypotensive action of medullin was cardiac or peripheral in origin. The results indicate that the hypotensive action of this substance is due to a direct vasodilator effect on peripheral arterioles, since a significant decrease was observed both in the peripheral resistance of the dog hind limb following intra-arterial injection of vasopressor substance, and in the total peripheral resistance of the dog following intravenous injection of this material. In addition, diastolic blood pressure reduction exceeded systolic, which indicates again a direct action of medullin on peripheral resistance vessels.

The hypotensive action of medullin does not appear to involve depression of cardiac rate or contractility, because cardiac output increased significantly and simultaneously with the maximum reduction of blood pressure. Moreover, studies on the isolated rabbit heart failed to demonstrate any significant change in cardiac rate or contractile force, supporting the conclusion that renomedullary depressor substance is devoid of a negative inotropic and chronotropic action. Although no direct measurements of the action of medullin on veins were obtained, it appears unlikely that any important venodilation was produced. If a significant degree of venodilation was present, pooling of blood in peripheral veins would be expected with reduction of venous return and a subsequent decrease of cardiac output. Actually, a rise in cardiac output and stroke volume occurred at the time of maximal blood pressure depression, suggesting increased venous return, which is most probably due to the delivery of a greater volume of blood to the venous bed by the dilated arterioles. It would appear, therefore, that the hypotensive action of medullin is attributable to a direct dilator effect on the smooth muscle of peripheral arterioles, resulting in a lowered total peripheral resistance and a subsequent decrease of diastolic and mean arterial blood pressure. Cardiac output is probably increased, with a reflex increase in heart rate and a rise in stroke volume secondary to increased venous return.

Prostaglandins have previously been shown to increase blood flow to the isolated hind limb of frogs and cats without a discernible effect on the contractile force of the isolated frog heart, suggesting a mechanism of vasodepression similar to that of medullin. By contrast, infusions of PGE into normal human beings were found to decrease cardiac output as well as lower blood pressure. In the present study, however, the vasodepressor effects of PGE-1 were indistinguishable from those of medullin, since vasodepression following administration of PGE-1 was the result of a decrease in peripheral resistance and was not associated with a demonstrable reduction of cardiac output. Furthermore, a comparison of the dose response curves of medullin and PGE-1, in lowering aortic blood pressure, revealed no difference in potency between the two substances. The only observed biological difference between medullin and PGE-1 was the relatively weak stimulation of nonvascular smooth muscle by medullin, which was in contrast to the relatively strong acetylcholine-like effect of PGE-1.

The "protective" role of intact functioning renal mass in prevention of various forms of experimental renal hypertension (partial renal artery occlusion, chronic compression of renal
parenchyma, and bilateral nephrectomy) is well established, and is illustrated by the difficulty of creating experimental hypertension by procedures involving one kidney without removal of an opposite intact functioning kidney. In addition, it has been shown that removal of both kidneys produces renoprival hypertension, which obviously cannot be ascribed to a renal renin-angiotensin mechanism. Introduction of a normally perfused kidney, and the oral administration of renomedullary tissue, and the oral administration of renomedullary extracts to bilaterally nephrectomized animals have been shown by Muirhead et al. to prevent the development of such renoprival hypertension. Furthermore, in human hypertension and in chronic established experimental hypertension resulting from renal compression or renal artery ligation, pressor agents have not been detected consistently in amounts sufficient to account for the observed degree of blood pressure elevation. These observations suggest that certain states of hypertension may result from a deficiency of renal vasodepressor substance(s), rather than solely from a relative increase in renal pressor mechanisms. In the present study, the isolation of medullin demonstrates that the renal medulla contains a potent, naturally occurring vasodepressor substance which lowers blood pressure by direct peripheral arteriolar beds without cardiac depression, because intra-arterial and intravenous injections of medullin reduced peripheral resistance markedly and increased cardiac output simultaneously. In addition, no effect on cardiac rate or contractility was observed in the isolated rabbit heart preparation. Comparative studies of medullin and PGE-1 indicated that the hypotensive activity of these compounds is similar with respect to potency and mechanism of action. The only biological difference between crystalline PGE-1 and medullin was a fiftyfold greater stimulation of nonvascular smooth muscle by PGE-1 in comparison to medullin. A second biologically active acidic lipid isolated from rabbit medulla was tentatively identified as PGE-1, and was found to possess both vasodepressor and nonvascular smooth muscle-stimulating properties. The third isolate from rabbit medulla possessed potent intestinal stimulating activity but weak to absent vasodepressor effects, properties similar to those of prostaglandin F compounds.

**Summary**

The chemical and physiological properties of renomedullary depressor substances were investigated utilizing the normotensive, vagotomized, pentolinium-treated rat for assay. By a combination of solvent extraction, column chromatography, and thin-layer chromatography, three biologically active acidic lipids were isolated from rabbit renal medulla. The first compound, called medullin, is an acidic lipid with potent vasodepressor and relatively weak nonvascular smooth muscle-stimulating properties. Ultraviolet analysis revealed spectra closely resembling that of prostaglandin E-1 (PGE-1). Infrared analysis showed the presence of carbonyl, methylene, and hydroxyl groups in addition to trans ethylene bonds. Although closely resembling PGE-1, medullin appears to be a more unsaturated carboxylic acid with less polar (hydroxyl) groups.

The hypotensive action of medullin is attributable to a direct effect on peripheral arteriolar beds without cardiac depression, because intra-arterial and intravenous injections of medullin reduced peripheral resistance markedly and increased cardiac output simultaneously. In addition, no effect on cardiac rate or contractility was observed in the isolated rabbit heart preparation. Comparative studies of medullin and PGE-1 indicated that the hypotensive activity of these compounds is similar with respect to potency and mechanism of action. The only biological difference between crystalline PGE-1 and medullin was a fiftyfold greater stimulation of nonvascular smooth muscle by PGE-1 in comparison to medullin. A second biologically active acidic lipid isolated from rabbit medulla was tentatively identified as PGE-1, and was found to possess both vasodepressor and nonvascular smooth muscle-stimulating properties. The third isolate from rabbit medulla possessed potent intestinal stimulating activity but weak to absent vasodepressor effects, properties similar to those of prostaglandin F compounds.

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