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PHARMACOLOGICAL ACTIONS OF PEPSITENSIN

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In confirmation of the results of Croxatto and his co-workers, plasma proteins incubated with pepsin yielded a substance (pepsitensin) with pressor activity. Euglobulin gave a much higher yield than the other plasma protein fractions. Incubation for 4 hr at *p*H 4 gave high yields of pressor activity (pepsitensin) but no antidiuretic activity; incubation of euglobulin for 4 hr at *p*H 2.5 yielded extracts with antidiuretic as well as pressor activity. Incubation for 8 to 11 hr at *p*H 2.5 produced the highest yield of both activities. Further incubation, at the same *p*H, up to 20 hr caused a rapid decline in the pressor activity of the extracts, but the antidiuretic activity was much more resistant to destruction by pepsin. Pepsitensin was found to be very soluble in water and poorly soluble in organic solvents. It is not inactivated by thioglycollate. In blood pressure assays some animals did not respond to pepsitensin, and nephrectomized (17 to 24 hr) rats were found to be more suitable preparations. Pepsitensin was shown to exert pressor effect by direct action on the blood vessels. Its pressor action could be differentiated from that of tyramine, dimethylphenylpiperazine, nicotine, noradrenaline and Pitressin but not from that of angiotensin. The isolated guinea-pig ileum and the rat uterus were equally sensitive to angiotensin and pepsitensin. In paper chromatograms, in the solvent system butanol-acetic acid-water, the R_F of pepsitensin was very similar to that of angiotensin.

Renin acts upon a substrate in the alpha-2 globulin fraction of plasma to produce angiotensin (hypertensin, angiotonin) (Braun-Menendez, Fasciolo, Leloir & Munoz, 1939, 1940; Kohlstaedt, Helmer & Page, 1938). Croxatto & Croxatto (1942) studied the action of pepsin on the same fraction of plasma proteins. They found that euglobulin incubated with commercial pepsin for 15 min at 38° C and *p*H 4.0 yielded an extract with pressor and vasoconstrictor properties. Croxatto & Croxatto named the pressor substance (or substances) "pepsitensin" and stated that it had many of the pharmacological properties of angiotensin (Alonso, Croxatto & Croxatto, 1943).

Studies on the formation and pharmacological actions of pepsitensin have been hindered by the simultaneous production of depressor substances (Braun-Menendez, Fasciolo, Leloir, Munoz & Taquini, 1946). It was also found that the same sample of pepsitensin may produce a pressor response in some rats but not in others.

Prolonged incubation of globulin with pepsin at *p*H 2.5 for 8 to 24 hr was shown by Croxatto, Rojas & Barnafi (1951a, b) to produce extracts with intense antidiuretic activity but with a relatively weak hypertensive action. The antidiuretic activity was called "pepsanurin." The oxytocic action of pepsitensin extracts was ascribed

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by Croxatto, Rojas & Barnafi (1950) to yet another principle which they called "pepsitocin." Pepsin, trypsin and carboxypeptidase abolished the vasoconstrictor potency of such extracts but not their oxytocic effect: chymotrypsin abolished both (Croxatto, 1955).

Since hydrolysis of serum globulins by pepsin liberates polypeptides which have many of the pharmacological actions of the neurohypophysial hormones, Croxatto (1957) suggested that the hypothalamo-neurohypophysial system forms oxytocin in a similar way. It may be convenient to call the active principle "pepsitensin," but it should be noted that it is left open whether the pharmacological activity of pepsitensin preparations is due to a mixture of several related substances or to a single substance.

The experiments to be described deal with the preparation and partial purification of "pepsitensin" and "pepsanurin"; a pharmacological analysis of the pressor action of pepsitensin and a comparison of its properties with those of vasopressin (Pitressin) and synthetic angiotensin.

METHODS

Preparation of protein substrates. Carotid artery blood was collected from cattle, immediately after killing, into polythene bottles, which contained enough 10% sodium citrate to make the final concentration 1%. After dialysing the plasma for 24 hr against running tap water, saturated ammonium sulphate solution (700 g/l.) was added to separate the plasma proteins into four fractions, viz., fibrinogen (20% saturation), euglobulin (30% saturation), pseudoglobulin (50% saturation) and albumin. The globulins were dissolved in 0.9% sodium chloride solution and dialysed for 36 hr against running tap water. The albumin fractions were also so dialysed.

Euglobulin from fresh and stored human plasma was prepared in the same way. Human plasma euglobulin (G2F) was prepared by the ether fractionation method of Kekwick & Mackay (1954). Horse serum euglobulin was prepared by diluting the serum in distilled water until the specific gravity reached 1090, at which point a precipitate appeared. This precipitate was collected and dialysed against running tap water for 24 hr. The solution obtained was freeze-dried.

Preparation of pepsitensin. Euglobulin from 100 ml. bovine plasma or 2 g horse euglobulin was dissolved in 50 ml. of 0.9% sodium chloride solution in a polythene beaker. The *p*H of the solution was adjusted to 4 by the addition of 10% hydrochloric acid; 50 mg of freshly dissolved pepsin was added and the solution incubated for 4 hr at 37° C. In some experiments (for details see Results) the conditions were varied, for example, the amount of pepsin added, the *p*H of the solution or the period of incubation were altered. Digestion was stopped by adding 4 vol. hot or cold ethanol. The solution was left to cool, centrifuged and the supernatant filtered through Whatman filter paper no. 1. The filtrate was evaporated under reduced pressure and at 45° C to 20 ml. Glacial acetic acid (20% v/v) was added until the final concentration was 0.25% and the solution boiled in a water bath for 5 min. It was then cooled and filtered through Whatman filter paper no. 42. After dialysing for 30 min against running tap water the *p*H of the final solution was adjusted with sodium hydroxide (10%) to 6.5 and stored in polythene bottles. A quantity of extract prepared in this way from horse serum euglobulin was freeze-dried and called crude pepsitensin standard one (S1).

Chemical procedures

Partial purification of crude pepsitensin. Following the method of Bumpus (personal communication), pepsitensin dissolved in 0.9% sodium chloride solution at *p*H 2.5, and containing toluene sulphonic acid (1%), was extracted with n-butanol. The combined butanol

extract was added to 0.01 N hydrochloric acid and petroleum ether (40/60). The aqueous layer was reduced to the original volume by evaporation under low pressure at 45° C. The *p*H of the solution was adjusted to approximately 5 with ammonia (s.g. 0.88) and filtered if necessary. After dialysis against running tap water for 30 min the solution was freeze-dried. A quantity of pepsitensin S1, the first standard prepared, purified in this manner was called crude pepsitensin standard two (S2).

Paper pulp chromatography. The method of Helmer (1950) was used. Pepsitensin solution (80 mg S1/ml.) was applied to the column at *p*H 4.0 or *p*H 6.5.

Ion exchange chromatography. Crude pepsitensin (40 mg S1/ml.) solution at *p*H 4.0 or *p*H 6.5 was added to a 8 × 2 cm. column of Amberlite CG-50 (type II) in the hydrogen form. Pepsitensin was eluted with 0.1 N sulphuric acid following the method of Bumpus, Green & Page (1954). In another experiment pepsitensin, applied at *p*H 4, was eluted with 50 ml. portions of ammonium acetate buffer at *p*H 5.0, 5.5, 6.0, 6.5 and 7.0. The effluent collected after each 50 ml. portion of buffer had passed through the column was reduced to 10 ml., freeze-dried and redissolved in 10 ml. water.

Paper chromatography. Whatman paper no. 3 MM was used in all experiments. Both ascending and descending techniques were used in the solvent system butanol-acetic acid-water (4:1:5 by vol.). The peptides were eluted with warm (40° C) 0.9% sodium chloride solution according to Heller & Pickering (1961).

Estimation of the nitrogen content of pepsitensin preparations. A Kjeldahl method (Mann & Saunders, 1952) was used.

Inactivation with sodium thioglycollate. Vogt's (1953) modification of the procedure of Ames & van Dyke (1951) was used.

Assay methods

Pressor assays. Adult male albino rats were used (180 to 220 g). Dekanski's (1952) method was used except that dibenamine was not injected. Experiments were performed in intact rats. in rats which had been nephrectomized for 17 to 24 hr, in rats which had been adrenalectomized for 17 to 24 hr, in rats 2 hr after adrenalectomy, in pithed rats and in spinal animals. In a few instances the electrocardiogram was recorded at the same time as the blood pressure from leads inserted into the front right and left paws of intact rats and rats nephrectomized for 17 to 24 hr. An Ediswan portable cardiograph was used.

Pressor assays were also performed in rats which had been treated with reserpine according to the procedure of Burn & Rand (1958): Reserpine 5 mg/kg body weight was injected intraperitoneally on the first day, on the second day the same dose was injected intravenously and on the third day the animal was prepared for blood pressure assay. The amount of urethane used was varied depending on the state of the animal. Larger doses of reserpine were not usually tolerated sufficiently well to provide sensitive bio-assay preparations.

Pressor assays in nephrectomized rats and intact rats were performed after the injection of the following substances: pentapyrrolidinium tartrate (with ephedrine) in polyvidone solution (25 mg/kg subcutaneously) 1 hr before the assay (Peart, 1955); bretylium tosylate (20 mg/kg subcutaneously) injected 6 hr before the start of the experiment; phenoxybenzamine (5 to 15 mg/kg intravenously) given after a preliminary assay and 30 to 60 min before a second assay; and dihydroergotamine (2 mg/kg intravenously) given repeatedly after a preliminary assay and 10 min before subsequent assays.

Hexamethonium (10, 20 and 40 mg/kg intravenously) and cocaine (in divided doses of 2.5 mg/kg intravenously at 10 min intervals) were used as antagonists against pepsitensin with angiotensin as control drug and nicotine and tyramine (respectively) as agonists. The potency relationship between these substances was determined before and after the injection of antagonist by (2+2+2) dose assays, 12 or 24 observations being made. The results of these experiments were expressed in terms of an "alteration in response index" (see below).

Estimation of pressor activity. Before the preparation of a laboratory standard of pepsitensin its pressor activity was expressed in terms of that of Pitressin; (2+2) dose assays were performed (8 or 16 observations, on 4 or more intact rats) and the mean potency calculated. The doses were chosen so that the effects fitted the steepest part of the log dose-effect curve. The potency of each extract was obtained by averaging the means obtained in several experiments. When a laboratory standard had been prepared each substandard was assayed against it. The results obtained in (2+2) assays were calculated according to Holton (1948). Pepsitensin S1 was compared with angiotensin and Pitressin in (2+2) dose assays in intact and nephrectomized rats.

Estimation of potency relationship. The potency relationship between a number of pairs of compounds was always estimated from several experiments.

The significance of the discrepancy between a set of results of potency relationship obtained from one preparation (for example, an intact rat) and the set of results obtained from another preparation (for example, a nephrectomized rat) was calculated according to Gaddum (1953).

Index for the expression of alteration in drug response. The results obtained with hexamethonium and cocaine were calculated as an index. Instead of determining the amount of agonist which was required to produce a given effect in the presence or absence of antagonist (Gaddum, Hameed, Hathway & Stephens, 1955), the change in potency relationship to pepsitensin was calculated. The index was defined as $\frac{B}{B_o}$, where B_o was the amount of agonist which had pressor activity equivalent to 1 unit pepsitensin (that is, 1 mg S1) in the absence of antagonist and where B was the amount of agonist which had pressor activity equivalent to 1 unit pepsitensin in the presence of the antagonist. This index does not differentiate between antagonism or potentiation. It does, however, indicate an alteration in response to any drug in relation to pepsitensin after the injection of the antagonist. The responses to the "control" drug showed whether there was an alteration in the sensitivity of the preparation to the pressor substances independent of the action of the antagonist. If the index was 1 the drugs (pepsitensin and agonist or control) were affected equally. If the index was >1, then the agonist (or control) could be assumed to be either antagonized preferentially to pepsitensin or pepsitensin was unaffected or even potentiated. The reverse applied if the index was <1. Experiments were repeated four or five times with the lowest dose of the antagonist. The standard error of the mean expressed in terms of the index was calculated.

Antidiuretic assays. Two methods were used: that of Ginsburg & Heller (1953) with the modification that the bladders of the assay animals were cannulated and the readings were taken every 4 min; and that of Jeffers, Livezey & Austin (1942), employing intravenous injections into ethanol-anaesthetized rats.

Isolated rat uterus. Virgin rats (160 to 200 g) were injected with stilboestrol 100 μg/kg. On the following day one horn of the uterus was suspended in a bath of 6 ml. capacity maintained at 35° C in de Jalon solution (de Jalon, Bayo and de Jalon, 1945). Atropine sulphate (10 μg/l.) and mepyramine maleate (10 μg/l.) were added; a frontal writing lever (Schild, 1947) was employed. The contractions were magnified about four times and the maximum excursion of the writing point was restricted to 14 cm. The load on the uterus was about 1 g.

The mean potency ratios were calculated from the results obtained in (2+2) assays (8 or 16 observations). After each assay a massive dose, 100 to 1,000 times larger than the doses used in the assay, of either pepsitensin or angiotensin, was added. When the muscle began to relax the bath fluid was changed, and the assay was repeated. 5-Hydroxytryptamine was used as control drug.

Isolated guinea-pig ileum. The terminal 3 to 4 cm (avoiding the patch of lymph tissue) of the ileum was suspended in a 16 ml. bath containing Tyrode solution at 35° C. Atropine sulphate (10 μg) and mepyramine maleate (10 μg) were added to each litre. The ileum was attached by a thread to a frontal writing lever giving a magnification of about 4 under a load of 0.5 to 1 g.

Specific desensitization. Gaddum's (1955) procedure of desensitization was applied both to the isolated rat uterus and the guinea-pig ileum. The results obtained have been expressed in terms of a "desensitization index" in much the same way as the index for the alteration in response already described. The potency relationship between angiotensin and pepsitensin was calculated before and after the administration of a massive dose 100 to 1,000 times larger than the doses used in the assay of angiotensin or pepsitensin. When the muscle began to relax despite the continued presence of the desensitizing dose the bath fluid was changed. The assay was then repeated. 5-Hydroxytryptamine was used as a control drug for unspecific desensitization of the tissue.

Desensitization index (DI_n). This was defined as the ratio of the amount of angiotensin equivalent in activity to 1 unit pepsitensin *after* administration of the massive dose, to the amount of angiotensin equivalent in activity to 1 unit pepsitensin *before* administration of the massive dose, where n is the ratio of the desensitizing dose to the larger dose (S_H) used in the assay. The desensitization index calculated for the control and pepsitensin showed whether there was any alteration in the sensitivity of the preparation to pepsitensin.

Isolated rabbit heart. Karp, Rinzler & Travell's (1960) modification of Langendorff's apparatus was used; the coronary flow was recorded with a Thorp impulse counter. Drugs was injected in volumes which did not exceed 1 ml. and were followed by 0.3 ml. of Ringer-Locke solution.

Perfused rabbit ear. Gaddum's (1950) modification of Page & Green's (1948) method was used.

Indices of discrimination. These were calculated according to Gaddum (1955).

Drugs.—The following preparations were used: Adrenaline chloride (Parke, Davis Co.), atropine sulphate (British Drug Houses), bretylium tosylate (Wellcome Research Laboratories), cocaine hydrochloride (Ferris), phenoxybenzamine (Smith Kline & French Laboratories, U.S.A.), dihydroergotamine methanesulphonate (Sandoz Ltd.), dimethylphenylpiperazinium iodide (Light), hexamethonium bromide (May & Baker), heparin (Evans Medical), 5-hydroxytryptamine creatinine sulphate (Sandoz), mepyramine maleate (May & Baker), nicotine hydrogen tartrate (British Drug Houses), noradrenaline hydrochloride (Bayer), pentolinium tartrate with ephedrine (May & Baker), dissolved in polyvidone (May & Baker), Pitressin (Parke, Davis Co.), reserpine (Serpasil and the pure base, Ciba), stilboestrol (British Drug Houses), sodium pentobarbitone (Abbott), tyramine hydrochloride (Light), urethane (May & Baker), angiotensin II-amide (val_5 octapeptide, Ciba) and natural horse angiotensin ($isoleu_5$ octapeptide 75% pure). Crystalline pepsin from pork mucosa was used in all experiments for preparing pepsitensin.

RESULTS

The conditions necessary for maximum yields of pepsitensin from a variety of substrates were examined. Because of its alleged similarity to the neurohypophysial principles, the pressor activity of the pepsitensin extracts was expressed in terms of that of Pitressin.

In intact rats pepsitensin caused a rapid rise in blood pressure followed by a quick recovery. A relatively large percentage of animals, approximately 35%, did not respond to pepsitensin. A period of rest after the operation (about 1 hr) increased the sensitivity in those animals which were already responsive, but the refractory rats remained completely insensitive. This difference in sensitivity of intact rats seemed an "all or nothing" phenomenon, since the responses/dose in sensitive animals were fairly consistent.

Extracts of pepsitensin prepared from salt-precipitated plasma proteins contained a hypotensive factor when only 2 vol. of ethanol were added to stop digestion. When 4 vol. were used very little hypotensive activity was present. Large doses

(equivalent to 18 m-u. Pitressin) had to be administered to cause a preliminary brief fall in blood pressure followed by a rapid rise. Pepsitensin obtained from horse euglobulin contained no detectable hypotensive activity. For this reason only the latter was used for the analysis of the pressor action. It was first shown that pressor activity was only produced by pepsin at acid *p*H.

Preparation of pepsitensin. Horse euglobulin (2 g) incubated at 37° C with pepsin (50 mg) at *p*H 3.5 for 4 hr yielded pressor activity equivalent to approximately 3,000 m-u. Pitressin (average activity of 5 extracts). Control experiments, namely, incubation at *p*H 7.3 with pepsin, incubation at *p*H 3.5 without pepsin or with inactivated pepsin, yielded less than 3% of the activity of the pepsin-euglobulin mixture at acid *p*H. The results of experiments, in which the amount of substrate, *p*H and temperature of incubation were kept constant (as above) but the period of incubation ($\frac{1}{2}$ to 4 hr) or the amount of pepsin added (10 to 50 mg) was varied, indicated that, provided sufficient time was allowed, the concentration of pepsin did not significantly affect the yield. The highest yields were obtained after incubation of euglobulin with 10 to 50 mg pepsin for 4 hr.

Table 1 shows the yield of pepsitensin from various substrates. Bovine euglobulin gave a much higher yield than any of the other protein fractions of bovine plasma. No detectable activity was obtained from fresh or stored human euglobulin obtained by salt precipitation, but ether-precipitated human euglobulin (G2F) yielded considerable activity.

TABLE 1

YIELDS OF PRESSOR ACTIVITY OBTAINED FROM VARIOUS SUBSTRATES, INCUBATED WITH 50 MG PEPSIN FOR 4 HR AT *p*H 3·5

The yields recorded are means of several extracts. (Number of extracts used in brackets)

Substrate	Amount of substrate	Method used to prepare substrate	Pressor activity in m-u. equivalent Pitressin
Bovine euglobulin	From 100 ml. plasma (approx. 2 g)	Ammonium sulphate precipitation	1,830 (9)
Bovine pseudoglobulin	From 100 ml. plasma	Ammonium sulphate precipitation	210 (2)
Bovine albumin	From 100 ml. plasma	Ammonium sulphate precipitation	420 (3)
Bovine gamma globulin (Armour Lab. Ltd.)	2 g (from approx. 500 ml.)	—	260 (4)
Fresh human euglobulin	From 100 ml. plasma (approx. 2 g)	Ammonium sulphate precipitation	None detectable [<50 (4)]
Stored human euglobulin	From 100 ml. plasma (approx. 2 g)	Ammonium sulphate precipitation	None detectable [<50 (2)]
Stored human euglobulin (G2F) (Lister Institute)	2 g	Ether precipitation	960 (6)
Horse euglobulin (Evans)	2 g	Precipitation with distilled water	2,970 (5)

There was a difference between the yield of pressor activity produced by incubating stored euglobulin and by incubating freshly prepared euglobulin at *p*H 3.5 and 37° C for 4 hr but without pepsin. The stored material yielded only 3% of the yield of the pepsin-treated substrate when tested in nephrectomized rats and in intact rats. In contrast the freshly prepared globulin yielded 27% of the pepsin-treated

incubate when tested in intact rats and 41% when tested in nephrectomized animals. Acid hydrolysis of freshly prepared euglobulin thus seems to yield more pressor activity than that of stored euglobulin and such activity is relatively more active than pepsitensin in nephrectomized animals.

Pepsitensin extracts were prepared according to the methods of several authors. The method of Braun-Menendez and Paladini (1958) yielded approximately 1,600 m-u. Pitressin equivalents from 2 g horse euglobulin incubated with 200 mg pepsin for 10 min at *p*H 4; that of Dengler (1956) yielded 1,900 m-u. Pitressin equivalents under similar conditions except that the period of incubation was 30 min; and those of Paiva (1954) and Croxatto (1957) yielded about 3,000 m-u. Pitressin equivalents from 2 g euglobulin incubated with 50 mg pepsin for 4 hr at *p*H 3.5 or *p*H 4 respectively. In the present work the method of Croxatto (1957) was used throughout.

Antidiuretic activity of peptic digests of plasma proteins

The pressor and antidiuretic activities of extracts prepared from 2 g horse euglobulin and incubated with 50 mg pepsin at *p*H 4 for 4 hr and at *p*H 2.5 for 8 hr or for 20 hr are shown in Table 2. Control experiments were carried out in the same

TABLE 2

EFFECT ON THE YIELD OF PRESSOR AND ANTIDIURETIC ACTIVITIES OF THE DURATION OF INCUBATION OF EQUINE EUGLOBULIN (2 g) AND OF BOVINE EUGLOBULIN (FROM 100 ML. PLASMA) WITH 50 MG PEPSIN AT *p*H 2·5 AND 37° C

The mean activities recorded were calculated from means estimated from several assays of the same extract (number of assays in brackets). The doses used in the antidiuretic assays did not affect the systemic blood pressure

Euglobulin used	Period of incubation (hr)	Pressor activity m-u. Pitressin equivalent with s.e. (P=0·95)	Antidiuretic activity m-u. Pitressin equivalent with s.e. (P=0·95)	Ratio pressor: antidiuretic activities
Bovine	4	435 (3)	24·1 (±2·6) (6)	18
Bovine	8	765 (±18) (5)	51·9 (±2·7) (5)	15
Equine	8	3,476 (±81) (4)	120 (±11) (6)	29
Bovine	11	1,000 (±41) (4)	30·9 (±1·5) (11)	32
Bovine	14	421 (±20) (5)	31·1 (±2·6) (4)	14
Bovine	20	Nil (3) (<40)	31·9 (±2·2) (10)	<1
Equine	20	152 (±11) (4)	21·6 (±1·1) (14)	7
Equine	4 hr at *p*H 4 (pepsitensin)	2,970 (±106) (5)	Nil (4) (<2·5)	—

manner except that no pepsin or inactivated pepsin was added. When testing the antidiuretic activity of extracts which also had pressor activity, three dose levels were used: (1) doses which were known to be too small to affect the systemic blood pressure of intact rats anaesthetized with urethane; (2) medium doses which could be expected to produce small (10 to 20 mm Hg) pressor effects; and (3) large doses which were expected to cause large increases in blood pressure (20 to 60 mm Hg). When the antidiuretic responses to a particular dose, that is, small, medium or large, were not consistent, the experiment was repeated in rats anaesthetized with ethanol.

After incubation of euglobulin with pepsin for 4 hr at *p*H 4 the mixture exerted strong pressor effects but had no antidiuretic action. Doses that were too small to raise the blood pressure did not affect water diuresis. Doses which did affect the

blood pressure had no consistent effects on urine output, which in conscious rats was either unaltered, slightly reduced or even increased. In anaesthetized animals medium doses caused an increase in urine flow and large doses caused marked diuresis (Fig. 1). In contrast to the pepsitensin sample, incubated for 4 hr at *p*H 4,

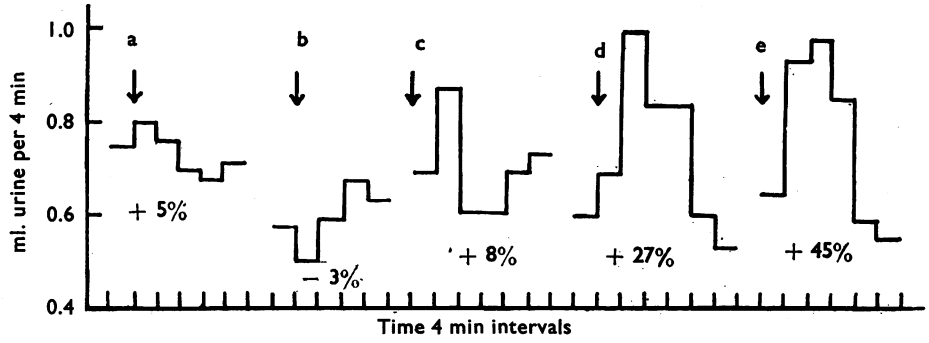

Fig. 1. Antidiuretic and diuretic responses to graded doses of peptic digest C 1 (incubated at *p*H 4 for 4 hr) given intravenously to an anaesthetized rat (180 g). Doses: a, b, c, d, e, represent the activity obtained respectively from 1, 2, 4, 8, and 16 mg equine euglobulin. The injections were given in the following order: a, c, e, d, b. The numerals give the % antidiuresis (−) and diuresis (+) for each response.

incubation for 8 hr at *p*H 2.5 produced considerable antidiuretic as well as pressor activity. Euglobulin-pepsin mixtures incubated for 20 hr at *p*H 2.5 had about 20 times less pressor activity than 8 hr samples and about 6 times less antidiuretic activity. The decline in pressor activity relative to antidiuretic activity with increased period of incubation emerges clearly from the last column of Table 2. The time-effect relationship of the antidiuretic responses observed is illustrated by Fig. 2. Mixtures incubated for 20 hr gave a good regression for antidiuretic activity over a large range of doses.

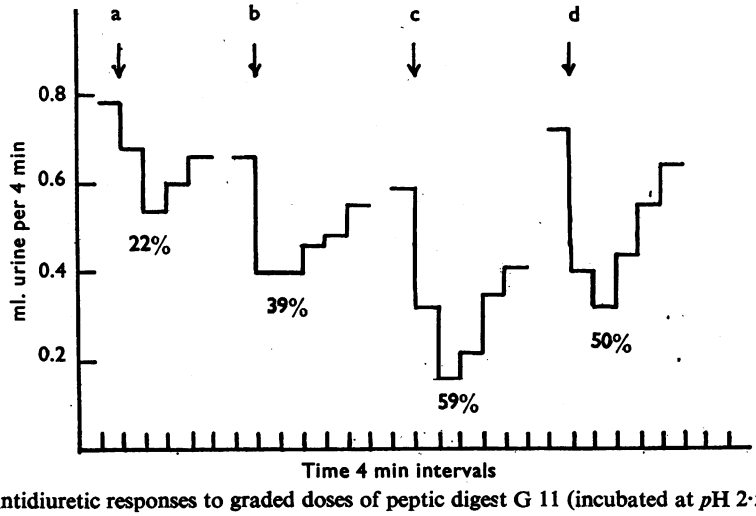

Fig. 2. Antidiuretic responses to graded doses of peptic digest G 11 (incubated at *p*H 2·5 for 11 hr) given intravenously to an anaesthetized rat (210 g). Doses: a, b, c, d, represent activity obtained respectively from 0·2, 0·4, 0·8 and 1·6 ml. bovine plasma. The injections were given in the following order: a, d, b, c. The numerals give the % antidiuresis for each response.

Control samples, incubated without pepsin or with inactivated pepsin, had very little pressor activity (0 to 3.0% of the pepsin treated incubates) and no detectable antidiuretic activity. When Pitressin was added to the globulin and incubated with pepsin for 20 hr, the mixture containing pressor activity consistent with the activity of the Pitressin added, the antidiuretic potency was, if anything, somewhat higher than could be expected from Pitressin alone.

Similar experiments were performed with extracts prepared by incubating bovine euglobulin obtained from 100 ml. plasma with 50 mg pepsin at *p*H 2.5 and 37° C for 4, 8, 11, 14 and 20 hr (Table 2). The pressor activity of these extracts increased with length of incubation with a peak after 11 hr; thereafter the pressor activity declined rapidly and none was detectable after 20 hr incubation. In contrast, antidiuretic activity was present, in approximately similar amounts, in all the extracts.

Preparation of a laboratory standard of pepsitensin

A large quantity of crude pepsitensin was prepared from horse euglobulin and freeze-dried. This material, called Crude Pepsitensin Standard One (S1), was assayed against Pitressin on the blood pressure of intact rats. Two grammes of horse euglobulin yielded 840 mg of material containing activity equivalent to 3,020 m-u. Pitressin or 3.6 m-u./mg. An extract prepared from 100 ml. bovine plasma yielded 440 mg of solid which had a pressor activity equivalent to 1,910 m-u. or 4.4 m-u./mg.

Definition of the unit of activity. One unit of pepsitensin was defined as the pressor activity contained in 1 mg crude horse pepsitensin standard one (S1).

Pepsitensin S1 contained 92 μg nitrogen/mg. It was insoluble in absolute methanol, ethanol and 90% phenol. It was soluble in trichloracetic acid and was precipitated from concentrated aqueous solutions only by full saturation with ammonium sulphate. The pressor activity was not abolished by sodium thioglycollate.

Since it has been suggested that pepsitensin resembles vasopressin (Croxatto, 1957) and angiotensin (Paiva, 1954), it was compared with Pitressin and angiotensin. In intact rats 1 unit of pepsitensin had a pressor activity equivalent to 19.4 ($\pm$2.3) ng angiotensin (P=0.95) and 2.88 ($\pm$0.67) m-u. Pitressin (P=0.85). The variations between pepsitensin and angiotensin due to deviation from parallelism (P>20%) and differences between the substances (P>20%) were not significant. The variation between pepsitensin and Pitressin due to differences between the two substances was highly significant (P<1%). In rats nephrectomized for 17 to 24 hr, 1 unit pepsitensin had a pressor activity equivalent to 20.7 ($\pm$2.5) ng angiotensin (P=0.95) and 7.9 ($\pm$1.4) m-u. Pitressin (P=0.95). The variations due to deviations from parallelism (P>20%) and the differences between angiotensin and pepsitensin (P>20%) were not significant. The variation due to deviation from parallelism between pepsitensin and Pitressin was significant (P<5%), but that due to differences between the substances (P>20%) was not. It thus seemed that pepsitensin resembled angiotensin more than Pitressin. Before the pharmacological analysis of the pressor action was continued attempts were made to obtain a purer preparation.

Partial purification of pepsitensin

Chromatography on paper pulp columns. No detectable pressor activity was recovered either from the total phenol wash or the total acid wash.

Chromatography on ion exchange columns. No detectable pressor activity was recovered from a column of Amberlite resin CG-50 after elution with ammonium acetate buffers of various hydrogen ion concentrations. All the pepsitensin, applied to the column at *p*H 4.0, was recovered after elution with 0.1 N sulphuric acid. 60% of the activity was contained in fractions 10 to 30 which were freeze-dried. Compared with the original sample this procedure led to an increase of 90% in the activity/unit weight. Approximately 75% of the pepsitensin, applied at *p*H 6.5, was eluted with sulphuric acid. The fractions 10 to 30 contained 56% of the original activity. There was an increase in activity/unit weight of 65%.

Extraction by the method of Bumpus. It was first shown that, provided sufficient butanol was used, the amount of activity extracted increased with the concentration of toluene sulphonic acid in the aqueous phase. The minimum concentration required to yield 90 to 100% recovery of activity was found to be 1%. No activity was lost in the process of freeze-drying. 840 mg pepsitensin S1 purified in this manner yielded 250 mg material which had pressor activity equivalent to 800 units S1, that is, 3.2 units/mg. The purified material was prepared in quantity and called Crude Pepsitensin Standard Two (S2). Pepsitensin S2 contained 153 μg nitrogen/mg, that is, 48 μg nitrogen/unit activity. This preparation was used in the pharmacological experiments described below.

Pharmacological analysis of the pressor action of pepsitensin

Pressor activity in intact rats. Table 3 compares the pressor activity of pepsitensin with that of other substances.

TABLE 3

ACTIVITY OF PRESSOR SUBSTANCES EQUAL TO THAT OF 1 UNIT PEPSITENSIN IN INTACT AND NEPHRECTOMIZED RATS

Number of animals in brackets

Pressor substances	Intact rats. Mean and s.e. (P=0·95)	Nephrectomized rats. Mean and s.e. (P=0·95)	P
Angiotensin	22·5 (±2·6) ng (6)	24·4 (±4·0) ng (6)	0·3
Noradrenaline	79·4 (±11·7) ng (6)	105·0 (±10) ng (6)	<0·01
Tyramine	14·4 (±1·7) μg (6)	38·0 (±5·7) μg (9)	<0·001
Nicotine	13·5 (±3·7) μg (5)	42·2 (±10·2) μg (7)	<0·001
Pitressin	3·01 (±0·37) m-u. (6)	8·05 (±1·43) m-u. (5)	<0·001

Pressor activity in nephrectomized rats. Exclusion of the kidneys from the circulation did not immediately increase the sensitivity to pepsitensin. However, after about 6 hr the animal was noticeably more sensitive. Rats which had been nephrectomized for 17 to 24 hr showed marked and consistent sensitivity to the

pressor action of pepsitensin and were very suitable for bio-assays (Fig. 3). Sensitivity to pepsitensin was even more pronounced 48 hr after removal of the kidneys, but such animals were moribund and unsuitable for bio-assays. Unilateral nephrectomy (17 to 24 hr) did not sensitize to pepsitensin. Similar results as in

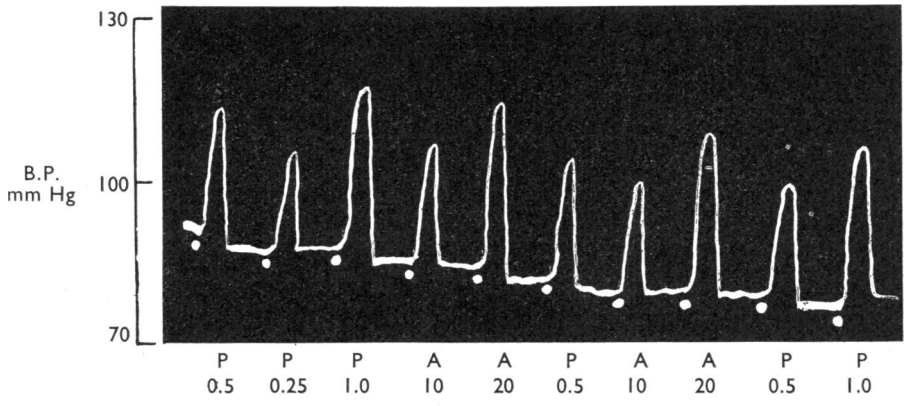

Fig. 3. Pressor responses of a nephrectomized (17 to 24 hr) rat (200 g) to pepsitensin (P) (0·25, 0·5 and 1·0 units) and angiotensin (A) (10 and 20 ng). The substances were injected every 4 min.

nephrectomized animals were obtained when the kidneys were not removed but only excluded from the circulation. Nephrectomized rats were 2 to 4 times more sensitive to the pressor action of pepsitensin than intact rats. After the injection of moderate or high doses of Pitressin there was a tendency for the blood pressure not to return completely to the pre-injection level.

The potency relationship between 1 unit of pepsitensin and other pressor substances was obtained both in intact and in nephrectomized rats (Table 3). Nephrectomized animals were more sensitive (2 to 4 times) than intact rats to the pressor action of pepsitensin and angiotensin, but relatively less sensitive to the pressor action of noradrenaline, tyramine, nicotine, and Pitressin. Significantly larger quantities of the latter substances were required to have a pressor activity equivalent to 1 unit of pepsitensin in nephrectomized animals than those required in intact rats. These results support the view that the mechanisms by which pepsitensin and angiotensin produce their pressor action are very similar, but different from those of noradrenaline, tyramine, nicotine, and Pitressin.

Pressor activity in pithed rats. Pepsitensin raised the blood pressure of rats after destruction of the spinal cord. One unit of pepsitensin was equivalent to 24.8 ng angiotensin.

Pressor activity in spinal rats. Spinal rats responded to the pressor action of angiotensin and pepsitensin but were much less sensitive than intact rats.

Pressor activity in rats treated with reserpine. In rats treated with reserpine 5 mg/kg for two days (see Methods) one unit of pepsitensin had pressor activity equivalent to 25.1 ± 4.1 ng angiotensin ($P = 0.95$), 227 ± 44 μg tyramine ($P = 0.95$), 50 ng noradrenaline, 86 μg nicotine and 3.9 m-u. Pitressin. There was no significant

difference in the potency relationship between angiotensin and pepsitensin in animals treated with reserpine and untreated animals ($P<0.2$). The difference in the potency relationship between pepsitensin and tyramine in treated and untreated rats was highly significant ($P<0.001$). Only a few rats tolerated treatment with higher doses of reserpine. In one rat treated with reserpine 7 mg/kg for three days, one unit of pepsitensin exerted pressor activity equivalent to 28.2 ng angiotensin, 800 μg tyramine, 390 μg nicotine and 2 ng noradrenaline.

Pressor activity of pepsitensin in rats treated with substances which modify the responses of other pressor agents

Intact rats treated with pentapyrrolidinium tartrate were as sensitive as nephrectomized rats to pepsitensin. One unit of pepsitensin had a pressor activity equivalent to 24.4 ng angiotensin, 122 ng noradrenaline and 3.6 m-u. Pitressin.

In intact rats bretylium tosylate did not affect the pressor responses to either pepsitensin or angiotensin.

The dose of dihydroergotamine methanesulphonate (2 mg/kg) which was sufficient to block the pressor response to noradrenaline completely had little effect on that to pepsitensin and angiotensin. Further administration of the adrenaline antagonist reduced the sensitivity of the preparations to both peptides but rather more (approximately twice) to pepsitensin than to angiotensin.

In rats, which had been injected with phenoxybenzamine 2 mg/kg 1 to 2 hr before the assay, one unit of pepsitensin had a pressor activity equivalent to 15.4 ng angiotensin, 1,052 ng noradrenaline and 4.0 m-u. Pitressin. Larger doses (5, 10, 15 mg/kg) of the antagonist reduced the sensitivity to pepsitensin and angiotensin. After the administration of phenoxybenzamine 15 mg/kg, one unit of pepsitensin had an activity equal to 10 ng angiotensin. The response to noradrenaline was completely blocked in these experiments (Fig. 4).

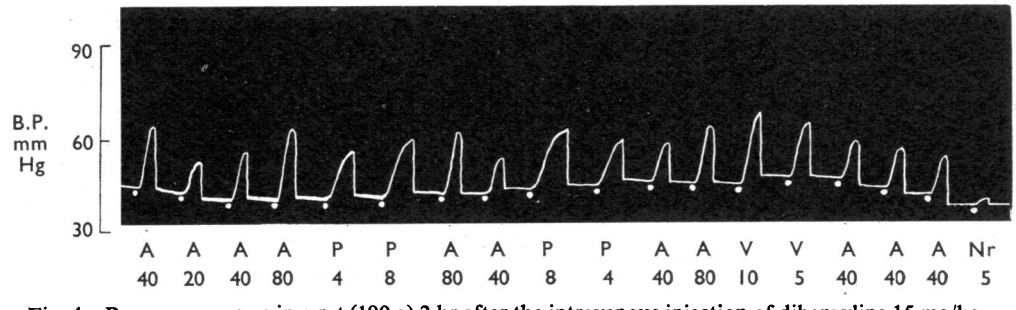

Fig. 4. Pressor responses in a rat (190 g) 2 hr after the intravenous injection of dibenzyline 15 mg/kg. The capital letters represent the substances and the numerals the quantities injected. Angiotensin (A) in ng, pepsitensin (P) in units, Pitressin (V) in m-u. and noradrenaline (Nr) in μg. The substances were injected every 5 min.

The potency relationship between pepsitensin and other pressor substances before and after hexamethonium was estimated in nephrectomized rats and has been expressed as an " alteration in response index " (see Methods). Index values after

the administration of hexamethonium 10 mg/kg were as follows: nicotine 2.98 (± 0.15, $P=0.95$), tyramine 1.13 (± 0.13, $P=0.95$), angiotensin 1.26 (± 0.11, $P=0.95$), dimethylphenylpiperazine 2.3 and noradrenaline 0.9. When hexamethonium 20 mg/kg was injected the index values obtained were nicotine 12, angiotensin 1.2 and tyramine 1.0. Since the response to nicotine was completely abolished, the index value approached infinity after administration of hexamethonium 40 mg/kg.

Values for the alteration in response index after administration of cocaine 2.5 mg/kg intravenously were: for angiotensin 1.12 (± 0.12, $P=0.95$), for tyramine 3.51 (± 1.46, $P=0.95$) and nicotine 0.73 (± 0.09, $P=0.95$). After cocaine 5 mg/kg thirteen times as much tyramine was required to produce the effect of one unit of pepsitensin (index = 13). When 10 mg/kg had been injected the index value for tyramine was 42 and for angiotensin 1.2.

Further studies of the site of the pressor action of pepsitensin

The adrenals. Pressor assays were performed in animals immediately after and 17 to 24 hr after adrenalectomy. The pressor action of pepsitensin could thus be studied in the absence of the hormones of the adrenal medulla and during adrenal insufficiency. Rats whose adrenals had been removed immediately before the assay seemed slightly more sensitive to the pressor action of pepsitensin and angiotensin than intact animals; one unit of pepsitensin had a pressor activity equivalent to 22.3 ng angiotensin and to 42.5 μg tyramine. Animals which had been maintained on a 5% glucose solution for 17 to 24 hr after the removal of the adrenals showed an unusual feature in that tachyphylaxis developed to the pressor action of angiotensin and pepsitensin (Fig. 5). The initial responses were 2 to 4 times weaker than

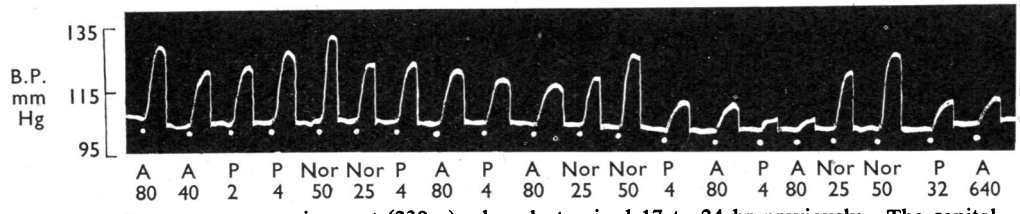

Fig. 5. Pressor responses in a rat (230 g) adrenalectomized 17 to 24 hr previously. The capital letters represent the substances and the numerals the quantities injected. Angiotensin (A) was given in ng, pepsitensin (P) in units and noradrenaline (Nor) in ng. The substances were injected every 5 min.

those produced in intact rats. In contrast these rats were about twice as sensitive to noradrenaline as intact animals; one unit of pepsitensin had a pressor activity approximately equivalent to 21 ng angiotensin and 10 ng noradrenaline. Rats which had been adrenalectomized and nephrectomized for 17 to 24 hr were as sensitive to pepsitensin and angiotensin as animals which had been nephrectomized only. Tachyphylaxis did not develop. One unit of pepsitensin was equivalent to 22.8 ng angiotensin, 349 ng noradrenaline, 6.99 m-u. Pitressin and 74.5 μg tyramine.

Heart in situ. No increase in frequency of heart beat or changes in the electrocardiogram were observed in intact or nephrectomized rats even when the blood pressure was raised by 60 mm Hg with pepsitensin or angiotensin.

Isolated heart. Large doses of pepsitensin (16 units) or angiotensin (2.5 μg) failed to alter the amplitude or frequency of the cardiac contractions or the coronary flow.

Isolated rat uterus. After pepsitensin was added to the bath there was a delay of about 40 sec before a slow contraction was produced, which was usually completed in about 60 sec. Contractions produced by angiotensin showed similar time relations. One unit of pepsitensin was equivalent to 21.7 ($\pm$3.5) ng angiotensin ($P=0.95$).

When a massive dose of angiotensin (100 times larger than that used in the preliminary assay) was applied, it was sufficient to desensitize the tissue to angiotensin without affecting the responses to 5-hydroxytryptamine. The " desensitization index " (DI) for angiotensin was 4.6 and that for 5-hydroxytryptamine 1.25. After a dose of angiotensin 50 times larger than that used in the preliminary assay had been added the desensitization index was 2.17. The results indicate that after a massive dose of angiotensin the sensitivity to that substance is more depressed than that to pepsitensin (Fig. 6).

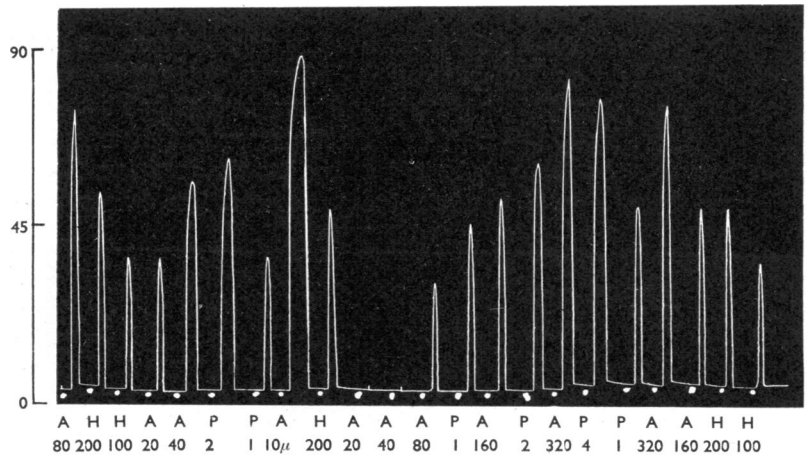

Fig. 6. Contractions of the isolated uterus of a rat, in response to angiotensin (A), pepsitensin (P) and 5-hydroxytryptamine (H), before and after the administration of a massive dose of 10 μg angiotensin. The numerals represent the quantities injected: angiotensin in ng, pepsitensin in units and 5-hydroxytryptamine in ng. The substances were administered every 5 min. Scale, mm.

Isolated guinea-pig ileum. One unit of pepsitensin was equivalent to 30.4 ($\pm$2.4) ng angiotensin ($P=0.95$). DI_{500} for angiotensin as auto-antagonist was 2.23 (mean of four estimations) and DI_{1000} 5.65. DI for 5-hydroxytryptamine in the latter experiment was 0.9. After 50 times the dose of pepsitensin used in the preliminary assay had been added the DI was 1.8. It appears that an ileum desensitized to angiotensin became relatively more sensitive to pepsitensin (Fig. 7) and vice versa.

The potency relationship between pepsitensin and angiotensin on the isolated rat uterus was not significantly different from that on the isolated guinea-pig ileum ($P<0.6$).

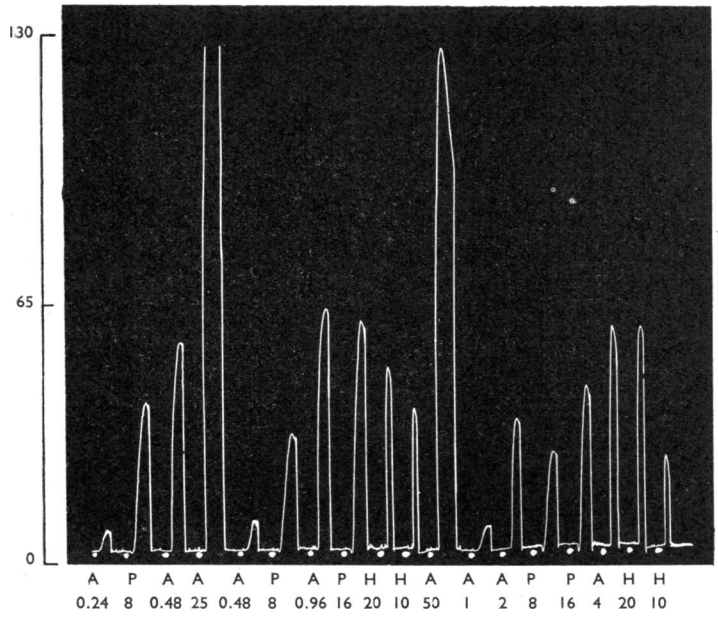

Fig. 7. Contractions of the isolated ileum of a guinea-pig in response to angiotensin (A), pepsitensin (P) and 5-hydroxytryptamine (H), before and after the administration of massive doses of 25 μg and 50 μg angiotensin. The numerals represent the quantities injected: angiotensin in μg, pepsitensin in units and 5-hydroxytryptamine in μg. The substances were administered every 5 min. Scale, mm.

Isolated perfused rabbit ear. Both pepsitensin and angiotensin constricted the vessels of this preparation. However, in equipressor doses angiotensin caused a more intense but shorter-lasting reduction in flow than pepsitensin. It is therefore difficult to estimate accurately the relative potency of the substances in this preparation.

Indices of discrimination

Potency relationships of pepsitensin and other pressor substances estimated from blood pressure assays in intact rats, nephrectomized (17 to 24 hr) rats and rats treated with reserpine were used to calculate the indices of discrimination (Table 4). The results indicate that pepsitensin is different in its mode of action from tyramine, nicotine, dimethylphenylpiperazine, noradrenaline and Pitressin, but resembles angiotensin.

Paper chromatography of pepsitensin. Descending chromatograms in the system n-butanol-acetic acid-water (4:1:5) gave the following R_F values: pepsitensin (preparation S2) 0.35 to 0.375, horse angiotensin (75% pure) 0.4 and synthetic angiotensin 0.4 to 0.5. About 30% of the activity of each substance applied was recovered. Ascending chromatograms in the same solvent system gave the following R_F values: pepsitensin (S2) 0.4 and horse angiotensin 0.3 to 0.4. Approximately 20% of the applied activity of each substance was recovered.

TABLE 4

INDICES OF DISCRIMINATION

Potency relationships were calculated from pressor assays in intact rats (I), nephrectomized rats (N) and rats treated with reserpine (R)

	$\frac{N}{I}$	$\frac{R}{I}$
$\frac{\text{Angiotensin}}{\text{Pepsitensin}}$	$\frac{24{\cdot}4}{22{\cdot}5}=1{\cdot}08$	$\frac{28{\cdot}2}{22{\cdot}5}=1{\cdot}25$
$\frac{\text{Nicotine}}{\text{Pepsitensin}}$	$\frac{42{\cdot}2}{13{\cdot}5}=3{\cdot}13$	$\frac{390{\cdot}0}{13{\cdot}5}=28{\cdot}9$
$\frac{\text{DMPP}}{\text{Pepsitensin}}$	$\frac{41{\cdot}0}{19{\cdot}0}=2{\cdot}16$	$\frac{391{\cdot}0}{19{\cdot}0}=20{\cdot}6$
$\frac{\text{Tyramine}}{\text{Pepsitensin}}$	$\frac{38{\cdot}0}{14{\cdot}4}=2{\cdot}64$	$\frac{800{\cdot}0}{14{\cdot}4}=55{\cdot}0$
$\frac{\text{Noradrenaline}}{\text{Pepsitensin}}$	$\frac{105{\cdot}0}{79{\cdot}4}=1{\cdot}32$	$\frac{2{\cdot}0}{79{\cdot}4}=0{\cdot}025$
$\frac{\text{Pitressin}}{\text{Pepsitensin}}$	$\frac{8{\cdot}05}{3{\cdot}01}=2{\cdot}67$	$\frac{3{\cdot}6}{3{\cdot}01}=1{\cdot}20$

DISCUSSION

In the experience of previous workers (Weber, Magor & Lobb, 1942; Braun-Menendez *et al.*, 1946; Paiva, 1954) the presence of depressor activity in crude preparations of pepsitensin has hindered studies of the pressor activity. In the present investigation it was found that euglobulin obtained by precipitation with distilled water, in contrast to salt-precipitated euglobulin, did not yield such substances.

In blood pressure assays some rats did not respond to pepsitensin. This may have been due to the stress of the operational procedure, since the same animals were also refractory to angiotensin and stress has been reported (Page, 1944) to lower the response to angiotensin. Rats which had been bilaterally nephrectomized 17 to 24 hr previously were 2 to 4 times more sensitive to pepsitensin than intact rats and proved very suitable for assays of pepsitensin. McCubbin & Page (1954) obtained similar enhancement to the pressor effect of angiotensin in nephrectomized dogs. In the present experiments the increase in sensitivity of nephrectomized rats to pepsitensin was progressive and was first noticeable after about 6 hr. Why nephrectomy should influence the pressor response to these polypeptides is not known. According to Collins & Harakal (1954) the " hypertensinase " content of dog plasma is decreased by approximately 34% two days after nephrectomy. This decrease may contribute to the increased pressor response to renin in nephrectomized animals, but angiotensin acts too quickly for enzymes to play a significant part in its pressor action. Gross & Lichtlen (1958) have suggested that three mechanisms may be responsible for the enhanced pressor actions of angiotensin in the nephrectomized animal: (*a*) a decrease in inactivation of hypertensive peptides; (*b*) a decrease in the content of renin and angiotensin in the body which may render it more sensitive to exogenous hypertensive peptides; and (*c*) an increase in vascular sensitivity to hypertensive stimuli due to higher sodium, potassium and water content of the arterial and arteriolar walls. McCubbin & Page (1954) believe

that increase or decrease in sodium uptake does not modify the effect of nephrectomy on the response to angiotensin. They suggest that the kidneys contain a substance inhibiting renin and angiotensin.

Croxatto, Pereda & Mellada (1959) and Croxatto & Barnafi (1960) have reported that freshly prepared serum incubated at *p*H 4.0 and 37° C for 1 to 30 hr, without pepsin, yields extracts with pressor activity. Croxatto called this substance "anephrotensin" and believes that it is liberated by enzymatic action. Anephrotensin was assumed to be different from vasopressin, since it raised the blood pressure markedly in nephrectomized (12 to 24 hr) animals but not in intact rats.

Incubation of euglobulin in an acid medium without pepsin yielded pressor activity in the present experiments. However, there was a difference in the yields from stored and freshly prepared euglobulin. Stored euglobulin produced only 3% of the activity of the pepsin-treated substrate when tested in intact and nephrectomized rats. In contrast, freshly prepared euglobulin yielded 27% of the activity of pepsin-treated substrates when tested in intact rats and 41% when tested in nephrectomized animals. This evidence supports Croxatto's suggestion that there is an enzyme in fresh plasma capable of releasing a pressor substance (or substances) after incubation at *p*H 4.0 which has greater pressor action in nephrectomized animals than in intact rats.

The identity of anephrotensin has not been established. The present work indicates that pepsitensin has two properties in common with anephrotensin, viz., a resistance to thioglycollate and enhancement of its pressor effect by nephrectomy. Croxatto believes that anephrotensin differs from pepsitensin by its antidiuretic action.

Pepsitensin, unlike vasopressin, was resistant to inactivation by thioglycollate. The analysis of variance of blood pressure assays in intact and nephrectomized rats indicated a significant difference between the two substances and a significant deviation from parallelism of the log dose-effect lines.

Information regarding the mechanism of the pressor action was obtained by comparison with other pressor substances in different preparations and after treatment with agents known to modify the responses to pressor substances. Parallel assays were performed in intact rats, in nephrectomized rats, in rats treated with reserpine, ganglion-blocking drugs, adrenaline antagonists, cocaine and bretylium tosylate, in pithed rats and in spinal rats. Both pepsitensin and angiotensin constricted the vessels of the perfused rabbit ear and were without significant action on the heart. The results differentiate pepsitensin from all other substances tested, except angiotensin, and permit the conclusion that it exerts pressor effect by direct action on the blood vessels.

The pressor responses to pepsitensin and angiotensin were unaffected by acute adrenalectomy, but rats adrenalectomized for 17 to 24 hr were less sensitive and showed rapid tachyphylaxis. Gross (1958) has suggested that there is a close relationship between the endocrine function of the kidney in releasing of renin and similar substances and the functional state of the adrenal cortex. He suggests that the lack of adrenocortical hormones stimulates renin secretion. It is of interest

that angiotensin has been shown to increase markedly the aldosterone and corticosterone production in the nephrectomized dog (Carpenter, Davis & Ayers, 1961). Nephrectomized and adrenalectomized rats were sensitive to pepsitensin and angiotensin.

In the guinea-pig ileum and the rat uterus exposed to angiotensin and pepsitensin similar potency relationships were found as in pressor assays, but by administering a massive dose of angiotensin both organs could be desensitized to angiotensin without reduction in the sensitivity to pepsitensin. However, since impure pepsitensin was used, these results cannot be regarded as conclusive evidence that pepsitensin is different from angiotensin.

Braun-Menendez & Paladini (1958) have reported that a given amount of hypertensinogen, treated with renin or pepsin under optimal conditions, yields equivalent amounts of pressor activity. A yield of not less than 100 μg of synthetic angiotensin can be obtained by the action of renin on euglobulin contained in 100 ml. plasma (Peart, personal communication). In the experiments here presented fresh euglobulin obtained from the same volume of plasma, when treated with pepsin, yielded pressor activity equivalent to that of only about 17 μg angiotensin. It is unlikely that hypertensinase, present in plasma proteins, influenced the yield of pepsitensin, since crude preparations are resistant to destruction by this enzyme (Braun-Menendez *et al.*, 1943) and the incubation was carried out at an acid *p*H. It is possible, however, that in altering the conditions for the action of pepsin (from those for renin) the low yield of pepsitensin may have been due either to denaturation of the substrate or to an alteration in the kinetics of the reaction.

Shortly after the discovery of pepsitensin, Alonso, Croxatto & Croxatto (1943) suggested that it was chemically identical with angiotensin. This suggestion cannot be verified until pure pepsitensin has been prepared. All that can be said at present is that crude pepsitensin is pharmacologically very similar to angiotensin and has a similar R_F value in the system butanol-acetic acid-water. These values are in agreement with those of Paiva (1954). It seems unlikely that pepsitensin has a physiological or even pathological significance. However, since pepsin, as well as renin, can produce substances with pharmacological activity there may be enzymes present in extravascular tissues which form similar polypeptides.

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