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A SPECTRUM OF PHARMACOLOGICAL ACTIVITY IN SOME BIOLOGICALLY ACTIVE PEPTIDES

BY

G. W. BISSET AND G. P. LEWIS

From the National Institute for Medical Research, Mill Hill, London, N.W.7

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The actions of bradykinin, angiotensin, oxytocin, vasopressin and substance P have been examined both on isolated smooth muscle preparations and *in vivo*. It was found that the isolated rat uterus and guinea-pig ileum can be used to distinguish between oxytocin and bradykinin and that the isolated rat colon and hen rectal caecum are almost specific test preparations for substance P. All the peptides were active on peripheral blood vessels, bradykinin, substance P and oxytocin causing vasodilatation and vasopressin and angiotensin vasoconstriction; bradykinin, substance P and angiotensin also caused an increase in capillary permeability in guinea-pigs. Only bradykinin and substance P were active in low concentrations in producing pain when applied to an exposed blister base. These two peptides were also active in causing bronchoconstriction. Oxytocin and vasopressin were the only peptides having milk-ejecting or antidiuretic activity which could be dissociated from cardiovascular effects. The spectrum of activity displayed by these peptides is in agreement with those functions which have been established for vasopressin and oxytocin and with those suggested, but not yet fully accepted, for bradykinin and angiotensin. It also indicates a possible function for substance P based on its vascular and permeability effects.

An analysis has been made of the pharmacological actions of synthetic bradykinin, angiotensin, oxytocin and vasopressin and of a laboratory preparation of substance P. The objects of the investigation were: firstly to reveal actions specific to a single peptide which might indicate its physiological or pathological significance; and secondly to assess the specificity of certain biological preparations used in bioassay work.

METHODS

Isolated smooth muscle preparations

Guinea-pig ileum. The terminal ileum from animals weighing 250 to 400 g was suspended in a 15 ml. bath containing oxygenated Tyrode solution at 34° C. Tests were made every 4 min and the contact time was 1 min.

Rat uterus and rat colon. Virgin rats weighing 120 to 200 g were injected subcutaneously with stilboestrol (100 μg/100 g) 16 to 18 hr before use. The uterine horn or a segment of colon was suspended in a 10 ml. bath containing de Jalon solution at 30° C. Tests were made every 4 min and the time of contact was 45 sec.

Hen rectal caecum. The proximal portion of the hen rectal caecum was suspended in a 15 ml. bath containing oxygenated Tyrode solution at 38° C. Tests were made every 10 min and the time of contact was 1.5 min.

In vivo *tests*

Simultaneous recordings of arterial blood pressure were made in tests for antidiuretic, milk-ejecting and bronchoconstrictor activities, in order to correlate these activities of the peptides with their cardiovascular actions. Blood pressure was recorded from the right carotid artery after cutting the right vagus nerve. Statham strain-gauge transducers were used to measure both arterial blood pressure and milk-ejection pressure in the cannulated milk duct. The transducers were arranged to write on a potentiometric recorder. This was either a single-channel recorder (Speedomax: Type H: Leeds & Northrup) or, for simultaneous recording of blood pressure and milk-ejection pressure, a double-channel recorder (Evershed & Vignoles).

Antidiuretic activity.. The method used, which is based on those of Ames & van Dyke (1952), Dicker (1953) and Dettelbach (1958), has recently been described in detail (Bisset, 1962). A water diuresis was induced in rats under ethyl alcohol anaesthesia. The rats were placed on a pair of scales and at regular intervals throughout the experiment injections of 2.5% ethyl alcohol were made through a stomach tube in order to maintain the water load at 7 to 8% of the body weight. Urine flow was recorded by a Thorpe impulse counter actuated by a 1 min time clock. The substances to be tested were injected intravenously. Continuous recording of the blood pressure did not inhibit diuresis, and repeated injections of small volumes of fluid into the stomach to maintain a constant water load did not produce any noticeable changes in the blood pressure record.

Milk-ejecting activity. Milk-ejection pressure was measured in the guinea-pig by a method based on that described by van Dyke, Adamsons & Engel (1955) for the rabbit. Lactating guinea-pigs weighing 750 to 1,000 g were taken from their litters about 7 days after parturition and anaesthetized by intraperitoneal injection of 0.5 to 0.7 ml./100 g of 25% urethane in saline. The trachea was cannulated, but artificial respiration was not applied. After excision of the tip of the nipple, nylon tubing, size oo of 0.5 mm internal bore, was inserted into a milk duct and connected with a transducer ; the system was filled with 3.8% sodium citrate to prevent clotting. Injections were given intravenously through a cannula in the external jugular vein at intervals of at least 5 min.

Bronchoconstrictor activity. The method used was that described by Konzett & Rössler (1940). Guinea-pigs were anaesthetized by intraperitoneal injection of 0.5 to 0.7 ml./100 g of 25% urethane in saline, and, immediately before artificial respiration was commenced, further injections of urethane were made intravenously until natural respiration ceased. All injections were made intravenously through a cannula in the external jugular vein.

Peripheral blood flow. This was examined in the hind limb of cats anaesthetized with pentobarbitone sodium 40 mg/kg. Venous outflow was recorded in the femoral vein using a photoelectric drop recorder and a Gaddum drop timer. Arterial injections were made through a cannula in a side branch of the femoral artery.

Capillary permeability. Guinea-pigs depilated 24 hr previously were injected intracardially, and rabbits intravenously, with Pontamine sky blue (60 mg/kg). A few minutes after injection of the dye the peptides in 0.1 ml. saline were injected intradermally into the abdominal skin. Thirty minutes later the diameter of the area of blueing was measured.

Pain production. The method used was that described by Armstrong, Dry, Keele & Markham (1953). Blisters were raised on the flexor surface of the forearms of human subjects by the application of cantharidin plasters 2×2 cm. The plasters were applied in the evening before the experiment and allowed to act for 6 hr. The area was covered with a sterile dressing and a blister allowed to form during the night. The skin of the blister was cut away and the blister base was washed with warm Ringer solution containing (g/l.) NaCl 9.2, KCl 0.4, $CaCl_2$ 0.24 and $NaHCO_3$ 0.15. Drugs to be tested were dissolved in this solution and kept at 37° C during the experiment.

The solution to be tested was applied to the blister area with a Pasteur pipette until the area was filled, and allowed to act until the pain reached a steady intensity, or began to subside, but not longer than 2 min. The area was then thoroughly washed with the Ringer solution and again periodically between successive tests which were made at intervals of

10 or 20 min. The subject was not told the nature of the applied solution ; he assessed pain intensity of each solution subjectively, grading it from 0 to + + +.

Materials. Synthetic bradykinin (Parke, Davis & Co.) and synthetic angiotensin (val^5-Hypertensin II-Asp-β amide) (Ciba) were used.

The preparations of oxytocin and vasopressin used were commercially available synthetic oxytocin (" Syntocinon " brand of injection of oxytocin, B.P. Sandoz, 10 u./ml.) and synthetic lys^8-vasopressin (Sandoz, 24 pressor u./ml.). Doses are given in units: the approximate corresponding weights of the pure substances are shown in parentheses. These weights were calculated on the basis of the estimate that pure synthetic oxytocin (Sandoz) contains 450 u./mg and synthetic lys^8-vasopressin (Sandoz) contains 270 u./mg (rat pressor activity) (Boissonnas, Guttmann, Berde & Konzett, 1961).

The sample of substance P was prepared from cow intestine by the method of Pernow (1953). In addition the final product was subjected to countercurrent distribution between butanol:acetic acid and water. The countercurrent distribution was kindly carried out by Dr D. F. Elliott, of the National Institute for Medical Research, Mill Hill, London. The preparation contained 15 u./mg. Doses are given in units ; the corresponding weights of pure substance P are shown in parentheses. These weights were calculated on the basis of the estimate of Franz, Boissonnas & Sturmer (1961) that pure substance P contains 30,000 u./mg.

RESULTS

Isolated smooth muscle preparations

The effective concentrations of the peptides on four isolated smooth muscle preparations are shown in Table 1. The rat uterus was contracted by relatively low concentrations of all the peptides. The guinea-pig ileum was as sensitive as

TABLE 1

CONCENTRATIONS (NG/ML.) OF PEPTIDES WHICH CAUSE CONTRACTION (C) OR RELAXATION (R) OF ISOLATED SMOOTH MUSCLE PREPARATIONS

The concentrations of oxytocin, vasopressin and substance P are expressed as ng/ml. of the pure substances and have been converted from units/ml. as described under methods

Peptide	Guinea-pig ileum	Rat uterus	Rat colon	Hen rectal caecum
Bradykinin	C 0·4–1·0	C 0·1–0·3	R+C 1,000	C or R 1,000
Angiotensin	C 0·4–0·8	C 0·4–0·7	C 500	C 1,000
Oxytocin	R 1,000	C 0·2–0·4	C 1,000	R 300
Vasopressin	C 30	C 0·8–1·0	C 1,000	R 70
Substance P	C 5	C 5–15	C 1·5	C 10

the rat uterus to angiotensin, less sensitive to bradykinin, a little more sensitive to substance P and relatively insensitive to vasopressin and oxytocin. The action of oxytocin in contrast with all the other peptides was to relax the ileum when the preparation was not already fully relaxed.

The rat colon and the hen rectal caecum were more sensitive to substance P than to any of the other peptides. The rat colon was particularly sensitive to substance P and contracted to the other peptides only in extremely high concentrations. With bradykinin the response was in fact a mixed one, the contraction being preceded by relaxation. The hen rectal caecum was regularly contracted by angiotensin, whereas bradykinin produced contractions in some preparations and relaxation in others, while both oxytocin and vasopressin caused relaxation. The actions of these four peptides were obtained only with high concentrations.

In vivo *tests*

Antidiuretic activity. Vasopressin inhibited urine flow in a dose as small as 0.025 m-u. (0.1 ng). Antidiuretic responses to doses ranging from 0.025 m-u. (0.1 ng) to 0.2 m-u. (0.8 ng) are illustrated in Figs. 1 and 2. These responses were characteristic of lys^8-vasopressin. The maximum intensity was reached in the third or fourth minute after the injection and urine flow returned to the pre-injection level in 10 to 15 min, depending on the dose. Oxytocin in doses of 2.5 m-u. (5 ng) and above produced an antidiuretic response similar in character to that of vasopressin, but with moderate rates of urine flow, this antidiuretic response was followed by a diuretic phase in which urine flow was increased above the basal level for as long as 20 min. It was calculated that 1 m-u. (2 ng) oxytocin was equivalent in antidiuretic activity to 0.016 m-u. (0.06 ng) of vasopressin. A dose of 20 m-u. (40 ng) oxytocin which gave a strong antidiuretic response did not have any effect on arterial blood pressure.

Angiotensin had only a transient antidiuretic action in doses which produced a considerable pressor response. The differences between the effects of angiotensin and vasopressin on urine flow and blood pressure are illustrated in Fig. 1. In a dose of only 2 ng angiotensin caused a rise of arterial blood pressure of about 10 mm Hg, but even when the dose was increased to 64 ng the only detectable effect on urine flow was an inhibition limited to the first min after the injection. By contrast 0.1 m-u. (0.4 ng) vasopressin inhibited urine flow almost completely for a period of 7 min with no change in blood pressure. The smallest dose which elicited a pressor effect in the rat was 0.5 m-u. (2 ng). Vasopressin produced a more gradual and prolonged rise of blood pressure than angiotensin. On the basis of the maximum rise produced, 1 m-u. (4 ng) vasopressin was equivalent to 8 ng angiotensin.

Bradykinin and substance P did not exhibit any significant antidiuretic activity except in doses which caused a profound fall of blood pressure. The effect of bradykinin is shown in Fig. 2. Doses of 2 to 4 μg caused a fall of 20 to 30 mm Hg with no effect on urine flow and a dose of 8 μg caused a fall of 40 mm Hg with a transient inhibition of urine flow. A dose of 16 μg produced a fall of 50 mm Hg, and, in spite of the fact that the blood pressure returned to the pre-injection level in 2 min, there was a prolonged inhibition of urine flow which did not return to normal until 40 min after the injection. In this preparation a typical antidiuretic response to vasopressin was obtained with doses of 0.05 to 0.2 m-u. (0.2 to 0.8 ng); the response to 0.2 m-u. was of greater intensity but more transient than that to 16 μg bradykinin. The effect of substance P is shown in Fig. 3. Doses of 3 and 6 u. (0.1 and 0.2 μg) lowered blood pressure by less than 25 mm Hg and caused only a transient inhibition of urine flow. A dose of 24 u. (0.8 μg) caused a fall of arterial blood pressure of about 70 mm Hg. The pressure returned to normal within 3 min, but urine flow was inhibited almost completely for about 20 min and then recovered slowly.

To test the association between fall of blood pressure and inhibition of urine flow, the effect of the vasodilator substance isoprenaline was examined. In the experiment illustrated in Fig. 3, a dose of 0.025 μg produced a small fall of arterial

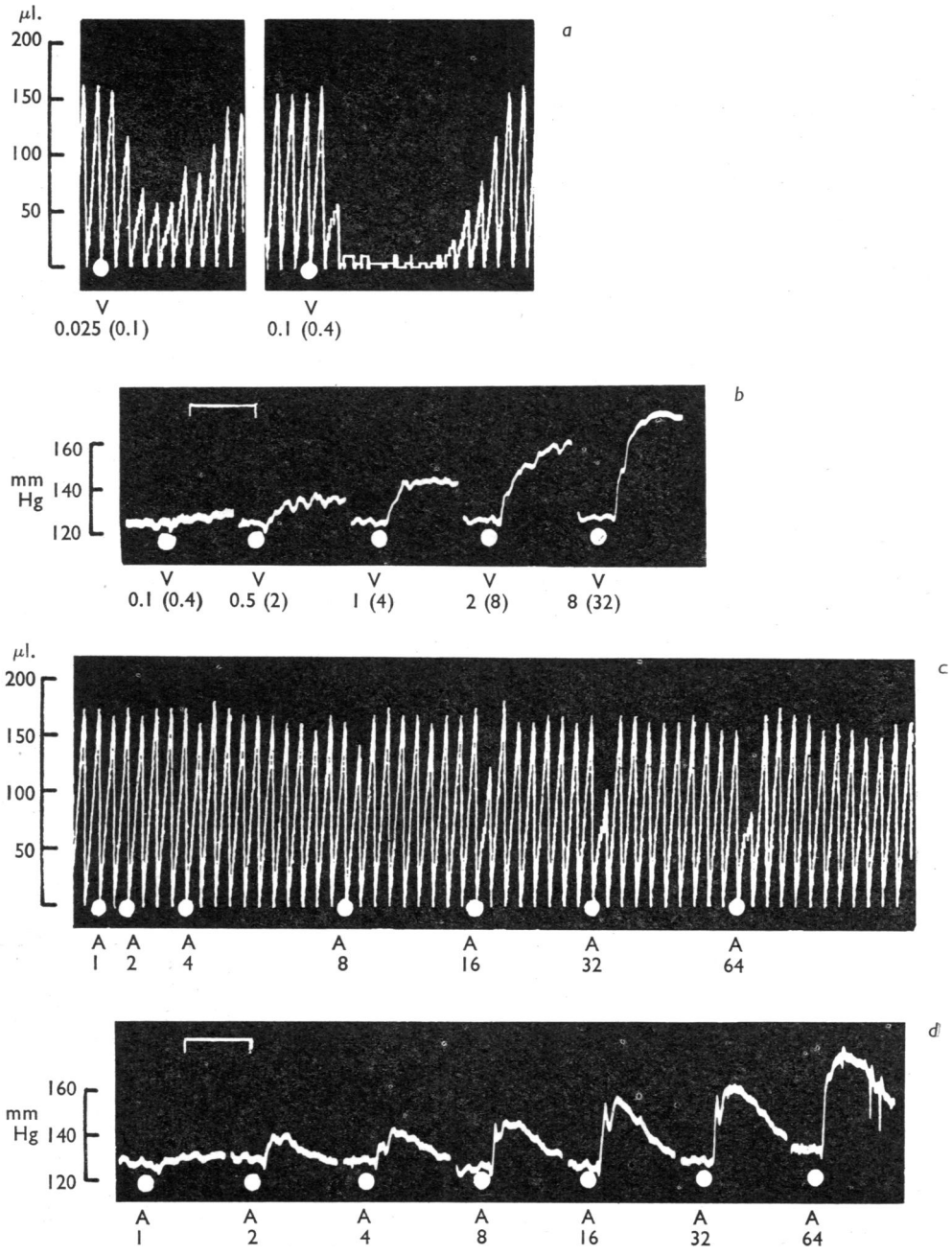

Fig. 1. Rat anaesthetized with ethyl alcohol. Records (*a*) and (*c*): urine flow recorded by Thorpe impulse counter actuated by a 1-min time clock. Records (*b*) and (*d*) (which are continuous): arterial blood pressure (time 1 min). Responses to intravenous injection of angiotensin (A; doses given in ng) and vasopressin (V; doses given in m-u. and ng in parenthesis).

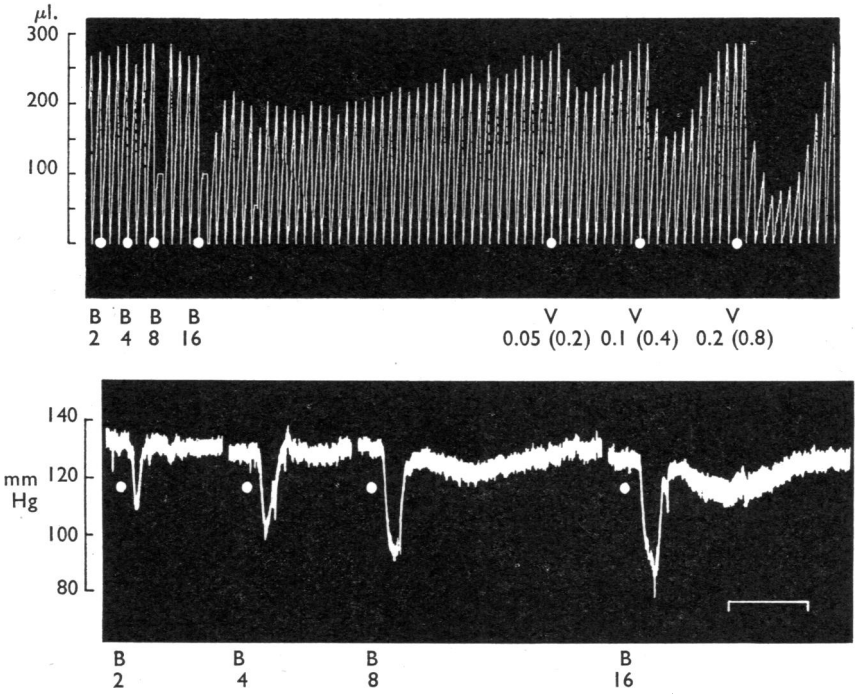

Fig. 2. Rat anaesthetized with ethyl alcohol. Upper record: urine flow recorded by Thorpe impulse counter actuated by a 1-min time clock. Lower record: arterial blood pressure (time, 1 min). Responses to intravenous injections of bradykinin (B; doses in µg) and vasopressin (V; doses in m-u. and ng in parenthesis).

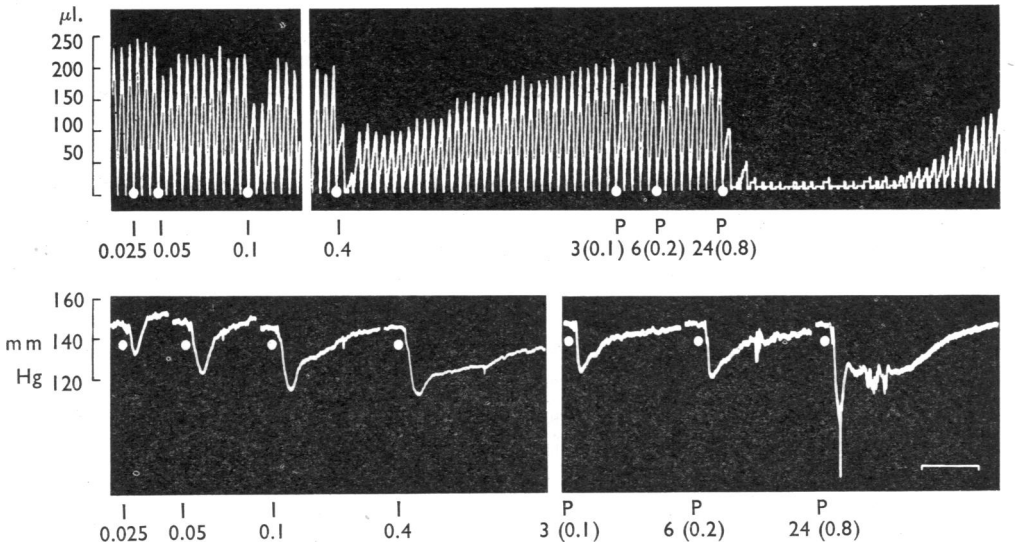

Fig. 3. Rat anaesthetized with ethyl alcohol. Upper record: urine flow recorded by Thorpe impulse counter actuated by a 1-min time clock. Lower records (which are continuous): arterial blood pressure (time, 1 min). Responses to intravenous injections of isoprenaline (I; doses in µg) and substance P (P; doses in u. and µg in parenthesis).

blood pressure with no effect on urine flow and doses of 0.05 and 0.1 μg larger falls with transient inhibition of urine flow during a period of 2 min after the injection. The effect of 0.4 μg, which lowered the blood pressure by about 35 mm Hg, was to produce a prolonged inhibition of urine flow similar in character to that observed after 16 μg bradykinin and 24 u. (0.8 μg) substance P. From this experiment it was concluded that the antidiuretic action of large doses of these two peptides is probably a direct result of the accompanying precipitous fall in blood pressure.

Milk-ejecting activity. In the lactating guinea-pig the smallest dose of oxytocin which produced a measurable increase of pressure in the cannulated milk duct was 1 m-u. (2 ng) and, of vasopressin, 5 m-u. (20 ng). The response to this dose of vasopressin was accompanied by a rise of arterial blood pressure of 25 mm Hg. Parallel log dose-response curves were obtained from which it was calculated that 1 m-u. vasopressin (4 ng) was equivalent in milk-ejecting activity to 0.20 m-u. (0.4 ng) oxytocin.

Bradykinin (up to 10 μg) and angiotensin (up to 25 μg) had no milk-ejecting activity. Bradykinin in a dose of 1 μg caused a fall of arterial blood pressure of 15 mm Hg and 0.1 μg angiotensin caused a rise of 40 mm Hg.

Substance P in a dose of 1.5 u. (50 ng), which produced a fall of arterial blood pressure of 15 mm Hg, did not cause milk ejection. In doses of 37.5 u. (1.25 μg) and above, small milk-ejection responses were obtained. It was calculated that 1 u. (33 ng) substance P was equivalent in milk-ejecting activity to 0.033 m-u. (0.07 ng) oxytocin. As a possible contaminant of the preparation of substance P, acetylcholine was tested in doses of 1 to 5 μg. An effect was discernible with 5 μg which was smaller than that obtained with 37.5 u. (1.25 μg) substance P but accompanied by a greater fall of blood pressure.

Bronchoconstrictor activity. Bradykinin was the most active peptide in causing bronchoconstriction. In confirmation of the results of Collier, Holgate, Schachter & Shorley (1960), it was found that the bronchoconstrictor response to bradykinin was different in character from the response to histamine. With bradykinin, and with the other peptides tested, the increase in bronchiolar tone was more gradual in onset and of longer duration than with histamine. The log dose-response curves for the peptides were flatter ; a doubling of the dose produced only a small increase in the amplitude of the response. For this reason, relative potencies could be expressed only approximately.

Bradykinin in many experiments exhibited tachyphylaxis. In one experiment in which this effect was absent and graded responses were obtained to increasing doses of the substances tested, it was found that 0.25 μg bradykinin was equipotent in bronchoconstrictor activity to 1 μg histamine. Although the initial blood pressure in the preparations used was only about 40 mm Hg, the bronchoconstrictor responses to bradykinin were accompanied by falls of blood pressure ranging from 5 to 15 mm Hg. There was no correlation between the bronchoconstrictor and depressor effects. Substance P, however, in doses of 3.75 to 7.5 u. (125 to 250 ng) caused a fall of blood pressure of 10 to 15 mm Hg without bronchoconstriction. Larger doses produced a small bronchoconstrictor effect, which, in contrast with bradykinin, did not exhibit tachyphylaxis. The bronchoconstrictor effect of 60 u. (2 μg) was

equivalent to that of 0.5 μg bradykinin. The bronchoconstrictor effects of bradykinin and substance P were not reduced by doses of mepyramine which abolished the response to histamine.

Angiotensin caused only a slight degree of bronchoconstriction in doses having a strong pressor effect. Although a rise of blood pressure was obtained with 50 ng, bronchoconstrictor responses to doses of 0.1 to 1 μg were negligible. In the experiment in which equivalent bronchoconstrictor responses to 0.25 μg bradykinin and 1 μg histamine were obtained, the equiactive dose of angiotensin was 8 μg.

Oxytocin and vasopressin had no significant bronchoconstrictor activity. Oxytocin in a dose of 2.5 u. (5 μg) produced a rise of arterial blood pressure of 40 mm Hg, but did not cause bronchoconstriction. In one experiment a small bronchoconstrictor response was obtained with 0.5 u. (2 μg) vasopressin, equivalent to that of 1 μg bradykinin, but in another experiment with a preparation responding to 0.25 μg bradykinin, 2.5 u. (10 μg) vasopressin did not cause bronchoconstriction. The minimum effective dose of vasopressin for a pressor response in the guinea-pig was 5 m-u. (20 ng).

Peripheral blood flow. On arterial injection into the cat hind limb, bradykinin, substance P and oxytocin caused vasodilatation and angiotensin and vasopressin vasoconstriction. These effects are illustrated in Fig. 4.

Both bradykinin and substance P were much more active than oxytocin. Vasodilator responses to 10 to 100 ng bradykinin were equivalent to those produced by 0.3 to 3 u. (10 to 100 ng) substance P. In order to produce responses equivalent to 20 to 30 ng bradykinin it was necessary to inject 1 to 1.5 u. (2 to 3 μg) oxytocin.

The vasoconstrictor responses to 20 to 30 ng angiotensin were equivalent to those produced by 7.5 m-u. (30 ng) vasopressin. On repeated injections the response to vasopressin decreased because of tachyphylaxis.

None of the vascular actions which have been described was affected by atropine (1 mg) or mepyramine (1 mg) injected intra-arterially.

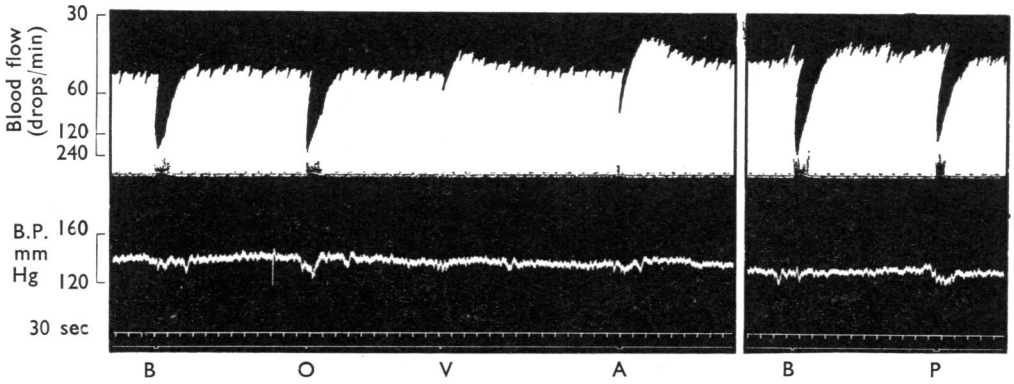

Fig. 4. Cat anaesthetized with pentobarbitone sodium 40 mg/kg. Upper record: venous outflow from the hind limb recorded with a Gaddum drop-timer. Lower record: arterial blood pressure. Responses to bradykinin 30 ng (B), oxytocin 1.4 u. (3 μg) (O), vasopressin 5.4 m-u. (22 ng) (V), angiotensin 20 ng (A) and substance P 0.75 u. (25 ng) (P).

Capillary permeability. When the permeability of the capillaries is increased after intradermal injection of drugs, protein-bound dye, injected into the circulating blood, seeps into the extravascular space and the affected area becomes coloured.

Oxytocin, vasopressin, and substance P were examined in guinea-pigs only, whereas bradykinin and angiotensin were also examined in rabbits.

Neither oxytocin 5 u./ml. (10 μg/ml.) nor vasopressin 0.25 u./ml. (1 μg/ml.) increased capillary permeability, and in concentrations down to 2.5 m-u./ml. (10 ng/ml.) vasopressin caused an area of constriction in the blued animal, as shown by the disappearance of the blue-coloured blood from the vessels in the injected area.

Both bradykinin and substance P increased capillary permeability. Bradykinin was examined in concentrations of 0.01 to 10 μg/ml. and substance P in concentrations of 0.075 to 7.5 u./ml. (2.5 to 250 ng/ml.). The blueing was less intense with substance P than with bradykinin and in the highest concentrations the area of blueing produced by substance P was more diffuse. The response to substance P thus resembled the response to histamine rather than that to bradykinin. This is illustrated by comparison of the dose-response curves shown in Fig. 5. Mepyramine (1 mg/kg) given intraperitoneally 5 min before the intradermal injection did not affect the response to bradykinin, reduced that to substance P and abolished the response to histamine. The effect on the response to bradykinin and histamine is illustrated in Fig. 6.

Angiotensin increased capillary permeability in the guinea-pig in concentrations of 0.01 to 10 μg/ml. (Fig. 6*a*), but had no such effect in rabbits. Like bradykinin,

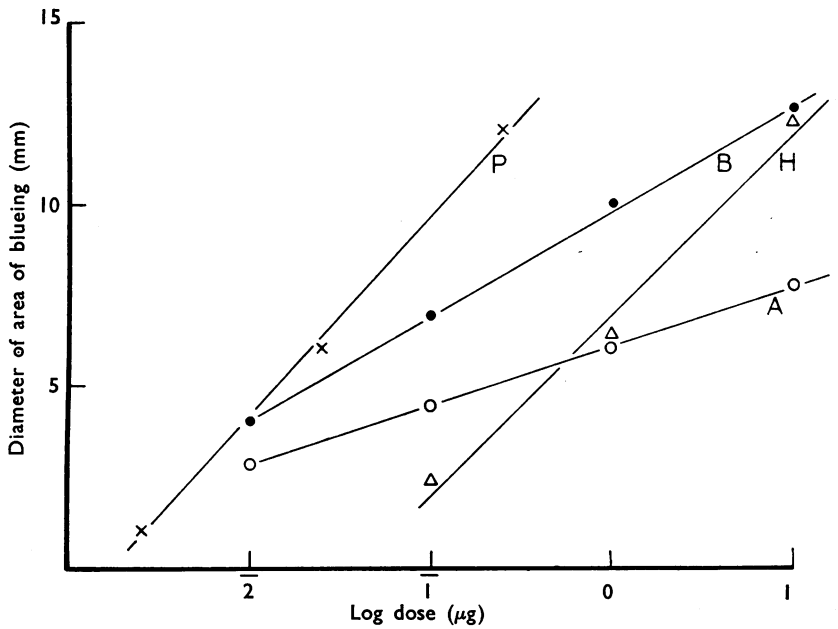

Fig. 5. Increased capillary permeability in guinea-pig skin. Log dose-response curves of bradykinin (B, ● —— ●), substance P (P, X —— X), angiotensin (A, ○ —— ○) and histamine (H, △ —— △).

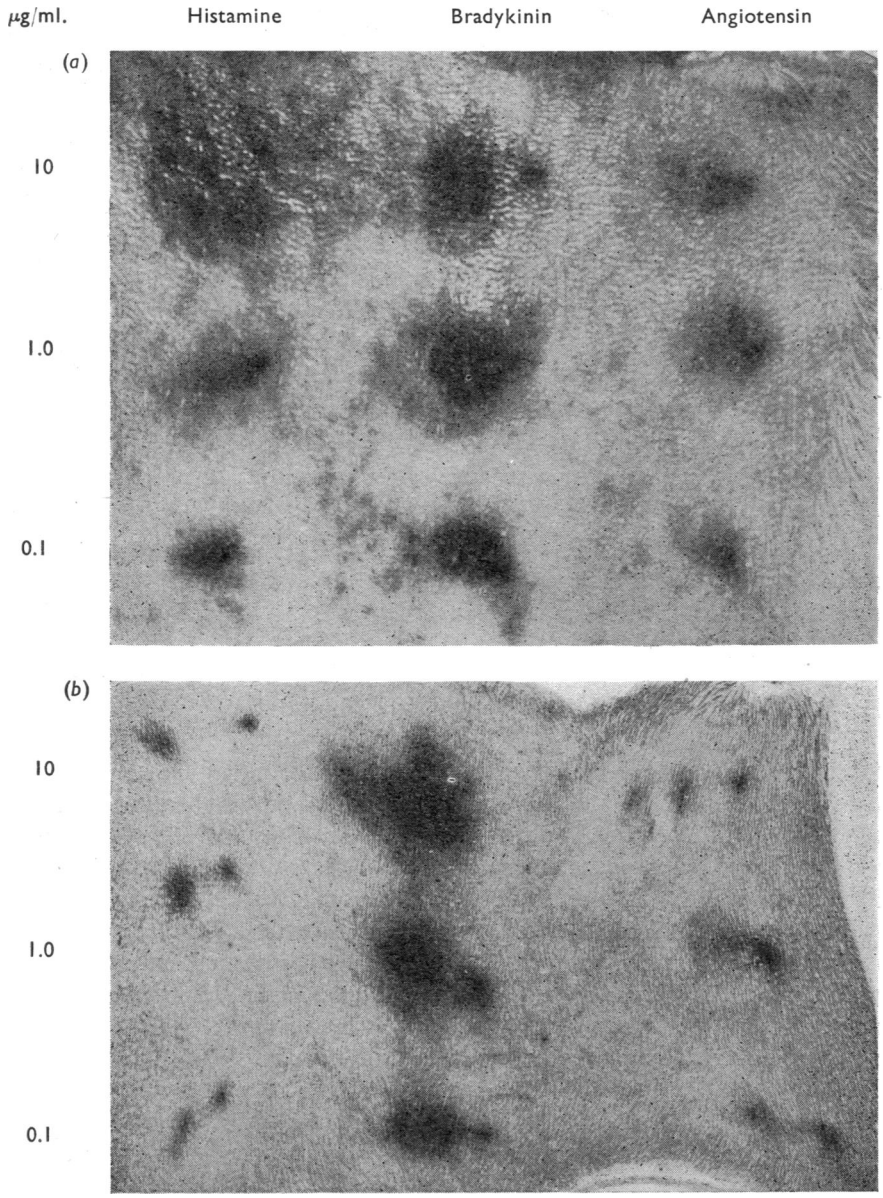

Fig. 6. Areas of blueing in the skin of guinea-pigs which had received Pontamine sky blue 60 mg/kg intravenously followed by intradermal injections of 0.1 ml. of solutions containing histamine, bradykinin and angiotensin 10 μg/ml., 1 μg/ml. and 0.1 μg/ml. Responses in a normal guinea-pig at (*a*) and in a guinea-pig treated with mepyramine maleate 1 mg/kg intraperitoneally 5 min before intradermal injections at (*b*).

angiotensin did not cause a large area of blueing and gave a flat dose-response curve (Fig. 5). Unlike the response to bradykinin, the blueing following injection of angiotensin was not intense, probably due to the accompanying vasoconstriction, and was abolished by previous administration of mepyramine. This effect of mepyramine is illustrated in Fig. 6. The antagonism was not due to the presence of histamine, as other effects of angiotensin were not affected by mepyramine and its activity on the guinea-pig ileum was completely eliminated after treatment with trypsin. Nor was the increased permeability due to histamine release, because angiotensin 50 μg did not release histamine when tested in one experiment on the perfused rat hind-quarter preparation (Mongar & Feldberg, 1954), while 100 μg compound 48/80 given after the angiotensin released 20 μg histamine.

Pain production. All the peptides produced pain when applied to an exposed blister base. Bradykinin 0.1 to 0.5 μg/ml. gave rise to a burning sensation unlike the intense itching sensation after histamine. On repeated administration of the peptide, the blister base became insensitive to bradykinin although not to the other peptides, histamine or 5-hydroxytryptamine.

Substance P produced a burning sensation in a concentration of 15 u./ml. (0.5 μg/ml.) but not of 7.5 u./ml. (0.25 μg/ml.).

Oxytocin 5 u./ml. (10 μg/ml.), vasopressin 2.5 u./ml. (10 μg/ml.) and angiotensin 50 μg/ml. also produced a burning pain but were ineffective in one-tenth of these concentrations. The blister base was blanched by vasopressin 2.5 u./ml. (10 μg/ml.) and by lower concentrations which did not produce pain.

DISCUSSION

The usefulness of smooth muscle preparations for discriminating between polypeptides is evident from the fact that the concentrations required to produce small effects differ greatly between the various peptides. Similar results have been obtained by Gaddum & Szerb (1961). The rat colon and the hen rectal caecum are particularly useful for the identification and assay of substance P, since the other peptides are either inactive or relatively inactive on these preparations. The isolated rat uterus is the least discriminating of the preparations tested. It is almost equally sensitive to oxytocin, bradykinin and angiotensin. On the other hand, the guinea-pig ileum, which has the same order of sensitivity as the rat uterus to bradykinin and angiotensin, responds to oxytocin only in high concentrations and the response is usually an inhibitory one. Parallel quantitative assays on the rat uterus and guinea-pig ileum would provide a means of distinguishing between oxytocin and bradykinin in a mixture of these two peptides.

Bradykinin and substance P show a remarkable similarity in their spectra of pharmacological activity. If the amount of substance P present in the impure preparation used in this investigation is calculated from the estimate of Franz, Boissonnas & Sturmer (1961), this substance would appear to be as active as bradykinin in its vasodilator action, in increasing capillary permeability and in producing pain. In their effects on capillary permeability a qualitative difference was observed between the two peptides. In the guinea-pig skin, bradykinin causes

a small intense area of blueing while substance P causes a more diffuse area of less intense blueing like that produced by histamine. It has been suggested (Lewis, 1960) that the diffuse nature of the response to histamine is due to its causing an axon reflex vasodilatation, and a similar mechanism might apply to substance P.

The action of both bradykinin and substance P on peripheral blood vessels is sufficiently potent to suggest a vascular function of these two peptides in physiological or pathological reactions. The formation of bradykinin at a given site is determined by the presence of an enzyme acting on a plasma globulin. Rapid destruction occurs in the blood and interstitial fluid so that its action is confined to its site of formation. Substance P is relatively stable in the body and can be extracted from the brain and intestine. Its possible physiological vasodilator function may be confined to these organs whereas bradykinin probably has a much wider vasodilator function as suggested by Hilton & Lewis (1957) acting at all sites where it is formed by plasma kinin forming enzymes (Lewis, 1959).

The finding that prolonged antidiuretic responses to bradykinin and substance P are preceded by a fall of arterial blood pressure of 40 mm Hg or more is of interest in connexion with an observation by Ginsburg & Brown (1957). These authors found that in rats anaesthetized with ethyl alcohol, which do not readily release vasopressin from the neurohypophysis, haemorrhage had this effect, but that the first great increase in the antidiuretic activity in the blood occurred only after the blood pressure had fallen by about 50 mm Hg. It is thus possible that the antidiuretic action of large doses of bradykinin and substance P is mediated by the release of vasopressin from the neurohypophysis in response to a sudden large fall of blood pressure. The fact that their antidiuretic action could be simulated by isoprenaline is consistent with this view.

The close similarity of substance P to bradykinin in its pharmacological actions may reflect a similarity of chemical structure as in the case of oxytocin and vasopressin.

Angiotensin is most potent in its effect on peripheral blood flow and blood pressure. Unlike vasopressin, it causes an increase in capillary permeability in the guinea-pig. This effect is abolished by mepyramine although it is not due to contamination of angiotensin with histamine or to histamine release. Since the effect of substance P is also reduced, mepyramine may have an effect on capillary permeability unrelated to its antihistamine action.

Pressor doses of angiotensin cause a fleeting inhibition of urine flow similar to that observed with the smaller doses of bradykinin and substance P. This inhibition is suggestive of an action on the smooth muscle of the ureter, or of a transient renal vascular action, rather than a direct effect, like that of vasopressin, on reabsorption of water by the renal tubules. Even with doses producing a rise in arterial blood pressure of 60 mm Hg the antidiuretic effect of angiotensin is only transitory. On the other hand, a prolonged antidiuresis has been obtained by intravenous infusion in non-anaesthetized dogs (Gross & Turrian, 1959) and in human subjects with normal blood pressure (Peart, 1960). The results obtained in the present investigation show that it is possible, at least in the anaesthetized animal, to obtain a pronounced pressor response to angiotensin with negligible antidiuretic action.

Oxytocin provides an interesting contrast with bradykinin. Although bradykinin is as potent as oxytocin on the isolated rat uterus it is relatively inactive on the uterus *in situ* on intravenous injection (Berde & Saameli, 1961) and it has no milk-ejecting activity. Both peptides cause peripheral vasodilatation, but oxytocin is only 1/100th as potent as bradykinin. Unlike bradykinin, oxytocin does not increase capillary permeability and it produces pain only in exceptionally high concentrations. The accepted physiological functions of oxytocin are milk-ejection and uterine contraction. In the spectrum of activity of bradykinin the emphasis lies on local actions which may be concerned with the control of blood flow and are possibly involved in the pathology of the acute inflammatory reaction (Lewis, 1962).

Vasopressin and angiotensin are about equally potent in their peripheral vasoconstrictor and pressor actions, but the effects of these two peptides on the kidney are strikingly different. The dose of vasopressin required to produce a detectable rise of blood pressure is about 40 times as great as that which produces antidiuresis. This high ratio of antidiuretic to pressor activity in vasopressin is consistent with its physiological function of controlling renal tubular reabsorption of water. In contrast with vasopressin, angiotensin in doses which produce a large rise of blood pressure causes only a transitory inhibition of urine flow. The predominance of the pressor action of angiotensin is in favour of the view that this peptide is involved in the pathology of hypertension.

The anaesthetized rat in alcohol diuresis and the mammary gland of the lactating guinea-pig provide preparations which are almost specific for neurohypophysial hormones. Vasopressin inhibits diuresis in a dose of less than 0.4 ng/kg. Oxytocin has a qualitatively similar antidiuretic effect, followed by a diuretic phase, in about 40 times this dose. None of the other peptides elicits an antidiuretic action which could be confused with that of vasopressin except in doses causing a profound effect on blood pressure. Oxytocin causes milk-ejection in a dose of 2 ng. This is of the same order as the dose required to cause contraction of the isolated rat uterus when added to an organ bath of conventional size. Vasopressin has milk-ejecting activity equal to about 1/10th that of oxytocin on a weight basis, but the milk-ejection responses to vasopressin are accompanied by a rise of blood pressure. The only other peptide which produces milk-ejection is substance P in doses having a marked depressor action. The absence of milk-ejecting activity in bradykinin and angiotensin confirms the findings of Fitzpatrick & Walmsley (1962) in the guinea-pig and those of Berde & Cerletti (1961) in the rabbit.

In order to assay neurohypophysial hormones in blood it is usually necessary to prepare concentrated extracts and in the preparation of these extracts it is difficult to exclude the formation of bradykinin or related plasma kinins. It has been found that acid alcohol extracts of blood contain a factor active on the isolated rat uterus, which can be distinguished from oxytocin by the fact that it is not destroyed by sodium thioglycollate (Bisset & Walker, 1954; Bisset & Lee, 1957; Hawker & Robertson, 1958). This factor does not cause milk-ejection in the lactating rabbit (Hawker & Robertson, 1958) nor does it inhibit urine flow in the anaesthetized rat (Bisset, 1961). The results of the present investigation would be consistent with the view that this factor is bradykinin or a closely related substance. The isolated

rat uterus is evidently unsatisfactory for the assay of oxytocin in blood unless lengthy procedures are carried out to separate oxytocin from bradykinin. The use of the lactating mammary gland makes such a separation unnecessary. The sensitivity of this preparation to oxytocin and of the anaesthetized rat in diuresis to vasopressin is so high that, especially if blood pressure is recorded simultaneously as in this investigation, it should be possible to distinguish these hormones even in relatively crude extracts from any other known pharmacological agent.

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