Br. J. Pharmac. (1977), **61**, 523–532

THE EFFECTS OF BRADYKININ AND THE BRADYKININ POTENTIATING PEPTIDE BPP_{5a} ON THE ELECTRICAL AND MECHANICAL RESPONSES OF THE GUINEA-PIG TAENIA COLI

P.N. AARSEN

Pharmacological Laboratory of the University of Amsterdam, Polderweg 104, Amsterdam (O), The Netherlands

1 By means of the double sucrose-gap technique, the effects of bradykinin and the bradykinin potentiating peptide BPP_{5a} were compared on the guinea-pig taenia coli under a number of experimental conditions.

2 In normal Krebs solution the response to bradykinin was mostly a slight stimulation, characterized by a depolarization, an increase in spontaneous spike activity and a contraction. If BPP_{5a} caused any effect at all, it was stimulation of the spike activity but without depolarization. Since the effect of bradykinin was little affected by an increase in dose, a potentiating effect of BPP_{5a} could not be determined.

3 Spontaneous spikes with a 5 to 7 s rhythm and prepotentials at their base were inhibited by bradykinin, whereas they were stimulated by BPP_{5a}.

4 Oscillatory potentials (slow waves) induced by a calcium and magnesium-free medium were also suppressed by bradykinin and stimulated by BPP_{5a}. This effect of bradykinin was accompanied by a depolarization and a decrease in membrane resistance, phenomena not found after administration of BPP_{5a}.

5 The amplitude of spontaneous spikes induced by potassium-depolarization was suppressed by bradykinin, even though the membrane resistance and potential had been decreased. BPP_{5a} produced either no effect or a small stimulatory effect without influencing the membrane resistance.

6 Reduction of the calcium concentration to 0.25 mM enhanced the stimulatory responses to both bradykinin and BPP_{5a}, especially the spike activity and depolarization. In this case the membrane resistance was increased by bradykinin as well as BPP_{5a}. These effects, especially those of BPP_{5a}, were inhibited by reduction of the sodium concentration to 15.5 mM. Reduction of the chloride concentration to 9.7 mM decreased rather than increased the stimulatory effects of both bradykinin and BPP_{5a}. Under these conditions bradykinin did not decrease the membrane resistance.

7 Bradykinin can have both inhibitory and stimulatory effects on the taenia coli whereas BPP_{5a} has only a stimulatory effect. Since under certain conditions both responses to bradykinin are accompanied by a sodium-dependent depolarization and decrease in membrane resistance, not influenced by lanthanum to any extent, it is suggested that bradykinin induces an increase in sodium conductance of the membrane. Under all the conditions investigated, except in low calcium, BPP_{5a} did not affect the membrane potential and resistance. Thus, the underlying cause of its stimulatory effect is probably different from that of bradykinin.

Introduction

Bradykinin causes a contraction of most isolated smooth muscle preparations (Stürmer & Berde 1963). However, bradykinin induces relaxation in the rat duodenum while a biphasic response consisting of an initial relaxation followed by an increase in tone (contraction) is found in the rat ileum and colon and in the rabbit duodenum (Horton, 1959; Elliott, Horton & Lewis, 1960). The bradykinin-induced contraction is thought to represent a direct action of bradykinin on the smooth muscle cells (Day & Vane, 1963; Gershon, 1967; Ohashi, Nonomura & Ohga, 1967), but less agreement exists concerning the bradykinin-induced relaxation. Bauer, Ziegler & Konzett (1966) demonstrated that the bradykinin-induced initial suppression of the spontaneous contractions accompanied by a decrease in the tone of the rabbit-ileum, could be inhibited by morphine, without affecting the secondary stimulatory effect of bradykinin. However, the relaxation caused by bradykinin on the rat duodenum, jejujum and ileum was not influenced by morphine. The bradykinin potentiating peptide Pyr-Lys-Trp-Ala-Pro (BPP_{5a}) (Stewart, Ferreira & Greene, 1971) from

the poison of the snake, *Bothrops jararaca* only potentiates the bradykinin-induced contractions of the isolated preparations of the rat intestine, and has no effect on their relaxation (Camargo & Ferreira, 1971; Ufkes, Aarsen & Van der Meer, 1976). This potentiating peptide also did not affect the dose-dependent relaxations induced by bradykinin on the rabbit isolated ileum, whereas the contractions were markedly potentiated (Ufkes *et al.*, 1976). The findings suggest that two different receptor sites may be involved in the biphasic bradykinin response and that only the stimulatory receptor is influenced by BPP_{5a}.

The present work describes both the electrical and mechanical effects of bradykinin and BPP_{5a} either alone or in combination, on the guinea-pig taenia coli under a number of experimental conditions.

Methods

Double sucrose-gap

The double sucrose-gap method was used as described by Bülbring & Tomita (1969). In this method only a small portion of the central part of a taenia preparation is exposed to various test solutions. Spontaneous and evoked electrical and mechanical activity as well as membrane resistance can be investigated by this method. The sucrose solution was made by dissolving 100 g sucrose (Analar, BDH) in 1 litre deionized and subsequently glass-distilled water. Strips of about 40 mm length and about 1 mm thickness were prepared from the superficial layers of the taenia coli taken from female guinea-pigs of 350 to 500 g weight. After insertion into the apparatus the right side of the strip was connected to a mechano-electric transducer (RCA 5734, in later experiments Pixie 8101) under a pretension of 0.5 gram. Constant current pulses (3 s duration, of the order of 4 to 8×10^{-7} A) with alternating polarities were applied to the tissue in the stimulating circuit at 15 s intervals.

The normal salt solution used was a Krebs solution, containing (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 15.5 and glucose 11.1, gassed with 5% CO_2 in O_2 at 36°C. When lanthanum was added to the Krebs solution KH_2PO_4 and $NaHCO_3$ were omitted and 5.8 mM Hepes buffer (pH 7.22) was added, because insoluble lanthanum salts were found. Lanthanum chloride was added as an isotonic solution to obtain the final concentration needed. In this case the salt solution was gassed with O_2. For the replacement of either sodium or chloride ions with a large ion, Tris-HCl buffer (pH 7.3) and sodium glutamate were used respectively.

The rate of flow of the test solution along the central part of the preparation was about 2 ml/min and remained constant throughout each experiment. The indicated doses of bradykinin and of the peptide BPP_{5A}, both dissolved in 0.9% w/v NaCl solution (saline), were added to the supply of the test solution over a period of 1 min and 4 min respectively by means of separate continuous infusion apparatuses at a speed of 0.2 ml and 0.05 ml per min respectively. In most experiments bradykinin was also injected during the last minute of the BPP_{5a} administration. Potassium depolarization was obtained either by adding twice the volume of KCl to the Krebs solution or by infusing isotonic KCl solution at a rate of 0.5 ml/min into the supply of the test solution. The latter procedure resulted in a final concentration of K^+ ions of 35.5 mM. Sodium-dependent slow wave-like potentials were induced by the method described by Golenhofen & Petrányi (1969). After a control period in normal Krebs solution, the taenia was superfused for 30 min with a calcium-free solution, containing 5 mM sodium fluoride. Afterwards this solution was exchanged for calcium- and magnesium-free Krebs solution without sodium fluoride.

Organ-bath preparation

A piece of the terminal part of the guinea-pig ileum (about 3 cm) was suspended in 5 ml organ bath and loaded with 0.5 gram. The isotonic contractions were recorded by means of a displacement transducer 7 DCDT, (Hewlett and Packard, California, U.S.A.).

Drugs

Synthetic bradykinin (BRS 640) was kindly supplied by Sandoz. The peptide BPP_{5a} (mol. wt. 629) was obtained from Spectrum Medical Industries Inc., Los Angeles, U.S.A.

Results

Effects of bradykinin and BPP_{5a} in normal Krebs solution

In accordance with Bülbring & Tomita (1970), it was found that spontaneous activity of the taenia coli in normal Krebs solution was sometimes absent or slow at the beginning of the experiment, probably due to the hyperpolarization of the membrane in the centre of the preparation caused by the fact that the adjoining portions were immersed in sucrose solution. Under these conditions, the responses to both BPP_{5a} and bradykinin were also absent. However, spontaneous activity usually appeared after administration of several doses of bradykinin at 5 min intervals. Spontaneous activity and reactions to bradykinin could also be induced by increasing the external potassium concentration from 5.9 mM to 10.6 mM. Excess potassium depolarizes the membrane and increases the

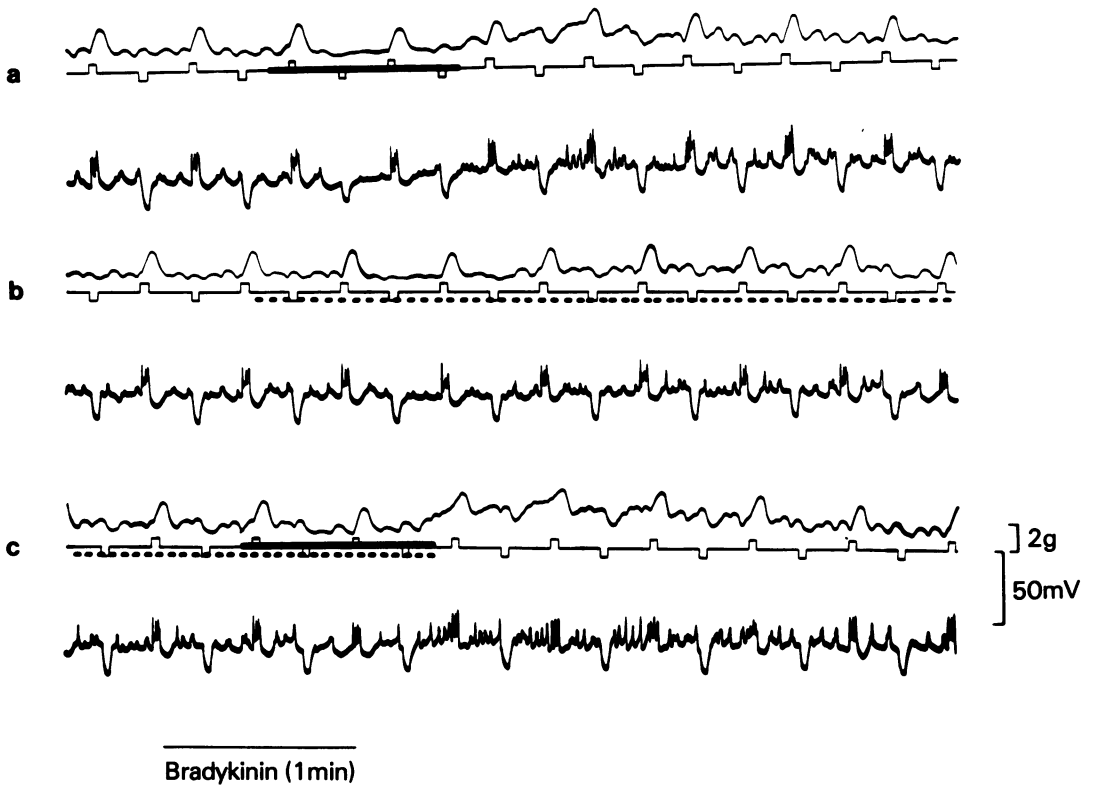

Figure 1 The effect of bradykinin and BPP_{5a} on the isometric tension (upper tracing) and the electrical potentials (lower tracing) of the taenia coli of the guinea-pig in normal Krebs solution. Current pulses with alternating polarity were applied as shown in the middle trace. (a) Bradykinin (2μg) added over a period of 1 min (see bar) caused a biphasic effect. (b) BPP_{5a} (0.2 μg/min) applied for 4 min (dotted line) had little effect. (c) The effect of bradykinin was only potentiated by BPP_{5a}

spontaneous spike discharge (Casteels & Kuriyama, 1966; Shimo & Holland, 1966).

When a response of the taenia in normal Krebs solution to bradykinin (2 μg) occurred it was usually a stimulation: a small depolarization was accompanied by an increase in spontaneous spike activity (Figure 1a). No apparent change of the electrotonic potential occurred under these experimental conditions. Sometimes the stimulation was preceded by a short-lasting inhibition of the spontaneous potentials. When BPP_{5a} (0.2 μg/min) caused an effect, it was always an increase in spontaneous activity without change of the membrane potential and the electrotonic potential (Figure 1b). Figure 1c shows that the response to bradykinin in the presence of BPP_{5a} was similar to the response to bradykinin alone. However, it was difficult to determine a potentiating effect of BPP_{5a} because of the small increase in effect with the dose of bradykinin under the conditions used.

Effects of excess potassium

Figure 2 shows an experiment in which spontaneous spike discharge had been induced by increasing the external potassium concentration to 10.6 mM. The spikes had a 5 to 7 s rhythm and showed prepotentials at their base. Under these conditions bradykinin alone caused predominantly an inhibition of the spontaneous spike activity (Figure 2a). BPP_{5a} stimulated the spontaneous activity which was suppressed by addition of bradykinin (Figure 2b). When electrical stimulation was applied both BPP_{5a} alone and BPP_{5a} plus bradykinin had no effect on the electrotonic potentials (Figure 2c).

A sudden increase in the external potassium concentration by an infusion of potassium chloride, decreased the membrane potential and increased the frequency of spontaneous spike discharge. This was accompanied by a decrease in electrotonic potential

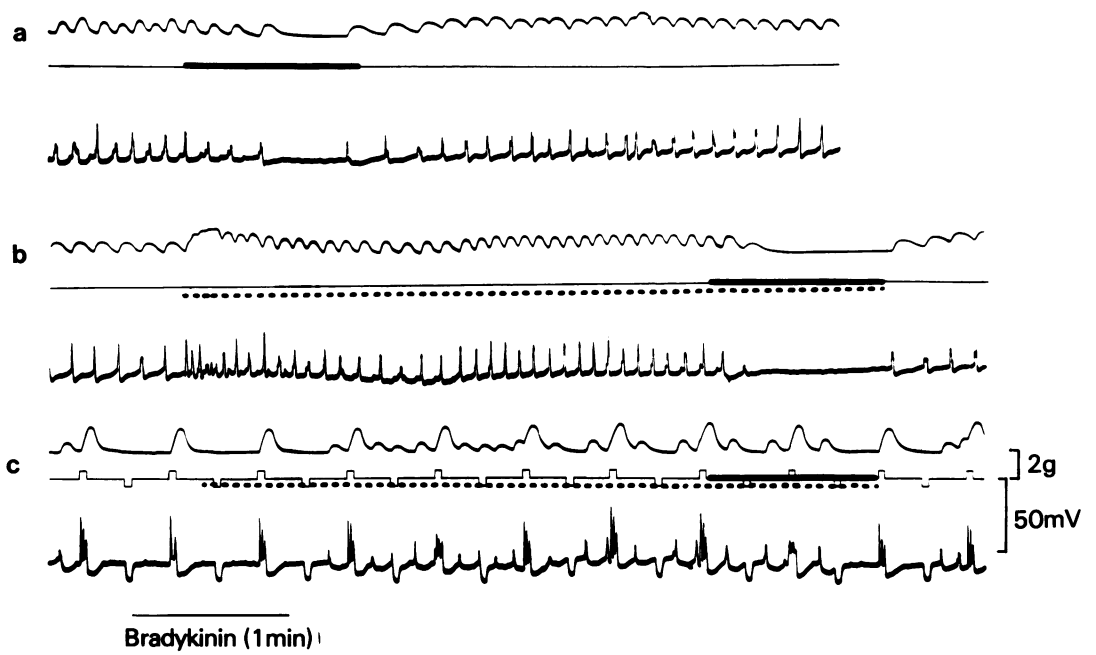

Figure 2 Effects of bradykinin (2 μg), indicated by bar, and BPP_{5a} (0.2 μg/min), indicated by dotted line, on spontaneous spikes with a 5 to 7 s rhythm and prepotentials at their base, induced in guinea-pig taenia coli by increasing the external potassium concentration to 10.6 mM. Upper tracing indicates tension, lower tracing the electrical potential, the middle tracing in (c) indicates the applied current pulses. Note the inhibitory effect of bradykinin alone in (a) and in combination with BPP_{5a} in (b) and (c). BPP_{5a} stimulated the generation of spontaneous spikes in (b) and (c).

(Figure 3b). In this 'spike' phase of the potassium-contracture, BPP_{5a} produced either no effect or a small stimulatory effect without decreasing the electrotonic potentials, whereas additional bradykinin caused a suppression of the spikes, particularly of their amplitude, and a considerable further reduction of the electrotonic potentials, accompanied by a decrease in membrane potential (Figure 3b, c). The potassium-induced contracture was hardly affected.

Calcium and magnesium-free medium

It is assumed that both calcium and magnesium reduce the sodium conductance in the taenia coli (Bülbring & Tomita, 1970). Omission of these ions from the solution evoked sodium-dependent oscillatory slow spike-like potentials in some preparations for more than 30 min, depending on the stability of the membrane potential. In all preparations bradykinin caused a marked suppression of the electrotonic potentials, accompanied by a greater or a smaller depolarization, according to the height of the resting potential. In spite of this, the amplitude of the oscillatory potentials progressively decreased under the influence of bradykinin (Figure 4a). On the other hand, BPP_{5a} caused only stimulation of the spontaneous activity, without affecting the electrotonic potential (Figure 4b). Figure 4c shows that addition of bradykinin to the BPP_{5a} infusion suppressed the slow wave activity and reduced the electrotonic potential for a longer period than bradykinin alone. In calcium- and magnesium-free medium the contraction was uncoupled from the electrical activity.

The influence of lanthanum

Lanthanum is able not only to replace calcium at superficial binding sites (Weiss & Goodman, 1969; Mayer, van Breemen & Casteels, 1972), but also to suppress calcium uptake into the cellular compartment of the taenia coli during the first 60 min of exposure to concentrations of 2 mM (Mayer *et al.*, 1972). In our experiments, lanthanum (2 mM) did not abolish either the spontaneous or the evoked spikes (Figure 5b). Furthermore Figure 5 shows that both the inhibitory and stimulatory effects of bradykinin were about equal

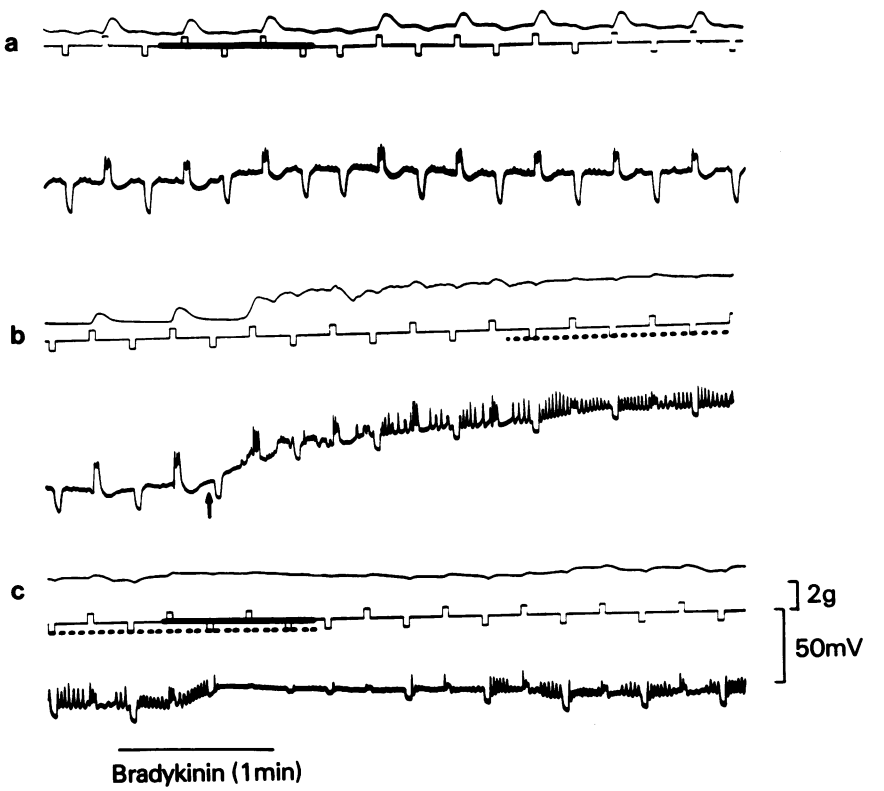

Figure 3 The influence of potassium-induced depolarization on the effect of bradykinin (2 μg/min), indicated by bar, and that of BPP_{5a} (0.2 μg/min), indicated by dotted line in guinea-pig taenia coli. Tracings as described in Figure 1. (a) Effect of bradykinin in normal Krebs solution. (b) At arrow the external potassium concentration was increased to 35.5 mM by continuing infusion of isotonic KCl solution at 0.5 ml/minute. BPP_{5a} stimulated the spike frequency during the spike phase of the potassium contracture without affecting the electrotonic potentials. (c) Addition of bradykinin caused suppression of the spikes, accompanied by a depolarization and decrease of the electrotonic potentials. Note that the tension is hardly decreased.

before, and 19 min after, the addition of lanthanum. The effect of BPP_{5a} was also unaffected by the presence of lanthanum. These findings are in contrast to those of Szurszewski & Bülbring (1973) on the guinea-pig myometrium with respect to the influence of lanthanum on the spike discharge.

To be quite sure that lanthanum did not affect the response to bradykinin, the influence of 10 mM lanthanum was investigated on the bradykinin-induced isotonic contraction of the guinea-pig ileum in an organ bath. The dose-response curve of bradykinin in the presence of 10 mM lanthanum was shifted a little to the right (Figure 6). However, no statistically significant difference ($P > 0.05$) was found for doses of bradykinin up to 40 ng/ml. After equilibration of the preparation in a solution contaning 10 mM lanthanum for 1 h the responses to the lower doses of bradykinin did not differ significantly, whereas those to doses greater than 20 ng/ml did.

Effects of low calcium and low sodium

The response of the taenia coli in normal Krebs solution to both bradykinin and BPP_{5a} was in most preparations small, so that a possible inhibitory influence of sodium-deficiency on these responses could hardly be determined (see Figure 7a and b). Lowering the calcium concentration to a tenth (0.25 mM) enhanced the stimulatory responses of both preparations. The spike activity was particularly enhanced; this was accompanied by an increase in depolarization and an abolition of the electrotonic potentials (see Figure 7c and d). When the sodium concentration was lowered to 15.5 mM and the calcium concentration

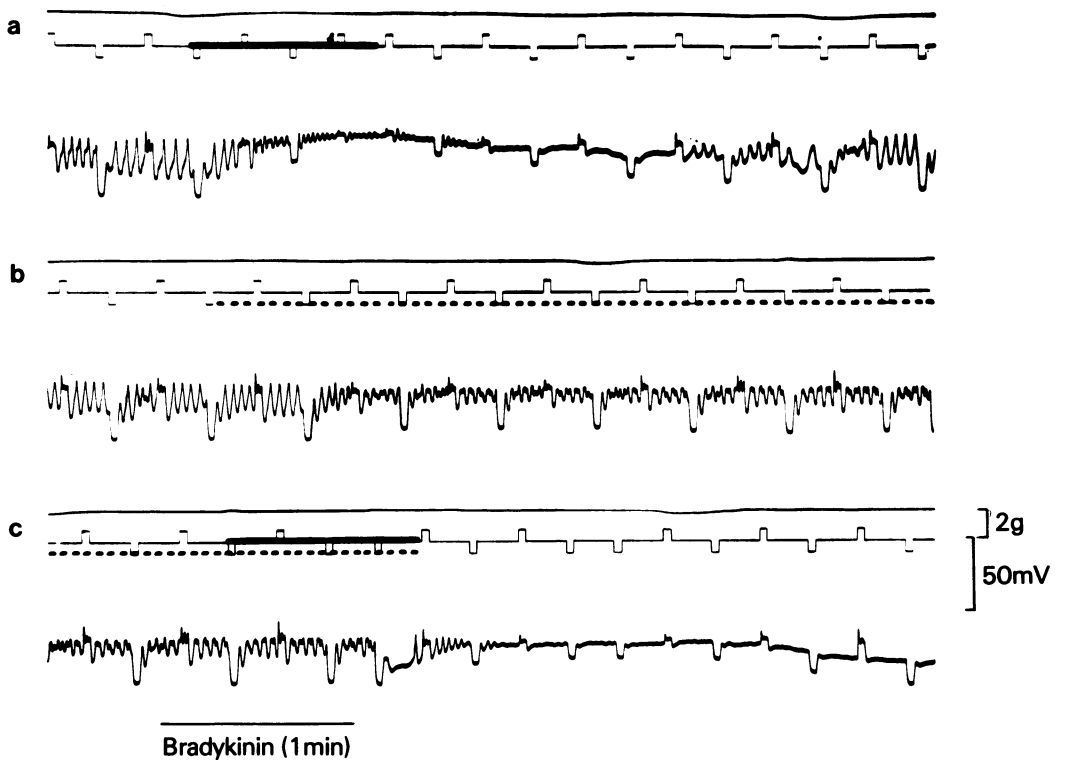

Figure 4 Effects of bradykinin (2 μg/min), indicated by bar, and of BPP_{5a} (0.2 μg/min), indicated by dotted line, on oscillatory slow spike-like potentials (slow waves), induced by a calcium- and magnesium-free medium in guinea-pig taenia coli. Tracings as indicated in Figure 1. (a) Note the inhibitory effect of bradykinin on the oscillatory potentials accompanied by a decrease in electrotonic potentials. (b) BPP_{5a} stimulated the oscillatory potentials without affecting the electrotonic potentials. (c) With a combination of BPP_{5a} and bradykinin the bradykinin effect lasted longer.

kept low, the response to bradykinin was almost completely suppressed within 5 min, as is shown in Figure 7e. Only a small depolarization and a small decrease of the electrotonic potentials remained. Administration of BPP_{5a} was now without effect (Figure 7f).

Effects of low chloride

If the depolarization induced by bradykinin is due to an increase in chloride permeability, reduction of the external chloride concentration should increase the efflux of chloride ions and thus enhance the depolarizing effect. However, Figure 8 shows that reduction of the chloride concentration to 9.7 mM rather decreased the depolarizing effect of bradykinin, while the stimulation of the spike activity was enhanced. Moreover, in these conditions the electrotonic potentials did not decrease in the presence of bradykinin, indicating that the membrane conductance was not enhanced. The response to BPP_{5a} was unchanged after reduction in the chloride concentration (Figure 8d).

Discussion

By the use of the double sucrose-gap technique, the response of the taenia coli to bradykinin was shown in most preparations to be a small contraction accompanied by a small increase in spike frequency and a decrease in membrane potential. Usually the membrane resistance remained unaltered. These findings are in contrast to those of previous experiments in which the single sucrose-gap technique was used, when the taenia-coli showed greater spontaneous activity and usually responded biphasically to bradykinin (Aarsen & van Caspel-de Bruyn, 1970). These results may be due to the hyperpolarization of the membrane in the centre of the preparation caused

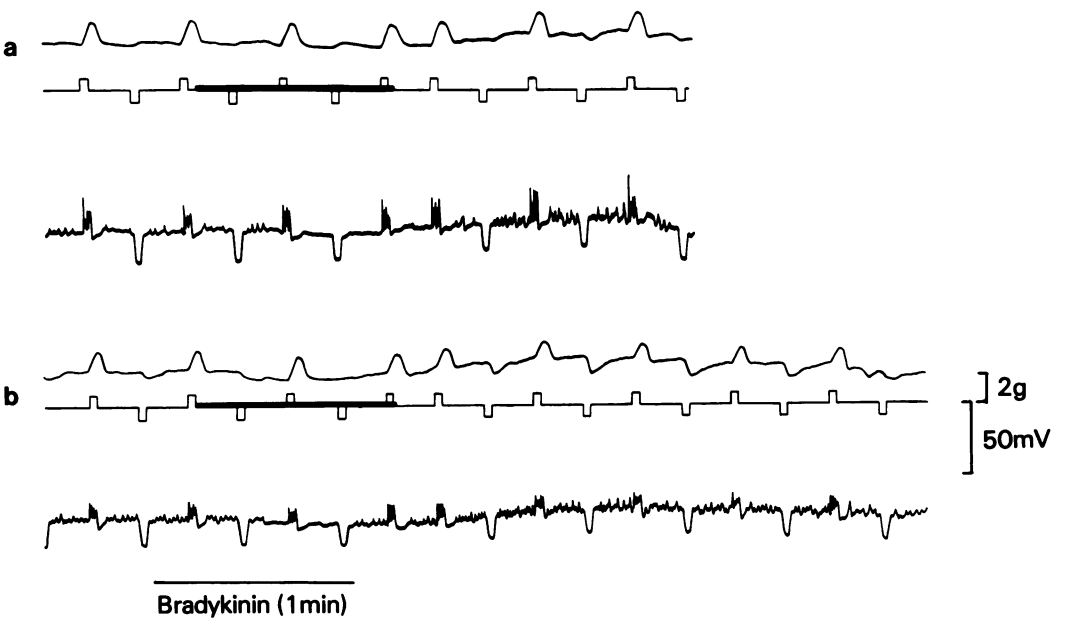

Figure 5 Effect of bradykinin (2.0 μg/min) in Krebs solution on guinea-pig taenia coli without (a) and 19 min after continuous addition of 2 mM lanthanum (b). Tracings as indicated in Figure 1.

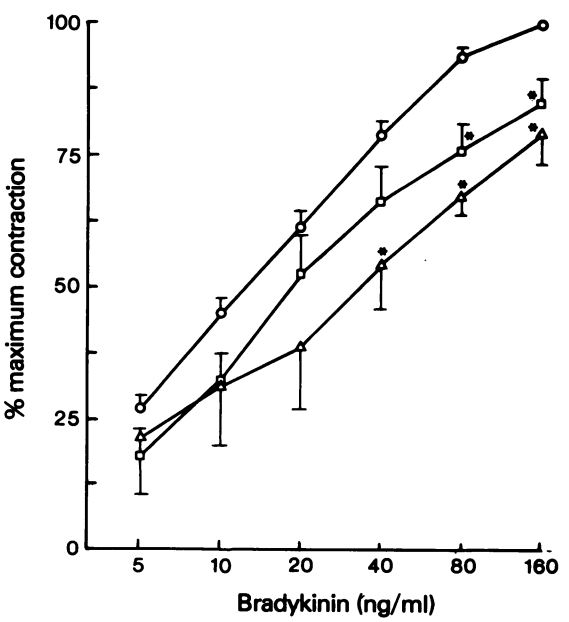

Figure 6 Dose-response curves of the taenia coli in an organ bath to bradykinin in the absence (○) and presence of lanthanum (10 mM) for 10 min (□) and 60 min (Δ). Number of experiments for the controls 10, and for the experiments with lanthanum, 5. Vertical bars indicate s.e. mean. The means which are statistically different from the controls at $P < 0.05$ are indicated by an asterisk.

by immersion of the adjoining portions in sucrose solution in the double sucrose-gap (Bülbring & Tomita, 1970). When spontaneous activity had been initiated by increasing the external potassium ion concentration from 5.9 to 10.6 mM, bradykinin inhibited the generation of the spikes without changing the membrane potential. In this case the membrane resistance was not influenced. Since the initiated spikes had a typical 5 to 7 s rhythm and showed prepotentials at their base, the inhibitory effect of bradykinin may be due to an influence on either the spontaneous oscillations of the membrane potential (slow waves) underlying the potentials, or on the action potentials themselves. Since it has been suggested that the prepotentials in the guinea-pig taenia coli are caused by rhythmic changes in sodium conductance, the effects of bradykinin were studied on experimentally evoked sodium-dependent oscillatory potentials. The amplitude of these oscillations was decreased by bradykinin, even though the membrane resistance and potential had been decreased. Another indication that bradykinin may inhibit slow wave activity is the finding that bradykinin produces only a relaxation in the guinea-pig ileum treated with carbachol or acetylcholine (Hall & Bonta, 1973; Ufkes, 1974). Acetylcholine and carbachol are known to produce sodium-dependent slow waves in the guinea-pig ileum (Bolton, 1971; Bolton, 1975).

When the membrane was suddenly depolarized by high potassium concentrations, (35.5 mM) repetitive

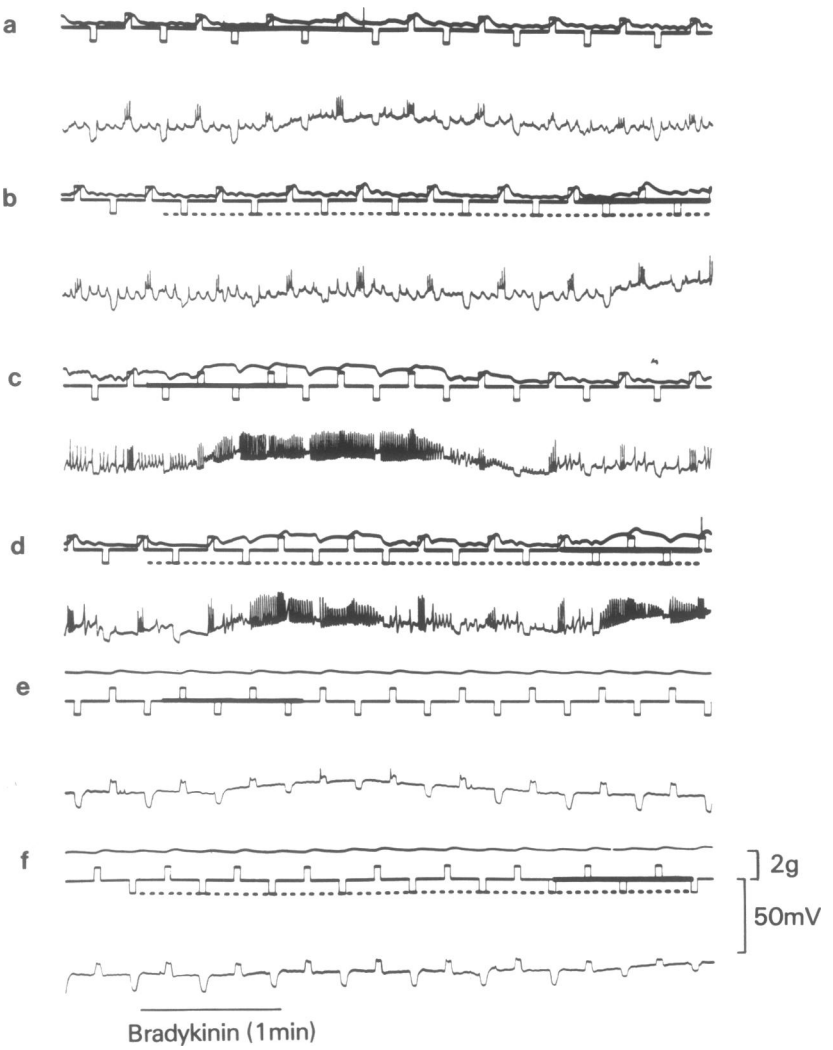

Figure 7 The effects of low external calcium (0.25 mM) and low external sodium (15.5 mM) on the responses to bradykinin (2 μg/min), indicated by bar, and BPP_{5a} (0.2 μg/min), indicated by dotted line in guinea-pig taenia coli. Tracings as described in Figure 1. The responses to bradykinin and BPP_{5a} which were small or absent in normal Krebs solution (see a and b), were strongly increased by lowering the external calcium concentration in (c) and (d). Both compounds caused a depolarization and suppression of electrotonic potentials, accompanied by an increase in spike activity. After lowering the sodium concentration as well, these effects were greatly abolished within 5 to 10 min in (e) and (f).

spiking occurred. During the phasic response to potassium, bradykinin depolarized the membrane further and reduced the electrotonic potential, indicating a decrease in membrane resistance. However, the amplitude of the spikes, which under these conditions are believed to be due to calcium entry, were suppressed by bradykinin. Since depolarization of the membrane by high potassium (29.5 to 72 mM) does not initiate slow wave activity (Bolton, 1971), the inhibitory effect of bradykinin on the spikes cannot be ascribed to an action on the slow waves. These results indicate that bradykinin increased the sodium conductance rather than that of calcium.

The idea that bradykinin increases the sodium con-

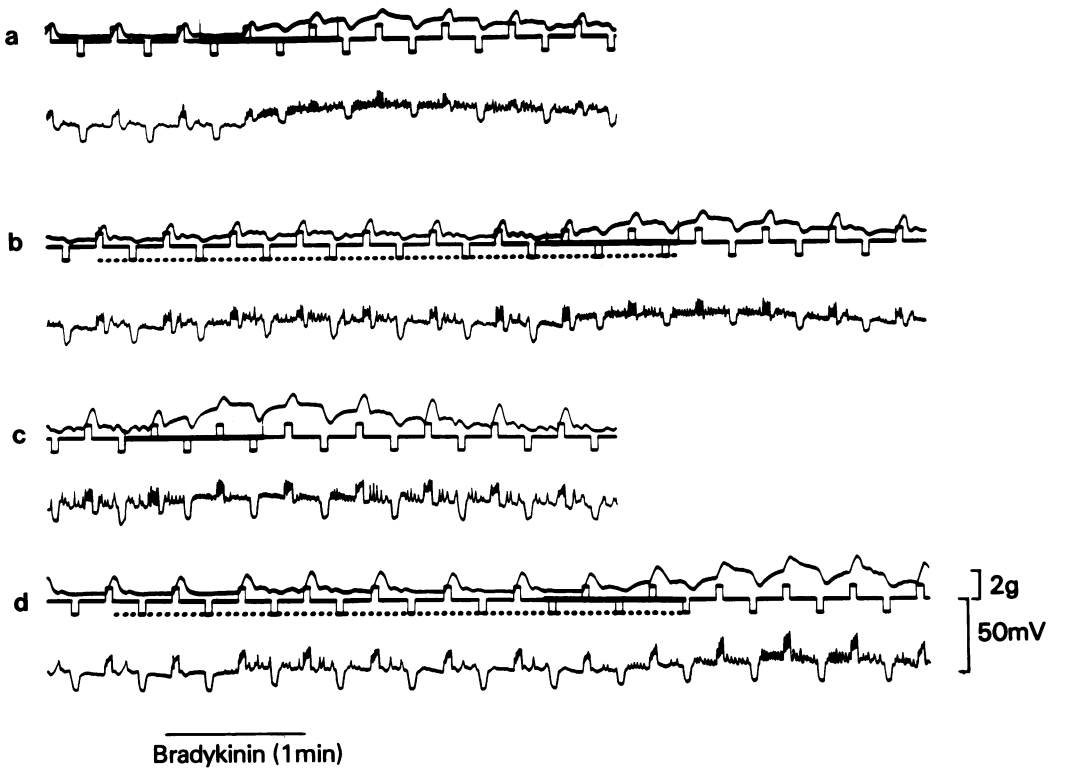

Figure 8 The effects of low external chloride (9.7 mM) on the responses to bradykinin (2 μg), indicated by bar, and to BPP_{5a} (0.2 μg/min), indicated by dotted line on guinea-pig taenia coli. Tracings as described in Figure 1. The depolarization and decrease in electrotonic potentials caused by bradykinin in normal Krebs solution (a) were suppressed by exposure of the taenia to low chloride for 6 min in (c), whereas the spike frequency activity increased. The small stimulatory effect of BPP_{5a} in normal Krebs solution in (b) was about equal to that in low chloride in (d). In both solutions no sign of a potentiating effect of BPP_{5a} on the bradykinin response could be detected.

ductance is also supported by the following findings: (1) Reduction of the calcium concentration to 0.25 mM, which increases the sodium conductance (Brading, Bülbring & Tomita, 1969; Bülbring & Tomita, 1970), enhanced the stimulatory effects of bradykinin. A higher contraction was produced, accompanied by a higher spike activity and depolarization, while the membrane resistance was markedly decreased. Moreover, when the external sodium concentration was reduced to 15.5 mM at a low external calcium concentration, the effects of bradykinin were suppressed. (2) Lanthanum (2 and 10 mM) did not influence either the inhibitory or stimulatory effects of bradykinin. (3) Reduction of the chloride concentration to 9.7 mM suppressed both the bradykinin-induced depolarization and decrease in membrane resistance, indicating that bradykinin does not increase the chloride permeability.

Thus it may be concluded that bradykinin enhances the sodium permeability. However, the effect obtained by this increase in permeability depends upon the condition of the preparation; whether the action potentials are triggered or not by slow-wave depolarizations, and further the degree to which sodium and calcium ions participate in the generation of the action potential. It seems that sodium-dependent oscillations of the membrane potential (slow waves) are suppressed by bradykinin, whereas action potentials in low calcium are strongly stimulated.

The effects of BPP_{5a} can be distinguished from those of bradykinin by a stimulation of both oscillatory potentials (slow waves) and action potentials. These findings are in agreement with those of Camargo & Ferreira (1971) and Ufkes *et al.* (1976) who found that BPP_{5a} potentiates only the bradykinin-induced contraction of isolated ileum of rat and rabbit,

without affecting the bradykinin-induced relaxation. However, in the present investigations no potentiating effect of BPP_{5a} could be demonstrated. This was obviously due to the very small increment in response to increasing doses of bradykinin under the present conditions. On the other hand, it seemed that spontaneous spikes with prepotentials at their base and oscillatory potentials (slow waves) were inhibited for a longer period by bradykinin after a preceding stimulation by BPP_{5a}. With regard to the effect on the membrane potential and resistance, BPP_{5a} also differs remarkably from bradykinin. BPP_{5a} does not cause a depolarization and a decrease in the membrane resistance, except in low external calcium concentration. From these findings it must be concluded that the mechanism of action underlying the effect of BPP_{5a} is different from that underlying the effects of bradykinin. This would imply that BPP_{5a} probably acts on a receptor other than that for bradykinin, and thus the basis for the hypothesis that two different receptor sites may be involved in the biphasic bradykinin response is undermined.

It is a pleasure to thank Professor Dr C. van der Meer for his helpful criticism during the preparation of this manuscript, and Mrs M. van Caspel-de Bruyn and Mr A. Zeegers for their technical assistance.

References

AARSEN, P.N. & van CASPEL-DE BRUYN, M. (1970). Effect of changes in environment on the action of bradykinin on the guinea-pig taenia coli. *Eur. J. Pharmac.*, **12**, 348–358.

BAUER, G., ZIEGLER, E. & KONZETT, H. (1966). Zur Hemmwirkung von Kininen an isolierten Darmpräparaten. *Naunyn Schmiedeberg's Arch. Pharmak. exp. Path.*, **254**, 235–244.

BOLTON, T.B. (1971). On the nature of the oscillations of the membrane potential (slow waves) produced by acetycholine or carbachol in intestinal smooth muscle. *J. Physiol., Lond.*, **216**, 403–418.

BOLTON, T.B. (1975). Effects of stimulating the acetylcholine receptor on the current-voltage relationships of the smooth muscle membrane studied by voltage clamp of potential recorded by micro-electrode. *J. Physiol., Lond.*, **250**, 175–202.

BRADING, A., BÜLBRING, E. & TOMITA, T. (1969). The effect of sodium and calcium on the action potential of the smooth muscle of the guinea-pig taenia coli. *J. Physiol., Lond.*, **200**, 637–654.

BÜLBRING, E. & TOMITA, T. (1969). Increase of membrane conductance by adrenaline in the smooth muscle of the guinea-pig taenia coli. *Proc. Roy. Soc. B.*, **172**, 89–102.

BÜLBRING, E. & TOMITA, T. (1970). Effects of Ca removal on the smooth muscle of the guinea-pig taenia coli. *J. Physiol., Lond.*, **210**, 217–232.

CAMARGO, A. & FERREIRA, S.H. (1971). Action of bradykinin potentiating factor (BPF) and dimercaprol (BAL) on the responses to bradykinin of isolated preparations of rat intestines. *Br. J. Pharmac.*, **42**, 305–307.

CASTEELS, R. & KURIYAMA, H. (1966). Membrane potential and ion content in the smooth muscle of the guinea-pig's taenia coli at different external potassium concentrations. *J. Physiol., Lond.*, **184**, 120–130.

DAY, M. & VANE, J.R. (1963). Analysis of the direct and indirect actions of drugs on the isolated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **20**, 150–170.

ELLIOT, D.F., HORTON, E.W. & LEWIS, G.P. (1960). Actions of pure bradykinin, *J. Physiol., Lond.*, **153**, 473–480.

GERSHON, M.D. (1967). Effects of tetrodotoxin on innervated smooth muscle preparations. *Br. J. Pharmac. Chemother.*, **29**, 259–279.

GOLENHOFEN, K. & PETRANYI, P. (1969). Spikes of smooth muscle in calcium-free solution (isolated taenia coli of the guinea-pig). *Experientia*, **25**, 271–273.

HALL, D.W.R. & BONTA, I.L. (1973). The biophasic response of the isolated guinea-pig ileum by bradykinin. *Eur. J. Pharmac.*, **21**, 147–151.

HORTON, E.W. (1959). Human urinary kinin excretion. *Br. J. Pharmac. Chemother.*, **14**, 125–132.

MAYER, C.J., van BREEMEN, C. & CASTEELS, R. (1972). The action of lanthanum and D600 on the calcium exchange in the smooth muscle cells of the guinea-pig taenia coli. *Pflügers Arch.*, **337**, 333–350.

OHASHI, H., NONOMURA, Y. & OHGA, A. (1967). Effects of angiotensin, bradykinin and oxytocin on the electrical and mechanical activities in the taenia coli of the guinea-pig. *Japan J. Pharmac.*, **17**, 247–257.

SHIMO, Y. & HOLLAND W.C. (1966). Effects of potassium on membrane potential, spike discharge, and tension in taenia coli. *Am. J. Physiol.*, **211**, 1299–1304.

STEWART, J.M., FERREIRA, S.H. & GREENE, L.J. (1971). Bradykinin potentiating peptide PCA-Lys-Trp-Ala-Pro; and inhibitor of the pulmonary inactivation of bradykinin and conversion of angiotensin I to II. *Biochem. Pharmac.*, **20**, 1557–1567.

STÜRMER, E. & BERDE, B. (1963). A comparative pharmacological study of synthetic eledoisin and synthetic bradykinin. *J. Pharmac. exp. Ther.*, **140**, 349–355.

SZURSZEWSKI, J.H. & BÜLBRING, E. (1973). The stimulant action of acetylcholine and catecholamines on the uterus. *Phil. Trans. R. Soc. Lond. B.*, **265**, 149–156.

UFKES, J.G.R. (1974). Effecten van bradykinine op glad spierweefsel en hun versterking door enige peptiden. *Thesis, University of Amsterdam*, pp. 57–62.

UFKES, J.G.R., AARSEN, P.N., & van der MEER, C. (1976). The bradykinin potentiating activity of two pentapeptides on various isolated smooth muscle preparations. *Eur. J. Pharmac.*, **40**, 137–144.

WEISS, G.B. & GOODMAN, F.R. (1969). Effects of lanthanum on contraction, calcium distribution and Ca^{45} movements in intestinal smooth muscle. *J. Pharmac. exp. Ther.*, **169**, 46–55.

(Received January 6, 1977
Revised August 3, 1977)