STUDIES ON THE PRESSOR RESPONSES PRODUCED BY BRADYKININ AND KALLIDIN

BY

W. J. LANG AND LEONIE PEARSON

From the Department of Pharmacology, University of Melbourne, Parkville, Victoria 3052, Australia

The hypotensive effect of bradykinin was first described by Rocha e Silva, Beraldo & Rosenfeld (1949), and has been found in all mammals so far investigated (Stürmer & Cerletti, 1961 ; Schröder & Hempel, 1964). A number of studies, however, have shown that bradykinin may also increase the blood pressure, or cause a biphasic response. Thus, Croxatto, Belmar, Pereda & Labarca (1962) reported a hypertensive effect of bradykinin in rats with low blood pressure, and Lecomte, Troquet & Cession-Fossion (1964) described biphasic responses in rats and rabbits after repeated administration of bradykinin. Using cross-circulation techniques in dogs, it was shown by Benetato, Hăulică, Muscalu, Bubuianu & Gălesanu (1964) that intra-cranial administration of bradykinin produced a biphasic response. A rise in blood pressure has also been described when bradykinin is injected intra-arterially to various organs, including the brain, in cats and dogs. It was considered to be a reflex component of the " pseudo-affective " response to pain (Guzman, Braun & Lim, 1962).

The related polypeptide kallidin resembles bradykinin in that it usually causes a fall in blood pressure (Stürmer & Berde, 1963). It too, however, may have more complex effects. Thus, like bradykinin, it has a biphasic effect after repeated injections in rats or rabbits (Lecomte *et al.*, 1964).

Feldberg and Lewis (1964) have shown that bradykinin is a potent releaser of catecholamines from the suprarenal medulla of cats ; a similar action has been shown for both bradykinin and kallidin on the suprarenal medulla of dogs (Staszewska-Barczak & Vane, 1965). These peptides also stimulate the superior cervical ganglion of the cat (Lewis & Reit, 1965). Stimulation of the adrenal medulla, or ganglion-cells, or both, could explain the pressor effects of these polypeptides. The pressor phase of the cardiovascular response to bradykinin and kallidin in rabbits and rats is accentuated by guanethidine, pronethalol and desmethylimipramine (Miele, 1966 ; Lecomte *et al.*, 1964), which suggests that it is caused by release of catecholamines from the adrenal medulla.

We have frequently observed biphasic responses to bradykinin and kallidin in the course of experiments on their cardiovascular action in conscious and anaesthetized dogs (Pearson & Lang, 1967b). We also observed biphasic responses in other anaesthetized animals and chose the cat to analyse the mechanism of the pressure effects to these polypeptides more fully. A preliminary communication has been given on the findings reported in this paper (Pearson & Lang, 1967a).

METHODS

The experiments were performed on cats of either sex weighing between 2 and 5 kg. Chloralose (60 mg/kg) or pentobarbitone sodium (40 mg/kg) was administered intraperitoneally for experiments on anaesthetized animals. Some cats were decerebrated by midcollicular section and others were made spinal as described by Burn (1952) after anaesthesia had been induced with ether.

Arterial blood pressure was usually recorded from a common carotid artery, either with a mercury manometer or with a Statham transducer coupled to an Offner pen-recorder. In some decerebrate animals, blood pressure was recorded from a femoral artery. For intravenous injections, a polythene cannula was tied into the right saphenous vein.

Intra-arterial injections to the common carotid artery were given by means of an Intramedic P.E. 10 polythene cannula inserted through a hole made by a 26 gauge syringe needle, the artery being temporarily occluded on either side during the insertion. The hole was of a smaller diameter than the cannula, so there was no leakage when the blood flow through the artery was restored.

In order to study the role of carotid sinus mechanisms, denervation was affected by careful dissection of the sinus from all surrounding tissue.

Cats were prepared for close-arterial injection to the suprarenal glands by the method described in Feldberg & Lewis (1964). The cats were eviscerated by removal of the large and small intestine, stomach and spleen ; the aorta below the kidneys and the renal arteries were tied, and a polythene cannula was tied into the central stump of the coeliac artery. The peptides were injected in a volume of 0.1–0.3 ml.

Bilateral adrenalectomy was performed using either the retro-peritoneal or the ventral approach.

The following drugs were used: bradykinin (synthetic bradykinin, BRS 640, Sandoz); kallidin (synthetic kallidin, KL 698, Sandoz); hexamethonium bromide (May & Baker); phentolamine hydrochloride (Ciba); and propranolol hydrochloride (I.C.I.). The doses mentioned in the text refer to these preparations.

RESULTS

Responses to intravenous injections of the polypeptides

Anaesthetized cats. The intravenous injection of bradykinin or kallidin produced a biphasic response in cats anaesthetized with either pentobarbitone or chloralose. The size of the pressor component was dependent on the level of the blood pressure, and increased as the blood pressure fell during the course of the experiment (Fig. 1).

In a series of thirty-three experiments, bradykinin (10 μg/kg) caused a secondary pressor response in all but two animals. When the greatest pressor response in each cat was taken, the mean rise in pressure was 34 mm Hg: the mean depressor response was 42 mm Hg. Kallidin (10 μg/kg) produced in fourteen cats a mean initial fall of 40 mm Hg and a mean secondary rise of 43 mm Hg.

Biphasic responses also occurred with smaller doses of the peptides, 2 μg/kg producing a secondary pressor response of the order of 10 mm Hg.

Ganglion blockade. After hexamethonium (10 mg/kg), the secondary rise was usually reduced, while the depressor response varied according to the level of the arterial blood pressure (Fig. 2B).

In eight out of eleven cats, a mean secondary rise of 39 mm Hg following bradykinin (10 μg/kg) was reduced to 7 mm Hg after hexamethonium, while in the others the pressor response was not significantly modified. Hexamethonium caused a reduction in the mean secondary rise in pressure produced by kallidin (10 μg/kg) from 37 mm Hg to 5 mm Hg in four cats. In a fifth cat, the pressor response was not reduced.
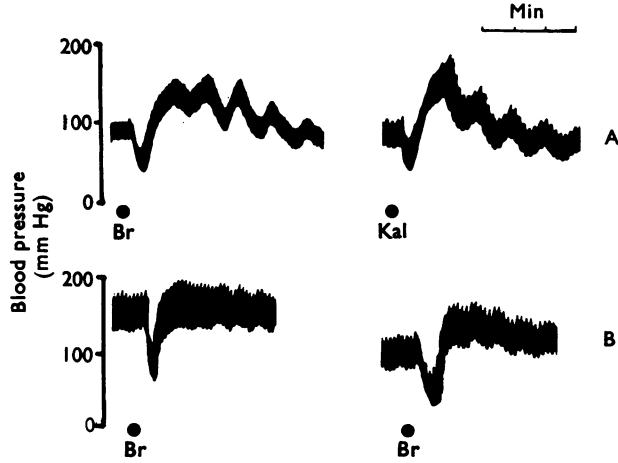
Fig. 1. A: Intravenous injections of bradykinin (Br) 10 μg/kg and kallidin (Kal) 10 μg/kg in an anaesthetized cat. B: Repeated intravenous injections of bradykinin (Br) 10 μg/kg in an anaesthetized cat during the course of an experiment. The pressor component is more pronounced at the lower blood pressure level.

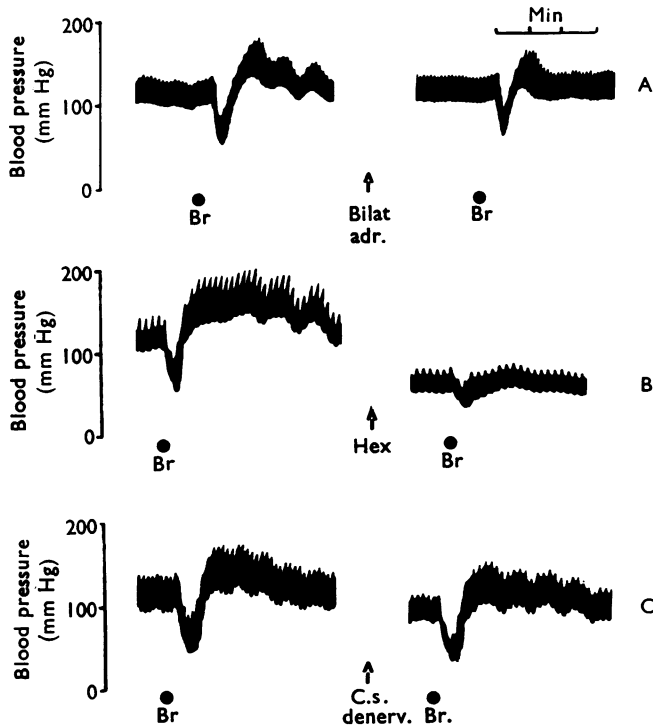Fig. 2. A: Intravenous bradykinin (Br) 10 μg/kg in an anaesthetized cat before and after bilateral adrenalectomy (Bilat. adr.). B: Intravenous bradykinin (Br) 10 μg/kg in an anaesthetized cat before and after ganglion blockade by hexamethonium (Hex) 10 mg/kg. C: Intravenous bradykinin (Br) 10 μg/kg in an anaesthetized cat before and after denervation of both carotid sinuses (C.s. denerv.).

Adrenalectomy. In ten cats, the pressor response to bradykinin (10 μg/kg) was reduced from a mean value of 50 mm Hg to 31 mm Hg after bilateral adrenalectomy (Fig. 2A). In one cat, however, the rise was increased from 20 mm Hg to 50 mm Hg. The mean depressor response to kallidin (10 μg/kg) in four cats was reduced from 91 mm Hg to 53 mm Hg by adrenalectomy. The depressor responses to bradykinin or kallidin were not significantly modified by removal of the adrenals.

Ganglion blockade and adrenalectomy. The combination of ganglion blockade with hexamethonium and adrenalectomy almost abolished the secondary pressor response in eight anaesthetized cats; the mean rise in pressure after bradykinin (10 μg/kg) was 5 mm Hg. In four further cats, a pressor response still occurred after adrenalectomy and hexamethonium, but was almost abolished after the administration of the adrenaline antagonists phentolamine (1 mg/kg) and propranolol (0.5 mg/kg). After hexamethonium and adrenalectomy, the pressor response to kallidin (10 μg/kg) was reduced to a mean value of 5 mm Hg in three cats. In a fourth cat, however, phentolamine was also needed to abolish the rise in blood pressure.

Carotid sinus denervation. The fall in blood pressure after the intravenous injection of the polypeptides was not modified by denervation of the carotid sinuses whereas the pressor phase of the response was enhanced (Fig. 2C). With bradykinin (10 μg/kg) in five cats, the mean secondary rise of 36 mm Hg was increased by 9 mm Hg; with kallidin (10 μg/kg) the mean rise of 58 mm Hg in three cats was increased by 8 mm Hg.

Decerebrate cats. In four decerebrate cats, bradykinin and kallidin produced only depressor responses at doses of 2 and 10 μg/kg. The mean falls in blood pressure were 20 and 40 mm Hg respectively, and no significant secondary pressor responses were observed (Fig. 3).
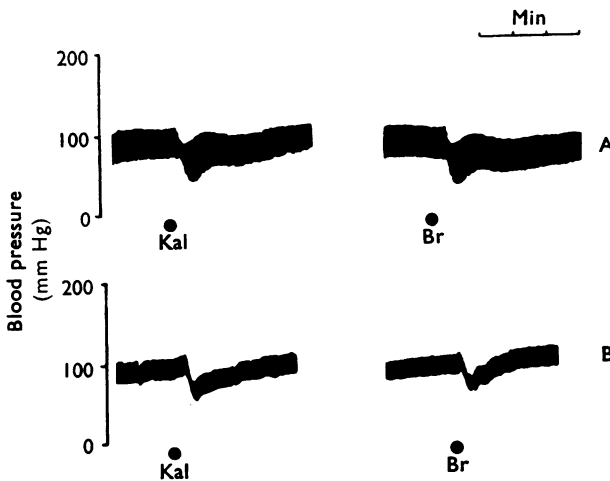

Fig. 3. A: Intravenous kallidin (Kal) 10 μg/kg and bradykinin (Br) 10 μg/kg in a spinal cat. B: Intravenous kallidin (Kal) 10 μg/kg and bradykinin (Br) 10 μg/kg in a decerebrate cat.

Spinal cats. In seven spinal cats, bradykinin in doses of 2 and 10 µg/kg produced a fall in blood pressure in all but one. The mean depressor values were 15 mm Hg and 20 mm Hg respectively. No secondary pressor responses were observed in six cats, but in the remaining one a small rise of 5 mm Hg occurred. Kallidin, 2 and 10 µg/kg, produced falls in blood pressure (mean values, 16 and 23 mm Hg) in four spinal cats. In two cats, secondary pressor responses occurred, but these were slight having a value of only 5 mm Hg. Typical responses are shown in Fig. 3.

Fig. 4. Intra-carotid injections of bradykinin (Br) 10 µg and kallidin (Kal) 10 µg in an anaesthetized cat.

Responses to intra-carotid injections of the polypeptides

When bradykinin and kallidin were injected into the carotid artery in anaesthetized cats, a biphasic response occurred in which the pressor component was more evident than that observed with intravenous injections (Fig. 4). Again, it was found that the pressor response increased as the arterial pressure fell during the course of the experiment.

In twenty-six cats, the mean of the largest pressor response in each cat to intra-carotid injections of 10 µg of bradykinin was 29 mm Hg ; the average rise in pressure produced by 10 µg of kallidin was 38 mm Hg in nine cats.

Ganglion blockade. Hexamethonium (10 mg/kg) almost abolished the pressor responses produced by bradykinin (10 µg) in eight cats ; before hexamethonium, the mean pressor response was 36 mm Hg and after hexamethonium it was less than 5 mm Hg (Fig. 5B). In one other cat, the rise increased from 20 mm Hg to 30 mm Hg following hexamethonium. A mean response of 30 mm Hg produced by kallidin (10 µg) was abolished by hexamethonium in three cats.

Adrenalectomy. Bilateral adrenalectomy markedly reduced the pressor responses to intra-carotid injections of bradykinin and kallidin (Fig. 5A).

In seven cats, the mean pressor response to bradykinin (10 µg) was reduced from 41 mm Hg to 8 mm Hg. In one cat, a pressor response to bradykinin of 46 mm Hg was increased to 54 mm Hg after adrenalectomy but was abolished following ganglion blockade with hexamethonium. Kallidin (10 µg) produced a mean rise in blood pressure of 51 mm Hg in four cats. This was reduced to 5 mm Hg after adrenalectomy.

Carotid sinus denervation. Denervation of the carotid sinus enhanced the pressor responses to the intra-carotid injection of bradykinin or kallidin (Fig. 5C).
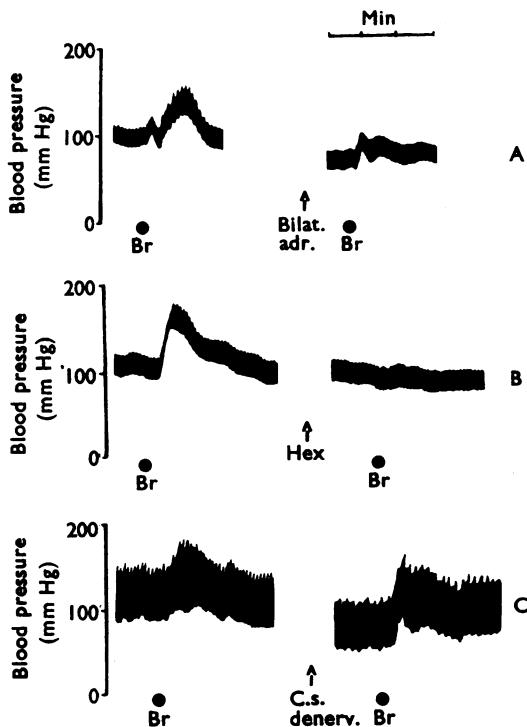
Fig. 5. A: Intra-carotid injections of bradykinin (Br) 10 μg in an anaesthetized cat before and after bilateral adrenalectomy (Bilat. adr.). B: Intra-carotid injections of bradykinin (Br) 10 μg in an anaesthetized cat before and after ganglion blockade by hexamethonium (Hex) 10 mg/kg. C: Intra-carotid injections of bradykinin (Br) 10 μg in an anaesthetized cat before and after denervation of the carotid sinus (C.s. denerv.).

The mean response in six cats to bradykinin (10 μg) was increased from 26 to 35 mm Hg. In four cats, the mean responses to kallidin 10 μg were 23 mm Hg before and 37 mm Hg after denervation of the carotid sinus.

Effects on the adrenal glands

Close-arterial injection of bradykinin 1 μg (eleven cats) or kallidin 1 μg (six cats) produced mean pressor responses of 12 mm Hg (Fig. 6).

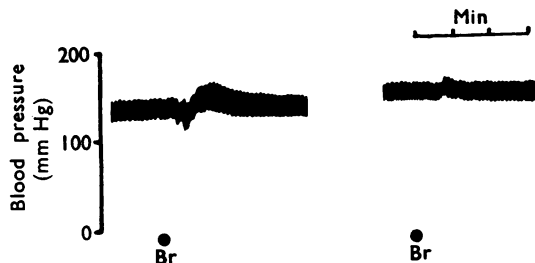

Fig. 6. Close-arterial injection to the adrenal glands of bradykinin (Br) 1 μg on the left and 0.06 μg on the right in an anaesthetized cat. The dose of 0.06 μg represents 0.25% of the intravenous dose of 10 μg/kg, which is the fraction calculated to reach the adrenals following an intravenous injection.

Studies on the adrenal gland of the sheep indicate that the blood flow is 5 to 10 ml./min (Wright, 1963). Because the cardiac output of the sheep is of the order of 4 l./min, it may be calculated that the fraction of an intravenous dose which passes through the adrenals is approximately 0.25%. In dogs, the average blood flow is 3.6 ml./min (Hume & Nelson, 1954). Allowing for the difference in cardiac output of the dog and the sheep, a similar fraction of an intravenous injection will pass through the adrenals. Assuming a comparable situation in the cat, we administered by close-arterial injection to the glands 0.25% of a dose which produced a substantial secondary rise when injected intravenously. As the kinins would be metabolized to a greater extent before they reached the adrenals after intravenous injection than when injected close-arterially, the calculated fraction seems adequate for the purpose of comparison. In seven cats with bradykinin and three cats with kallidin, this calculated fraction did not produce significant pressor responses. The occasional rises in blood pressure observed were only of the order of 5 mm Hg.

DISCUSSION

The hypotensive responses after the intravenous injection of bradykinin and kallidin are well documented ; indeed, these substances are known as depressor kinins. They have, however, been shown regularly to produce a secondary pressor response in the cat. When they are injected into the carotid artery, the pressor response is more evident, and the initial depressor effect may be absent altogether. The pressor responses often become more pronounced after repeated administration as the basal blood pressure level falls. It has been reported that the pressor responses to bradykinin and kallidin are significantly increased in rats with low blood pressure (Croxatto et al., 1962) and in rats and rabbits after repeated administration of the kinins (Lecomte et al., 1964). Our results indicate that the level of the blood pressure is the more important factor in cats in the enhancement of the pressor response, because the size of the pressor responses correlated better with the decrease of blood pressure levels than with the number of doses of the polypeptides.

The effects of ganglion blockade and adrenalectomy indicate that the pressor responses are caused both by sympathetically induced vasoconstriction and by release of catechol-amines from the adrenals. The occasional enhancement of the secondary pressor responses after ganglion blockade alone can be explained by the effect of hexamethonium in potentiating the pressor action of even small amounts of released adrenaline.

After the combination of ganglion blockade and adrenalectomy a pronounced pressor response was still present in some experiments. This was probably the result of release of catecholamines, for the response was reduced by adrenaline antagonists. The source of the catecholamines may be extra-medullary chromaffin tissue as suggested by Lecomte et al. (1964).

Our results on the direct release of catecholamines from the adrenal medulla by brady-kinin and kallidin agree with the findings of Feldberg & Lewis (1964, 1965). The fraction of the intravenous dose calculated to reach the adrenal glands, however, produced only slight pressor effects when it was administered by close-arterial injection to the glands. The magnitude of these responses could not account for the secondary pressor responses observed after intravenous or intra-carotid injection.

The absence of a significant secondary pressor response after intravenous injection of bradykinin or kallidin in spinal and decerebrate cats indicates that a central component is involved in this response. The occasional small pressor effect observed in these animals is probably the result of the direct action of the polypeptides on the adrenal medulla and other chromaffin tissue.

The pronounced pressor responses produced by the intra-carotid injection of the polypeptides support the view that their pressor effects are centrally mediated. Denervation of the carotid sinus did not reduce the pressor responses, so stimulation by the kinins of sensory receptors in the carotid sinus can be excluded as the cause of the centrally mediated pressor response. In fact, it was observed that the pressor response produced by intra-carotid injection of bradykinin and kallidin was enhanced after denervation. Benetato *et al.* (1964) concluded from their experiments in cross-circulation dogs that the stimulation by bradykinin or kallidin of the carotid sinus caused a fall in blood pressure. The enhancement of the pressor responses after denervation may be explained by the absence of this effect.

Whether the central effects are the result of a direct action on the brain, or stimulation of pain receptors as believed by Guzman and his colleagues (1962) cannot as yet be determined from our results. Bradykinin has, however, been reported to have a direct effect on the brain. It was shown that administration of bradykinin into the cerebral ventricles of the cat causes behavioural changes (Corrado, 1960 ; Čapek, 1961). Cortical desynchronization occurred in unanaesthetized cats when this kinin was injected into the lateral ventricle of the brain (Čapek, Corrado, Ferreira & Rocha e Silva, 1966).

Bradykinin injected into the carotid artery of the cat initially produced cortical desynchronization and associated behavioural changes in the " encéphale isolé " preparation, but no effect on the electroencephalogram in the " cerveau isolé " preparation (Čapek *et al.*, 1966). If these effects result from stimulation by the kinin of the brain stem structures caudal to the midcollicular section and this stimulation results in a pressor response, then a secondary pressor response to the polypeptides might have been expected in the decerebrate cat. The absence of this response suggests that the presence of a pathway rostral to the section may be necessary to link the central stimulant effect of bradykinin with the sympathetic outflow.

It has been established that the secondary pressor responses to bradykinin and kallidin are mediated by sympathetic mechanisms and involve a central component. Whether this is a result of a direct effect on the brain or of stimulating pain receptors, however, cannot be conclusively stated. Further studies are in progress to clarify this situation.

SUMMARY

1. The polypeptides bradykinin and kallidin produced biphasic blood pressure responses when injected intravenously or into the carotid artery in anaesthetized cats.

2. The pressor component was reduced or abolished after adrenalectomy or ganglion blockade.

3. After denervation of the carotid sinuses the pressor responses were enhanced.

4. In spinal and decerebrate cats, intravenous bradykinin and kallidin produced only depressor responses.

5.   The close-arterial injection of the polypeptides to the adrenal glands releases catecholamines.   This direct effect on the adrenal medulla does not, however, explain their pressor effects after intravenous or intra-carotid injections.

6.   It is concluded that the pressor responses to bradykinin and kallidin are mediated by sympathetic mechanisms and involve a central component.

7.   Whether the pressor responses are a result of the stimulation of pain receptors by the peptides or are caused by a direct action on the brain has yet to be established.

REFERENCES

BENETATO, G., HĂULICĂ, I., MUSCALU, I., BUBUIANU, E. & GĂLEȘANU, S. (1964).   On the central nervous action of bradykinin. *Rev. Roum. Physiol.*, **1**, 313–322.

BURN, J. H. (1952).   *Practical Pharmacology*. Oxford: Blackwell.

ČAPEK, R. (1961).   Some effects of bradykinin on the central nervous system. *Proc. 1st. Int. Pharmac. Meeting*, **9**, 61–65.

ČAPEK, R., CORRADO, A. P., FERREIRA, S. H. & ROCHA e SILVA, M. (1966).   The effect of bradykinin on the electroencephalogram. *Activitas. nerv. sup.*, **8**, 419–420.

CORRADO, A. P. (1960).   *Techniques for the study of psychotropic drugs*, p. 27. Bologna: G. Tonini.

CROXATTO, H., BELMAR, J., PEREDA, T. & LABARCA, E. (1962).   Hypertensive effects of bradykinin and anephrotensin in normal and nephrectomized animals. *Acta. physiol. latinoamer.*, **12**, 19–21.

FELDBERG, W. & LEWIS, G. P. (1964).   The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. *J. Physiol., Lond.*, **171**, 98–108.

FELDBERG, W. & LEWIS, G. P. (1965).   Further studies on the effects of peptides on the suprarenal medulla of cats. *J. Physiol., Lond.*, **178**, 239–251.

GUZMAN, F., BRAUN, C. & LIM, R. K. S. (1962).   Visceral pain and the pseudoaffective response to intra-arterial injection of bradykinin and other algesic agents. *Archs int. Pharmacodyn. Thér.*, **136**, 353–384.

HUME, D. M. & NELSON, D. H. (1954).   Adrenal cortical function in surgical shock. *Surg. Forum*, **5**, 568–575.

LECOMTE, J., TROQUET, J. & CESSION-FOSSION, A. (1964).   Sur la nature de l'hypertension artérielle provoquée par la bradykinine et la kallidin. *Archs int. Pharmacodyn. Thér.*, **147**, 518–524.

LEWIS, G. P. & REIT, E. (1965).   The action of angiotensin and bradykinin on the superior cervical ganglion of the cat. *J. Physiol., Lond.*, **179**, 538–553.

MIELE, E. (1966).   On the mechanism of the potentiating of the angiotensin pressor effect by guanethidine and desmethylimipramine. *Med. Pharmac. exp.*, **15**, 35–44.

PEARSON, L. & LANG, W. J. (1967a).   The nature of the pressor responses produced by bradykinin and kallidin. *Aust. J. exp. Biol. med. Sci.*, **45**, P-35.

PEARSON, L. & LANG, W. J. (1967b).   A comparison in conscious and anaesthetized dogs of the effect on blood pressure of bradykinin, kallidin, eledoisin and kallikrein. *Europ. J. Pharmac.*, **2**, 83–87.

ROCHA e SILVA, M., BERALDO, W. T. & ROSENFELD, G. (1949).   Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and trypsin. *Am. J. Physiol.*, **156**, 261–273.

SCHRÖDER, E. & HEMPEL, R. (1964).   Bradykinin, kallidin and their synthetic analogues. *Experientia*, **20**, 529–544.

STASZEWSKA-BARCZAK, J. & VANE, J. R. (1965).   The release of catecholamines from the adrenal medulla by histamine. *Br. J. Pharmac. Chemother.*, **25**, 728–742.

STÜRMER, E. & BERDE, B. (1963).   A pharmacological comparison between synthetic bradykinin and synthetic kallidin. *J. Pharmac. exp. Ther.*, **139**, 38–41.

STÜRMER, E. & CERLETTI, A. (1961).   Bradykinin. *Am. Heart. J.*, **62**, 149–154.

WRIGHT, R. D. (1963).   Blood flow through the adrenal gland. *Endocrinology*, **72**, 418–428.