Br. J. Pharmac. (1973), **47**, 517–528.

Antinociceptive action of bradykinin and related kinins of larger molecular weights by the intraventricular route

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Summary

1. It was shown previously that bradykinin (Bk) when given intraventricularly increases the threshold of electrical stimulation of dental pulp in the rabbit.

2. Bradykinin derivatives with increasing molecular weights (lysyl-Bk (L-Bk), methionyl-lysyl-Bk (ML-Bk) and glycyl-arginyl-methionyl-lysyl-Bk (GAML-Bk)) were found to produce effects of decreasing intensity on the threshold of electrical stimulation according to the following scale: Bk (1·00)>L-Bk (0·28)>ML-Bk (0·060)>GAML-Bk (0·047).

3. The four peptides had similar relative activities on the guinea-pig ileum but an inverse relationship in their effects on vascular permeability and rat blood pressure.

4. The discrimination index, increase in vascular permeability/antinociceptive effect rose to values of the order of 170 to 550, taking Bk as the reference peptide (potency=1·00).

5. We conclude that the increase in threshold of electrical stimulation could not be due to an increase in vascular permeability or decrease of blood pressure.

Introduction

Central effects of bradykinin given by the intraventricular route have been reported in previous papers from this laboratory (Corrado, Ramos & Rocha e Silva, 1959; Graeff, Corrado, Pelá & Čapek, 1967; Da Silva & Rocha e Silva, 1971). The increase in threshold of electrical stimulation applied to the tooth pulp of the rabbit produced by intraventicular bradykinin was also described by Ribeiro (1970) and Ribeiro, Graeff & Corrado (1971). This antinociceptive effect of bradykinin can be obtained with doses that are at least 100 times smaller than those of morphine given by the same route to produce a similar analgesic effect (Cube, Teschemacher, Herz & Hess, 1969; Herz, Albus, Metys, Schubert & Teschemacher, 1970; Ribeiro *et al.*, 1971).

On the other hand, a comparative analysis of the pharmacological actions of bradykinin and related peptides of larger molecular weights, such as lysyl-Bk (L-Bk), methiony-lysyl-Bk (ML-Bk) and glycyl-arginyl-methionyl-lysyl-Bk (GAML-Bk) showed that they behave as a series of homologous compounds on smooth muscle preparations (guinea-pig ileum, rat uterus, rat duodenum), on the rat blood pressure and in increasing vascular permeability (Reis, Okino & Rocha e Silva, 1971; Roche e Silva, Reis & Okino, 1972). In the present paper we analyse the

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effects of this series of compounds administered intraventricularly by measurement of the threshold of electrical stimulation of the rabbit tooth pulp; a comparison is made with the data already obtained for bradykinin (Ribeiro *et al.*, 1971).

Methods

Synthetic peptides related to bradykinin

The peptides used in the experiments described below were the same as those used in previous experiments in this laboratory (Reis *et al.*, 1971; Rocha e Silva *et al.*, 1971): bradykinin (Bk); lysyl-bradykinin (L-Bk); methionyl-lysyl-bradykinin (ML-Bk) and glycyl-arginyl-methionyl-lysyl-bradykinin (GAML-Bk). As shown in previous experiments, their effects decrease with increasing molecular weight when acting on the guinea-pig ileum, the rat uterus and the rat duodenum (Bk>L-Bk> ML-Bk>GAML-Bk) but increase when tested on rat blood pressure and the vascular permeability of the rat's skin (GAML-Bk>ML-Bk>L-Bk>Bk).

The discrimination index, increase in vascular permeability/potency on guinea-pig ileum, rose to values of 180 to 300 when GAML-Bk was compared with bradykinin. The effects of all four peptides on the smooth muscle, blood pressure and vascular permeability were qualitatively similar (Rocha e Silva, 1970).

Intraventricular administration

A modified Collison cannula (Feldberg & Sherwood, 1953) was implanted in the skull of rabbits (2–2·5 kg weight) with a Labtronix, Model 4, Stereotaxic Instrument, according to the technique described previously (Graeff, Pelá & Rocha e Silva, 1969; Ribeiro *et al.*, 1971). The correct position of the cannula was confirmed at the end of the experiments by injecting 0·2 ml of Evans blue. The volume of the intraventricular injections was always 0·05 ml. As a control, the same volume of artificial cerebrospinal fluid was injected 24 h before or after the main experiments. When morphine was used, the same routine procedure was followed.

Tooth pulp electrical stimulation

The technique used has been described previously (Cheymol, Montagne, Paeile, Dalion & Duteil, 1959; Alonso Verri, Graeff & Corrado, 1968). Electric parameters were the same as described for the rabbit's tooth pulp stimulation by Paeile, Guerrero & Mardones (1962), i.e., 5 ms duration, at a frequency of 5 Hz, and variable voltage applied during 1 s at intervals of 2 seconds. In order to determine the threshold, the voltage of the electrical stimulus delivered by an electronic stimulator (Grass Model S_4) was increased stepwise from a subthreshold value until eliciting a response, characterized by chewing movements, sometimes associated with licking of the incisor teeth and electrodes. The mean of 3 successive measurements was calculated for each threshold determination.

The drugs used were bradykinin (BRS-640; Sandoz, Switzerland); capsaicin, kindly supplied by Dr. N. Janscó; lysyl-bradykinin (L-Bk) (kallidin, KL-698, Sandoz, Switzerland); methionyl-lysyl-bradykinin (ML-Bk) (Schwarz & Co.); glycyl-arginyl-methionyl-lysyl-bradykinin (GAML-Bk) kindly supplied by Dr. L. J. Greene of Brookhaven National Laboratories, L.I., New York; morphine hydrochloride (Merck, Germany). The drugs were dissolved in artificial cerebrospinal fluid (Elliott & Jasper, 1949), for intraventricular injection.

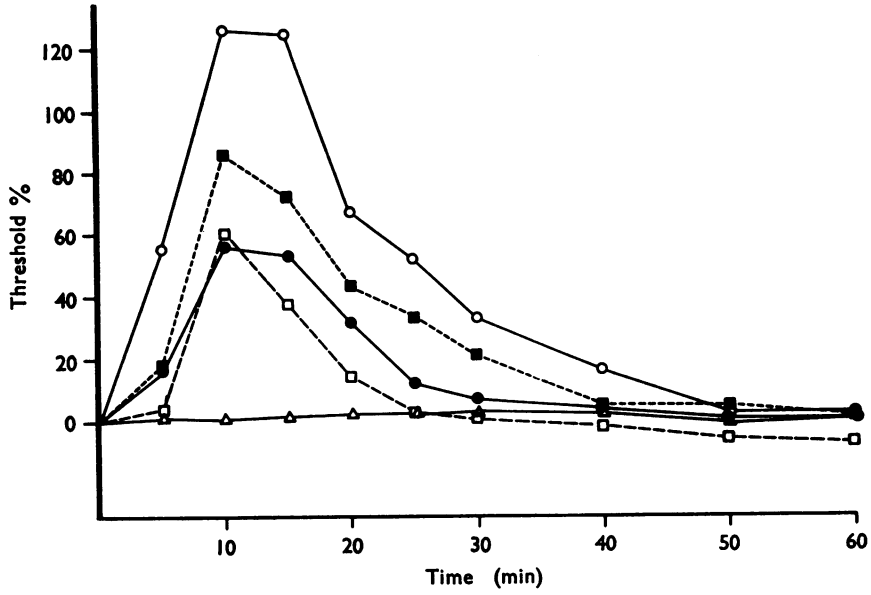

FIG. 1. Increase in threshold voltage of electrical stimulation of tooth pulp after the intraventricular injection of bradykinin (Bk) and lysyl-bradykinin (L-Bk) into the lateral cerebral ventricle. For Bk mean results from 6 and for L-Bk from 8 rabbits are shown. ●——●, Bk 0·5 μg; ○——○, Bk 2·0 μg; □- - -□, L-Bk 2·0 μg; ■- - -■, L-Bk 4·0 μg; △——△, 0·05 ml artificial spinal fluid, as a control.

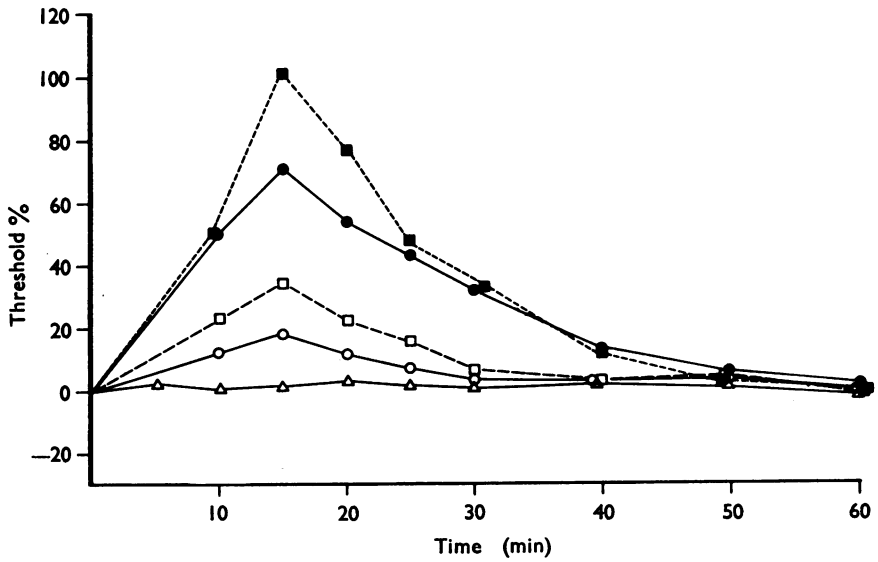

FIG. 2. Increase in threshold voltage of electrical stimulation of tooth pulp after the intraventricular injection of methionyl-lysyl-bradykinin (ML-Bk) and glycyl-arginyl-methionyl-lysyl bradykinin (GAML-Bk). Results are the mean from 6 animals for ML-Bk and from 8 to 10 animals for GAML-Bk. □- - -□, ML-Bk 8·0 μg; ■- - -■, ML-Bk 16·0 μg; ○——○, GAML-Bk 8·0 μg; ●——●, GAML-Bk 16·0 μg; △——△, 0·05 ml artificial spinal fluid, as control.

Results

Increase of threshold voltage caused by the intraventricular administration of peptides

The four peptides, Bk, L-Bk, ML-Bk and GAML-Bk, produced an increase in threshold voltage of stimulation of the tooth pulp when given by the intraventricular route, in rabbits. Figure 1 shows the effects of Bk and of L-Bk, on the threshold voltage observed during 60 min by stimulation repeated every 5 or 10 minutes. Each point represents the mean result from 6 animals for Bk and 8 for L-Bk, the former being about 4 times more potent than L-Bk in increasing the threshold. This fact will be analysed below by comparing ratios of potency.

Figure 2 shows the increase in threshold of electrical stimulation of the rabbit tooth pulp, after intraventricular administration of 2 doses of ML-Bk and GAML-Bk. The same effects were observed as with Bk, although much larger doses of ML-Bk and GAML-Bk had to be used.

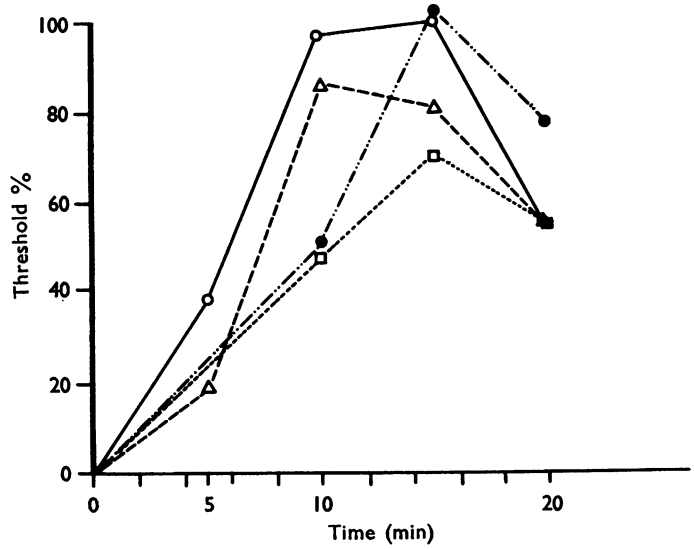

FIG. 3. Comparative effects on the onset and duration of the maximal antinociceptive action of approximately equipotent doses of bradykinin (Bk), lysyl-bradykinin (L-Bk), methionyl-lysyl-bradykinin (ML-Bk) and glycyl-arginyl-methionyl-lysyl bradykinin (GAML-Bk. ○——○, Bk (1·0 μg), △- - - -△, L-Bk (4·0 μg); ●—··—●, ML-Bk (16·0 μg); □- - -□, GAML-Bk (16·0 μg).

Figure 3 shows the comparative time-course of approximately equipotent doses of the four peptides, measured as per cent increase in threshold of electrical stimulation of the tooth pulp of rabbits receiving intraventricular injection of the peptides in the amounts indicated. When compared with Bk, the larger kinins had a delayed onset of action, which was evident with L-Bk and increased to a maximal effect with ML-Bk and GAML-Bk. The return to normal, however, followed a similar time-course, which may indicate that the larger kinins act as such and not because of their eventual transformation into Bk; such a transformation is possible for enzymes have been detected in the brain tissue that can release Bk from L-Bk (Camargo, Ramalho Pinto & Greene, 1972).

Comparative effects of bradykinin and morphine administered intraventricularly

As indicated before (Ribeiro, 1970; Ribeiro *et al.*, 1971) some of the effects of Bk are similar to those produced by morphine when these substances are administered by the intraventricular route. These effects include catatonia (Banergee, Burks, Feldberg & Goodrich, 1968; Da Silva & Rocha e Silva, 1971), depletion of brain noradrenaline (Vogt, 1954; Graeff *et al.*, 1969), hyperglycaemia (Borison, Fishburn, Bhide & McCarthy, 1962; Moore, McCarthy & Borison, 1965; Ribeiro, Da Silva, Camargo & Corrado, 1970), release of antidiuretic hormones (Goodman & Gilman, 1965; Rocha e Silva Jr. & Malnic, 1964; Harris & Rocha e Silva Jr., 1965; Rocha e Silva Jr. & Harris, 1970), and an antinociceptive effect as measured by the increase in threshold of stimulation of the tooth pulp, shown for morphine and analogues by Cube *et al.*, 1969; Herz et al., 1970, and for Bk by Ribeiro *et al.*, 1971.

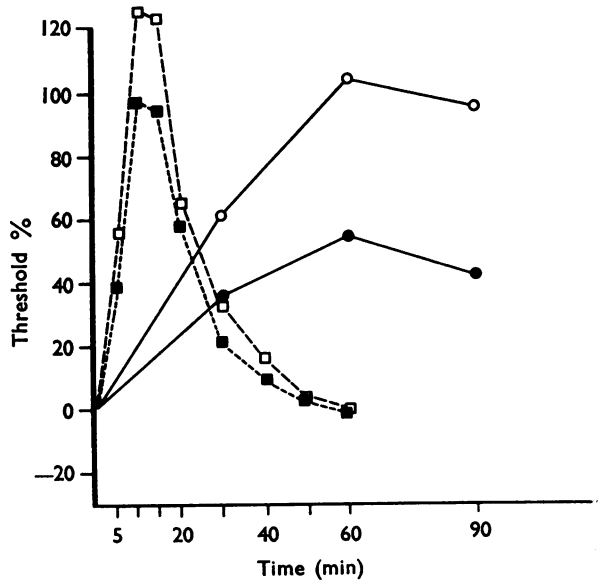

FIG. 4. Increase in threshold voltage of electrical stimulation of tooth pulp after intraventricular injection of bradykinin (Bk) and morphine. The data for Bk are taken from experiments in Fig. 1, the data for morphine are from a group of 11 rabbits, where 50 to 100 μg of morphine were injected by the intraventricular route; the slow onset and sustained maximal effect obtained with morphine is contrasted with the rapid and evanescent effect produced by Bk. ■- - -■, Bk 1·0 μg; □- - -□, Bk 2·0 μg; ●——●, morphine 50 μg; ○——○, morphine 100 μg.

Figure 4 shows the time-course of the antinociceptive effect of two doses of Bk (1·0 and 2·0 μg) and morphine. It has to be stressed that morphine must be given in much larger doses (50 and 100 μg) and elicits a more sustained effect, the onset of which is much delayed when compared to that of Bk.

There is a remarkable similarity between the time-course observed with Bk and that with 20 times larger doses of fentanyl (*N*-[1-phenethylpiperid-4-yl]propionanilide) (Cube *et al.*, 1970; Herz *et al.*, 1970). The latency both for fentanyl and Bk is very short, and the return to normal similarly abrupt. The behavioural changes produced by fentanyl were described by Herz *et al.* (1970) 'following an initial phase characterized by motor excitation, with occasional myoclonic and stretch

movements, that lasted about 2 min a catalepsia-like condition (with marked respiratory depression) developed'. This description is remarkably similar to the behavioural changes described for Bk by Graeff *et al.* (1969) and Da Silva & Rocha e Silva (1971).

Discrimination coefficients of the four kinins in different preparations

It appears that each peptide acts as such, without transformation of the larger peptides into Bk. Since the four peptides used in the present experiments have already been assayed upon several other preparations (guinea-pig ileum, rat uterus, duodenum, rat blood pressure and vascular permeability) by Reis *et al.* (1971) and Rocha e Silva (1971), a comparison has been made of the data presented by these authors with the results described in the present paper, to ascertain whether the relative responses of the ventricular lining were similar to those of the smooth muscle preparations or to those of the blood vessels in producing changes in blood pressure and vascular permeability. Figure 5 gives the dose-response curves of

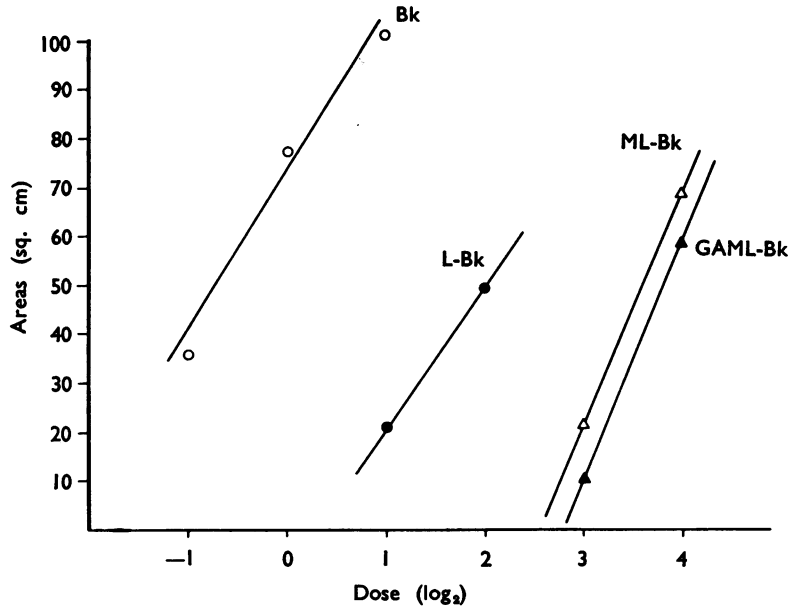

FIG. 5. Dose-response lines for the antinociceptive action of bradykinin (Bk), lysyl-bradykinin (L-Bk), methionyl-lysyl-bradykinin (ML-Bk) and glycyl-arginyl-methionyl-lysyl bradykinin (GAML-Bk). The doses are given in a $\log_2$ scale and the effects as areas under the curves of the increase in threshold of electrical stimulation of tooth pulp as given in the previous figures.

the four peptides, taking the 'areas' of increase in the threshold time curves, for two doses of Bk, L-Bk, ML-Bk and GAML-Bk. It can be seen that the lines so obtained are approximately parallel and therefore the relative potencies can be deduced from their horizontal distances. Tables 1 and 2 give the relative potencies and the discrimination indexes, taking Bk as 1·00. Although the activities on the guinea-pig ileum and on the threshold of electrical stimulation were not strictly parallel, the general trend was the same. Table 2 shows the same parameters in relation to the increase in vascular permeability and the antinociceptive effect. It can be seen that the activities of the four peptides run in the opposite direction,

TABLE 1. *Relative potencies and discrimination indexes of the peptides, on the guinea-pig ileum and on threshold of stimulation (antinociceptive effect)*

Bradykinin (Bk) and derivatives	Relative potencies* Guinea-pig ileum†	Relative potencies* Antinociceptive effect	Discrimination indexes Antinociceptive effect / Guinea-pig ileum
Bk	1·00	1·00	1·00
Lysyl-Bk	0·30	0·28	0·94
Methionyl-lysyl-Bk	0·08–0·10	0·060	0·76–0·60
Glycyl-arginyl-methionyl-lysyl-Bk	0·06–0·07	0·047	0·78–0·66

* All data were corrected for molar concentrations; † Data from Reis *et al.*, (1971).

TABLE 2. *Relative potencies and discrimination indexes of the peptides on vascular permeability and threshold of stimulation (antinociceptive effect)*

Bradykinin (Bk) and derivatives	Relative potencies* Vascular permeability (blue test)†	Relative potencies* Antinociceptive effect	Discrimination indexes Vascular permeability / Antinociceptive effect
Bk	1·00	1·00	1·00
Lysyl-Bk	1·00	0·28	3·60
Methionyl-lysyl-Bk	10·00	0·060	170·00
Glycyl-arginyl-methionyl-lysyl-Bk	11·0–20·0	0·047	235–550

* All data were corrected for molar concentrations; † Data from Reis *et al.*, (1971).

since the effect on vascular permeability increases 10 to 20 fold when the larger peptides are used, although the analgesic effect, as shown above, decreases conspicuously to 1/17 for ML-Bk and to 1/21 for GAML-Bk. The discrimination indexes thus rise to the highly significant values of 170 for ML-Bk, and 235–500 for GAML-Bk.

Absence of desensitization by previous injection of capsaicin

To decide whether the antinociceptive effect of Bk and derivatives is due to a direct effect on pain receptors, capsaicin was used since Jancsó (1968) showed that when capsaicin is applied to the rat saphenous nerve it produces an initial stimulatory effect followed by desensitization to capsaicin itself and refractoriness to other chemical agents (nicotine and acetylcholine) but not to thermal or mechanical stimulation. Similarly, Riccioppo Neto (1971) showed that capsaicin can render pain receptors insensitive to Bk (in a reflex involving paravascular plexes of the posterior meningeal vessels) when the polypeptide is injected through the occipital artery in dogs. Figure 6 gives the results obtained in five rabbits each of which received two injections of 500 μg of capsaicin intraventricularly before the injection of the smaller dose of Bk. There was no observable effect of capsaicin on the increase in threshold voltage of stimulation produced by 0·5 μg Bk, nor did capsaicin alone produce any antinociceptive effect when administered intraventricularly in rabbits.

Discussion

The series of bradykinin derivatives, of increasing molecular weights, L-Bk, ML-Bk, GAML-Bk and Bk itself, have already been studied in relation to their relative potencies upon smooth muscle structures (guinea-pig ileum, rat uterus and rat duodenum), and on rat blood pressure and vascular permeability (Reis *et al.*, 1971 ; Rocha e Silva *et al.*, 1972 ; Rocha e Silva, 1971). As regards their action upon smooth muscle structures, they showed decreasing potencies, as the amino

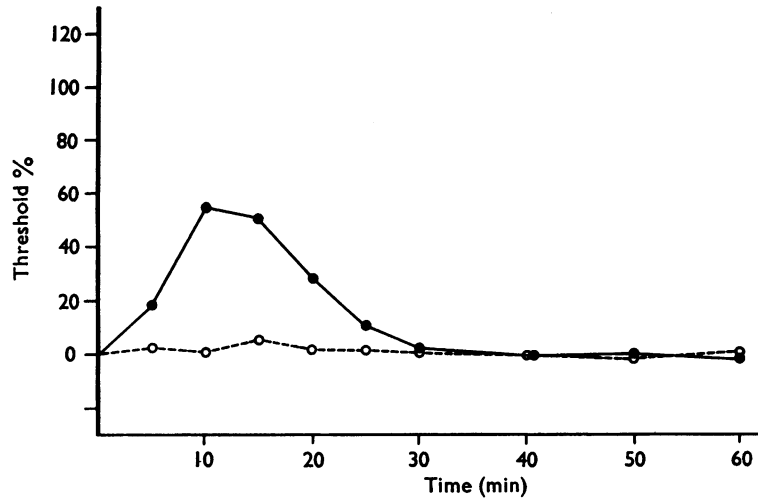

FIG. 6. Increase in threshold of tooth pulp stimulation produced by intraventricular bradykinin (●——● 0·5 μg), after two injections of intraventricular capsaicin (500 μg each); in five rabbits capsaicin alone (○- - -○, 500 μg) did not produce any important threshold change.

acid chain at the NH_2 terminal group of Bk was increased, from Bk to GAML-Bk. When given by the intra-arterial route in rats, their effects on blood pressure were about the same, but if given by the intravenous route, they displayed increasing activities starting from Bk with a 'vein/artery' ratio of 0·12, to GAML-Bk with a ratio approaching unity; on the other hand, previous treatment of the animal with a kininase inhibitor such as BAL which increased the ratio 'vein/artery' of Bk to approximately one, did not affect the ratio of the larger kinin, GAML-Bk, indicating that this compound is resistant to the action of the lung kininases, (Stewart, Roblero & Ryan, 1970; Ryan, Roblero & Stewart, 1970). The most striking differences were observed when the peptides were compared in relation to their activities on smooth muscle and on vascular permeability by the Evans blue test. When the indexes of discrimination, increase in vascular permeability/potency on the guinea-pig ileum were calculated, taking the potencies of Bk as 1·00, the values for GAML-Bk, rose to 180–300 (Reis *et al.*, 1971; Rocha e Silva *et al.*, 1972).

The peptides were tested on the stimulated tooth pulp in order to ascertain whether their potency on the cerebral ventricular lining resembled that on smooth muscle preparations, or on vascular permeability. Bradykinin itself was found to produce a sharp increase in threshold of stimulation (antinociceptive effect) in doses that were at least 100 times smaller than doses of morphine needed to produce a similar increase in threshold, and 10 to 20 times smaller than the dose of the potent synthetic analgesic, fentanyl, required to produce a similar effect, according to data from Herz *et al.* (1970).

The experiments presented in this paper allow the following conclusions: The antinociceptive effect of the 4 peptides run parallel with their effects on smooth muscle preparations, and if the indexes of discrimination in relation to the guinea-pig ileum are calculated, they do not show variations far from unity. It seems obvious, therefore, that the 4 peptides owe their action on the lateral ventricular lining to

their chemical nature, and do not depend upon any breakdown into the smaller peptide (Bk). If their potencies in producing the antinociceptive effect are compared with their activities upon vascular permeability, it becomes apparent that the effects run in opposite directions, the ratios, effect on vascular permeability/antinociceptive effect reaching values as high as 235 to 550 for the largest kinin assayed (GAML-Bk). Such a result would exclude any possibility of the peptides acting as antinociceptive agents by any effect upon the blood supply or the vascular permeability of neighbouring vessels, which according to Feldberg (1963) might occur with drugs injected into the lateral ventricle. We can assume that the kinins stimulate directly the nervous structures lining the lateral ventricles and that the different kinins have decreasing stimulatory effects.

The mechanism by which polypeptides and morphine (and analogues such as fentanyl) produce the antinociceptive effect when given intraventricularly is still controversial. Both types of compounds produce behavioural changes that can be remarkably similar: an initial phase of motor excitation, with flight reactions, vocalization followed by a phase of sedation (depression) ending with a more or less prolonged catatonic-like condition (Graeff *et al.*, 1969 ; Da Silva & Rocha e Silva, 1971 ; Herz *et al.*, 1970). The differences in the time of reaction to the kinins and morphine appear to depend more on the chemical nature of the materials than on any essential difference in mechanism and locus of action. The antinociceptive effect produced by Bk and L-Bk starts after a very short latency attaining a peak after 10 min, but with morphine the onset is much slower, reaching a peak 60 min after the injection. However, the narcotic analgesic fentanyl, when given by the intraventricular route, attains the peak after 5 to 10 min, as kinins do. The explanation that the delayed onset with morphine and the rapid start with fentanyl might be due to differences in lipid solubility would not account for the quick onset after Bk or L-Bk which are sparingly soluble in lipids. It might be suggested that the delay in onset observed with the larger kinins (M-L-Bk and GAML-Bk) indicates a less superficial locus of action. However, after producing their antinociceptive effect, all four peptides behave in a similar way, with a prompt return to normal levels 20 to 40 min after the injection. The fact that the return to normality takes about the same time for the 4 peptides is another reason for thinking that they act *per se*, and not through a conversion into Bk. It is to be noted that after morphine there is a much slower return to normal, than with kinins or fentanyl.

It is difficult to think of a common mechanism underlying the effects of such dissimilar compounds as morphine, fentanyl and the kinins. We know that morphine and Bk are able to mobilize the stocks of catecholamines in the brain (Vogt, 1954 ; Moore *et al.*, 1965 ; Graeff *et al.*, 1969), and noradrenaline was found to produce an analgesic effect when given by the intraventricular route in doses of 5 to 10 μg (Feldberg, 1954 ; Gardella, Izquierdo & Izquierdo, 1966). However, the release of noradrenaline by Bk was obtained with much higher concentrations than those needed to produce a strong analgesic effect by the intraventricular route, and there is no available evidence that fentanyl is able to release catecholamines from their central stores to produce the rapid onset of action shown by Herz *et al.* (1970).

We have, however, to stress the difference that morphine and analogues have an analgesic effect when given systemically whereas the kinins when given intra-

arterially produce a noxious effect by stimulating pain receptors located in the walls of such vessels (Guzman, Braun & Lim, 1962; Guzman, Braun, Lim, Potter & Rodgers, 1964; Lim, 1966). or produce a pain sensation when applied to the cantharidin blister area (Armstrong, 1970). As far as we can tell from other experiments, the central effects of bradykinin when given intraventricularly do not depend upon the actual production of pain, since morphine itself did not abolish the changes in behaviour produced by the intraventricular injection of Bk (Graeff *et al.*, 1969), nor of any desensitization to pain, since capsaicin did not affect the antinociceptive effect of Bk, in contrast to the desensitizing effect produced on the reflex mediated by stimulation of the meningeal occipital plexus, as shown by Riccioppo Neto (1971).

With such limitations in mind, we are left with the possibility that the antinociceptive effect of Bk, like that of morphine, might depend on the action upon specific structures of the central nervous system, producing a more or less rapid increase in concentration of noradrenaline in the synaptic cleft as suggested by Radouco-Thomas, Singh, Garcin & Radouco-Thomas (1967).

The longer duration of the effect observed when morphine is injected in comparison to that of the polypeptides could be due to the longer persistence of the alkaloid at its site of action in the CNS structures, in contrast to the rapid inactivation of Bk by the nervous tissues. The evidence for this possibility is the fact that bradykinin potentiating factor and aprotinin (trasylol), known to inhibit brain kininases (Camargo & Graeff, 1969), strongly potentiated the antinociceptive effects of Bk given by the intraventricular route (Ribeiro *et al.*, 1971).

This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Pesquisas (CNPq), Rio de Janeiro, GB.

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(*Received May* 30, 1972)