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REDUCTION OF THE BRADYKININ-INDUCED ACTIVATION OF FELINE GROUP III AND IV MUSCLE RECEPTORS BY ACETYLSALICYLIC ACID

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SUMMARY

1. In chloralose-anaesthetized cats, the influence of systemically or locally applied acetylsalicylic acid (ASA) on the responses of thin-fibre muscle receptors to close-arterial injections of bradykinin was studied.

2. Many of the slowly conducting (group III and IV) muscle afferents had a background activity of low frequency. This discharge was either unaffected or slightly increased by the ASA doses used. In two units which had a very high discharge rate ASA led to a marked decrease in background activity.

3. On local (I.A. or I.M.) injection of ASA, doses below 1 mg were sufficient for reducing the bradykinin-induced activations of group III and IV muscle receptors. The reduction lasted for about 15–30 min.

4. On systemic (I.V.) administration of ASA (50 mg/kg body weight) the reduction in response magnitude to bradykinin became significant 8 min after injection of the analgesic. The effect was maximal about 10 min later and lasted for more than 60 min.

5. Five receptors were found which gave a repeated response to 5-hydroxytryptamine (5-HT) injected at 10 min intervals. The 5-HT-induced activations could not be reduced by ASA (50 mg/kg I.V.).

6. Most of the receptors responding to bradykinin had a high threshold on mechanical stimulation and thus were probably nociceptors. It is concluded that the reduction of their bradykinin-induced activations reflects the suppression of nociceptive information by an analgesic. Since the recordings were obtained from primary afferent units the data constitute direct evidence for a peripheral action of ASA.

INTRODUCTION

Previous publications of this laboratory have shown that the endogenous algesic substance bradykinin is a powerful excitant for muscle receptors with group III and IV afferent fibres (Franz & Mense, 1975; Mense, 1977) and that 5-hydroxytryptamine (5-HT) and prostaglandin (PG) E_2 enhance this action (Mense, 1981).

In the above studies many of the receptors appeared to be nociceptive since in addition to responding to bradykinin they had a high threshold on mechanical stimulation. The bradykinin-induced discharges of such receptors in skin and deep

tissues probably elicit the painful sensations that follow an intra-arterial administration of this substance in human beings (cf. Burch & DePasquale, 1962; Coffman, 1966).

The bradykinin-evoked pain in man and pseudaffective responses in animals (e.g. vocalization, increase in blood pressure, writhing) can be reduced by acetylsalicylic acid (ASA) (Emele & Shanaman, 1963; Collier, Hammond, Horwood-Barrett & Schneider, 1964; Guzman, Braun, Lim, Potter & Rodgers, 1964; Coffman, 1966), an action which has been shown to be peripheral, i.e. not mediated by the central nervous system (Guzman *et al.* 1964; Lim, Guzman, Rodgers, Goto, Braun, Dickerson & Engle, 1964; Lim, Miller, Guzman, Rodgers, Rogers, Wang, Chao & Shih, 1967). Similar results have been obtained with sodium salicylate (Taira, Nakayama & Hashimoto, 1968). Since ASA acts at the site of the peripheral nerve endings, and the group III and IV receptors are the only afferent units in skeletal muscle which are substantially activated by bradykinin (Mense, 1977), the analgesic should have an influence on the chemically induced discharges of these receptors.

Until now, only a few investigations have been performed in which the effect of a peripherally acting analgesic upon the discharges of presumable nociceptors was studied in single fibre recordings. Uchida & Murao (1974*a*, *b*) described a reduction of the potassium- and bradykinin-induced excitation of sympathetic cardiac afferents by ASA. In an investigation on the sensitization of cutaneous nociceptors King, Gallant, Myerson & Perl (1976) and Perl, Kumazawa, Lynn & Kenins (1976) studied the effect of indomethacin or ASA on the responsiveness and heat sensitization of these receptors. Kumazawa & Mizumura (1980) tested seven polymodal receptors of the scrotal contents of dogs with indomethacin and observed a significant decrease of their bradykinin-induced activation.

The present study was undertaken to find out whether ASA in therapeutic doses is effective in reducing the responses of group III and IV muscle receptors to bradykinin, and to obtain some data on the time course of the effect after systemic and local administration of the analgesic.

The results show that by a single injection of ASA the excitatory action of bradykinin on thin-calibre muscle afferents can be significantly decreased for periods of more than one hour. This effect might play a role in the therapy of painful tissue lesions or inflammations, in the course of which considerable amounts of bradykinin are released (Rocha e Silva & Rosenthal, 1961; Rocha e Silva, 1964; Brocklehurst, 1971). Preliminary communications on this subject have been published (Mense, 1976; Mense & Schmidt, 1977).

METHODS

Data were obtained from thirty cats weighing 2·4–4·2 kg. The animals were deeply anaesthetized with chloralose alone (70 mg/kg I.P.) or with a combination of ketamine (Ketanest®) 15–20 mg/kg I.M. followed by chloralose 10–30 mg/kg I.V. Additional doses of chloralose were given I.V. as required in order to maintain a deep anaesthesia.

After induction of muscular relaxation with gallamine triethiodide (Flaxedil®) the cats were artificially ventilated and the end-tidal P_{CO_2} adjusted to 3·5–4·0 %. As Flaxedil was given in single doses of 10–20 mg I.V. the level of anaesthesia could be tested about every two hours after the effect of the drug had worn off. Body core temperature and arterial blood pressure were continuously measured and kept at physiological levels.

The receptors studied were all situated in the gastrocnemius-soleus (g.s.) muscle; recordings of fibre activity were made from dorsal rootlets or the peripheral muscle nerve. In the latter case the left g.s. muscle with its nervous and vascular supplies was dissected free and a pool formed over the dorsal aspects of the hind limb. A side branch of the sural artery was cannulated for close-arterial injections of chemicals into the muscle. For dorsal root recordings, the lumbo-sacral spinal cord was exposed by a laminectomy and, in addition to the hind limb pool, a second one was formed covering the laminectomy wound. Both pools were filled with liquid paraffin at 37 °C.

Single fibre recordings were obtained by splitting dorsal rootlets or filaments of the muscle nerve with watchmaker's forceps until only one or a few clearly distinguishable units were left. The dissected strand of nerve fibres was placed over a hook electrode. As a searching stimulus electrical stimulation of the g.s. nerve was used. Dorsal root fibres were classified as group IV if they conducted at less than 2·5 m/s and as group III if their conduction velocity was 2·5–30 m/s. In the case of recordings from the muscle nerve a group III or IV fibre was accepted as afferent if it displayed resting activity and/or responded to natural stimulation of the g.s. muscle. Before injecting bradykinin into the muscle most units were tested with local pressure to determine their approximate mechanical threshold. According to an earlier publication (Franz & Mense, 1975) where the methods used are described in more detail, units were labelled low-threshold pressure sensitive (l.t.p.) if they responded already to light, innocuous pressure, and high-threshold pressure sensitive (h.t.p.) if they required noxious pinching of the muscle to be excited.

The bradykinin solution used was prepared from bradykinin triacetate and contained 86–430 μg of the peptide per millilitre Tyrode; the standard dose for intra-arterial injections was 26 μg bradykinin in 0·3 ml Tyrode. Only units that gave a reproducible response to repeated administrations of the substance were evaluated. ASA was dissolved in a small amount of N-NaOH, the pH adjusted to 7·3–7·4, and the solution diluted with Tyrode to a final concentration of 10 mg ASA/ml for I.V. application, 2–3 mg ASA/ml for I.A. injections and 1·5–3 mg ASA/ml for I.M. injections. The I.M. injections were given into the mechanosensitive receptive field of the unit. The needle was advanced until a short burst of neural activity occurred, then the muscle area close to the needle tip was infiltrated with 0·1–0·2 ml of ASA solution. 5-HT (serotonin creatinine sulphate) and methysergide (methysergide bimaleate) were dissolved in Tyrode solution; the doses refer to the base only.

Since after I.V. injection of ASA the time course of the elimination of the drug was not known, only one receptor per experiment was tested with systemic administration of the analgesic. Usually the last nerve preparation of a given experiment was used for this purpose, the other ones were evaluated for a sensitization study (Mense, 1981).

The impulse activity of the single- or few-fibre preparations was amplified and stored using conventional electronic equipment. If more than one active unit was present in a given filament, they were counted separately by means of a window discriminator. A computer with 1024 addresses constructed time histograms of the fibre activity using bin widths of 1–6 s.

RESULTS

ASA effects on background activity

Of the twenty-seven group IV and eleven group III units tested with injections of ASA, twenty of the former and nine of the latter showed a background activity, i.e. they generated action potentials in the absence of intentional stimulation. As described earlier (Franz & Mense, 1975) the impulse pattern was irregular, sometimes showing grouped impulses.

Since the background activity might represent discharges of nociceptors signalling tissue damage due to prior stimulation and/or surgical exposure of the muscle, the influence of ASA on this type of nervous activity was studied.

Of six single group IV receptors which were tested with local (I.M., I.A.) or systemic (I.V.) injections of ASA, three were unaffected (cf. Fig. 1*A*), one exhibited a slight increase in background discharge (Fig. 1*B*), another one was transiently activated,

and the sixth – which had a high discharge rate – depressed by injection of the analgesic.

In addition, three few-fibre preparations, each containing three to five group IV units, were tested with I.V. injections of ASA 50 mg/kg. Of these, two preparations were unaffected, and the third showed a slight increase in impulse activity.

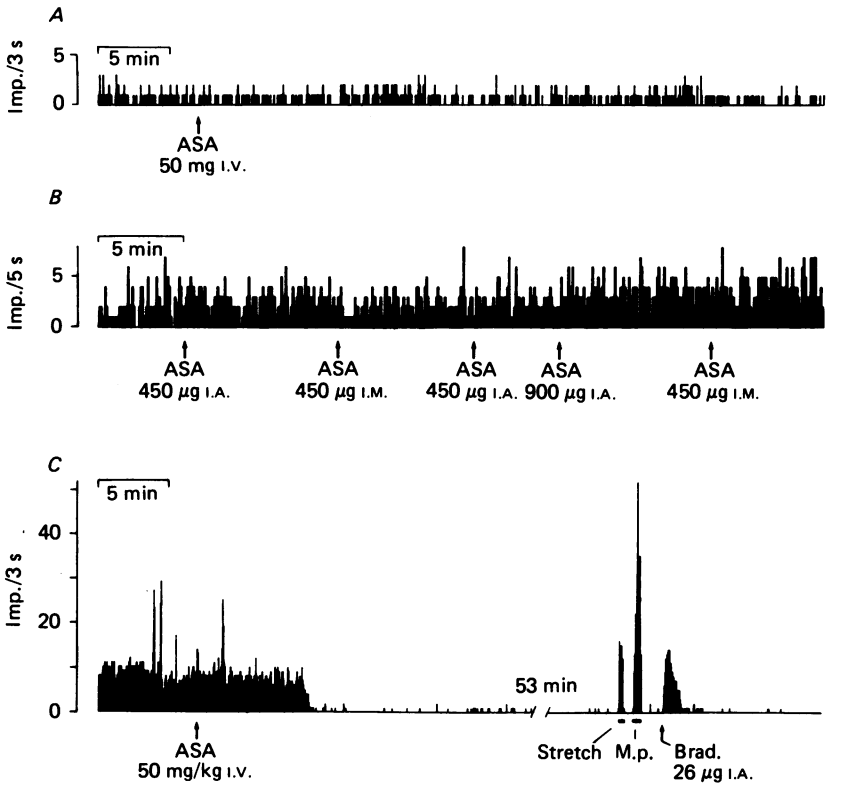

Fig. 1. Effects of ASA on background activity. *A*, lack of effect on discharges of a group IV receptor, h.t.p., conduction velocity (c.v.) 0·94 m/s. Bin width of histogram: 3 s. *B*, slight increase in background activity after several I.A. and I.M. injections of ASA. Group IV receptor, h.t.p., c.v. 1·00 m/s. Bin width of histogram: 5 s. *C*, removal of unusually high background discharge. Group III receptor, l.t.p., c.v. 24·50 m/s. About 80 min after administration of ASA the receptor responded to forceful stretch of the g.s. muscle (Stretch), to moderate local pressure applied to the receptive field (M.p.) and to I.A. injection of 26 µg of bradykinin. Bin width of histogram: 3 s.

Group III receptors usually had a background discharge of very low frequency (a few impulses per minute). However, one unit showed an exceptionally high level of activity; about 7 min after injection of ASA (I.V.) the discharge decreased sharply to the usual low frequency. This effect was not due to an unspecific deterioration of the unit since its ending still reacted to mechanical and chemical stimulation (Fig. 1*C*).

Thus, with the exception of the two highly active units that showed a marked depression after application of ASA, the effects of the analgesic on background activity appeared to be negligibly small.

Whether ASA changes the mechanical responsiveness of the receptors is not known since no quantitative tests with mechanical stimuli were performed in this study.

Effects of ASA on the responses of muscle receptors to intra-arterial injection of bradykinin

Intra-arterial administration of ASA. In these experiments both bradykinin and ASA were injected into the artery supplying the g.s. muscle. Injections were done at an interval of 5 min; after the second or third response to bradykinin ASA was given. Single doses of ASA in the milligram range were effective in reducing the bradykinin-induced activation of group III and IV muscle receptors. In the case shown in Fig. 2*A*, 1·5 mg ASA abolished the bradykinin effects almost completely, but sometimes a reduced response was still visible, even after intra-arterial infusion of high doses of ASA (Fig. 2*B*).

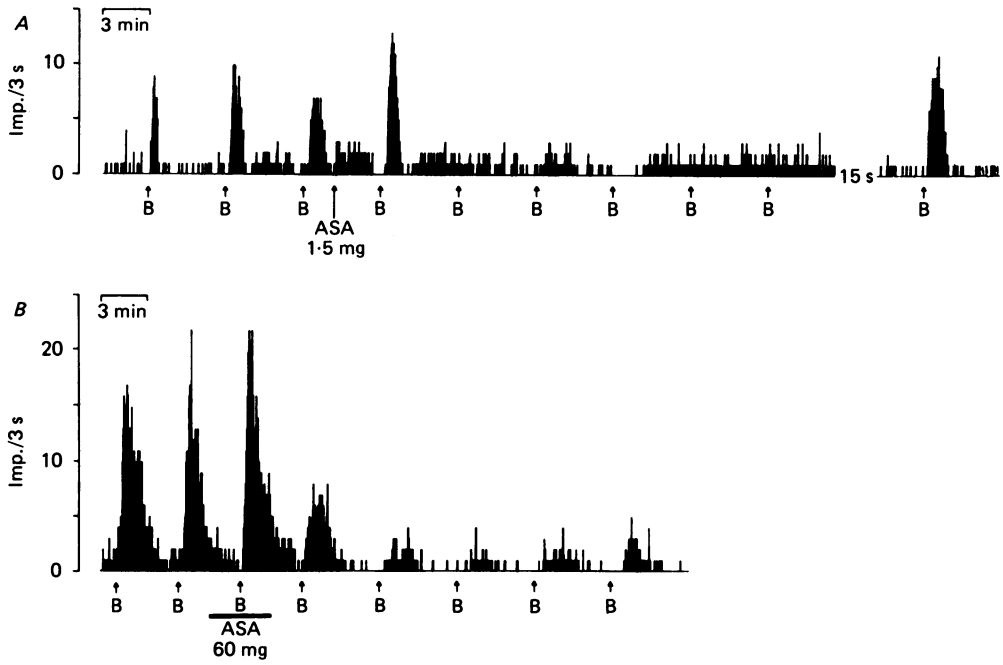

Fig. 2. Reduction of the excitatory action of bradykinin by ASA. Both substances were injected close-arterially. The arrow heads mark the time of injection of bradykinin 26 μg (B) or of ASA. *A*, group IV unit, h.t.p., c.v. 1·23 m/s. *B*, group III unit, h.t.p., c.v. 9·38 m/s. During the period indicated by the black bar (4 min) an intra-arterial infusion of ASA (3 mg/ml) was given at a rate of 5 ml/min. The histograms were constructed using a bin width of 3 s.

The quantitative evaluation of the responses of four group IV units and one group III unit shows that reductions of the bradykinin effects were already obtained with ASA doses of 0·6 mg (Fig. 3). Three additional units were tested with 0·4–0·6 mg ASA; in only one a dose of 0·45 mg ASA led to a decrease in the response to bradykinin. Thus, on intra-arterial application the minimal effective dose for ASA was approximately 0·5 mg.

In units that showed a recovery in their responses to bradykinin during the recording period, the duration of the ASA effect could be determined. From Fig. 3

it can be seen that the bradykinin responses of some receptors increased again 13–33 min after administration of 0·6–1·5 mg ASA.

Intravenous administration of ASA. The intra-arterial injection of ASA was sometimes followed by an increase in discharge frequency of the group III and IV muscle receptors (cf. Fig. 2*A*), possibly due to an irritant action of the ASA solution on the receptive ending. Therefore, in later experiments the intravenous route of administration was preferred. The dosage used (50 mg/kg body weight) was adopted from the work of other authors who had shown that it was effective in reducing bradykinin-induced pain in man and pseudaffective responses in animals (Guzman *et al.* 1964; Coffman, 1966).

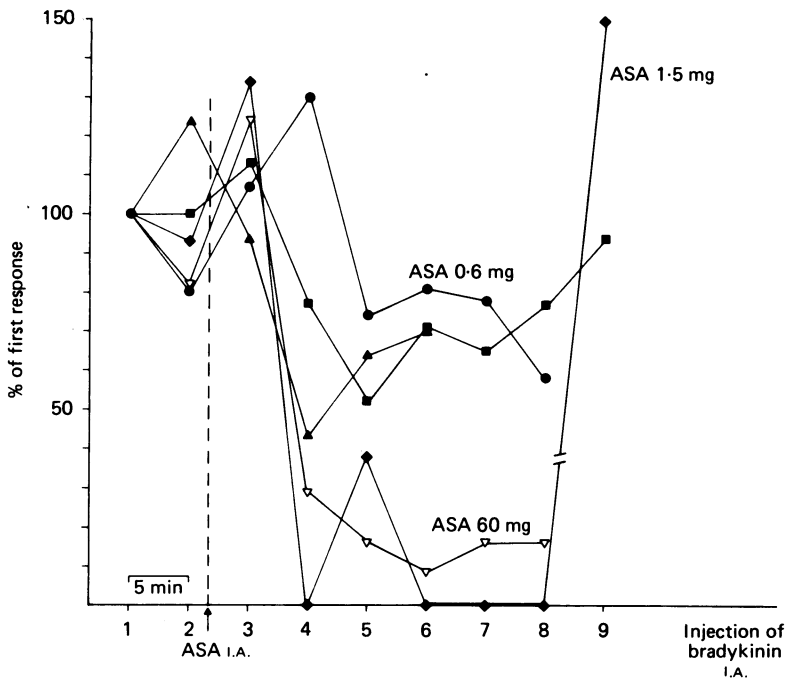

Fig. 3. Quantitative evaluation of data as shown in Fig. 2. Ordinate: response magnitude (impulses per injection) in percent of first response; existing background activity prior to injection of bradykinin was extrapolated for the duration of the response and deducted. Abscissa: number of injections given at intervals of 5 min. Two min after the second injection of bradykinin ASA was applied. Filled symbols indicate group IV receptors, the open triangle the group III unit of Fig. 2*B*. The unit marked with (◆) is identical with that shown in Fig. 2*A*. Its first response to bradykinin is not shown; the interval between the 8 and 9 injection was 7 min.

This dose of ASA led to a marked decrease of the bradykinin-evoked excitations in most group III and IV muscle receptors. An example is shown in Fig. 4. Eighteen minutes after injection of ASA the bradykinin response of the group IV unit was totally abolished (Fig. 4*A*). In the course of the following two hours it recovered to a certain extent but did not reach the initial magnitude (Fig. 4*B* and *C*). In Fig. 4*C* an attempt is shown to restore the bradykinin sensitivity with an intra-arterial injection of 5-HT. 5-HT indeed caused an increase in the response magnitude to

bradykinin (cf. Mense, 1981), but this increase was only transient and about 25 min later the bradykinin effect was reduced as before.

As can be seen from the quantitative evaluation in Fig. 5, the onset of the ASA effect was rather rapid. Already 8 min after I.V. injection of the analgesic the bradykinin responses were significantly reduced when compared to a control series in which, instead of ASA, an identical volume of Tyrode solution was given intravenously. The ASA effect was maximal 18 min after I.V. injection of the analgesic; it clearly outlasted the observation period of 33 min after ASA administration.

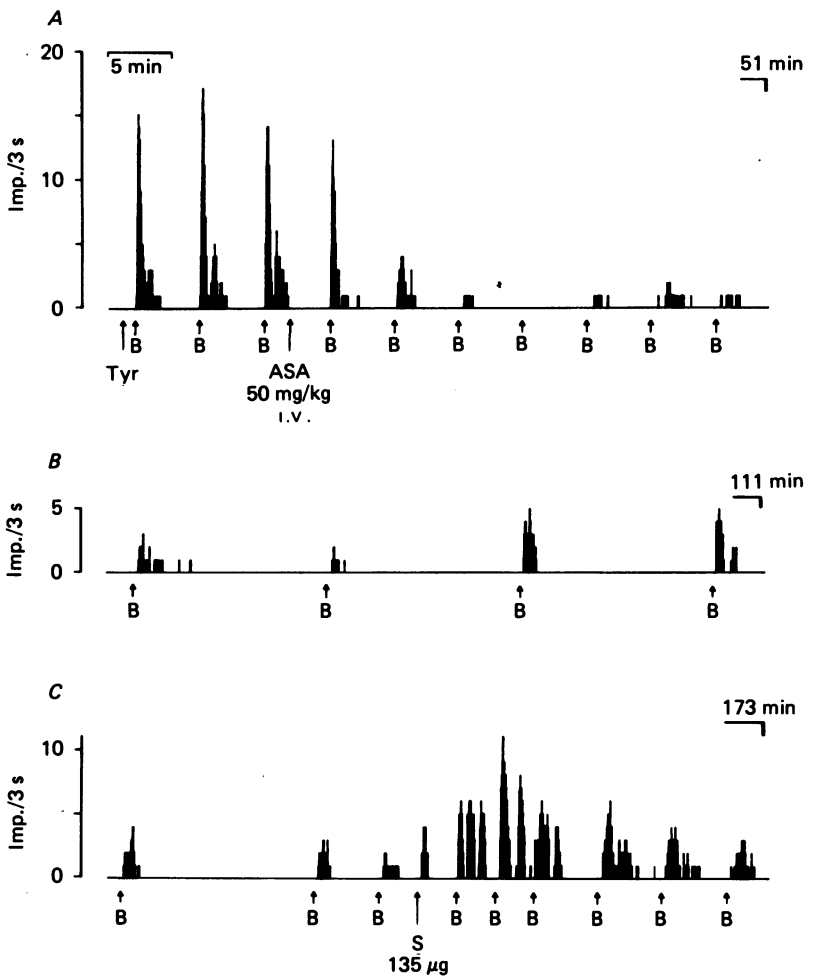

Fig. 4. Reduction of bradykinin-induced activations by ASA. Bradykinin was administered intra-arterially, ASA intravenously. Group IV receptor not excited by the mechanical stimuli used, c.v. 2·05 m/s. Arrow heads indicate injections of the following solutions: Tyrode (Tyr) I.A. as a control, bradykinin 130 µg (B) I.A. as the test stimulus (the high dosage was chosen because for this unit the standard dose of 26 µg was just above threshold), ASA I.V. and 5-HT (S, serotonin) I.A. Bin width of histograms: 3 s. The rectangular brackets above each histogram mark the time from the beginning of panel *A*.

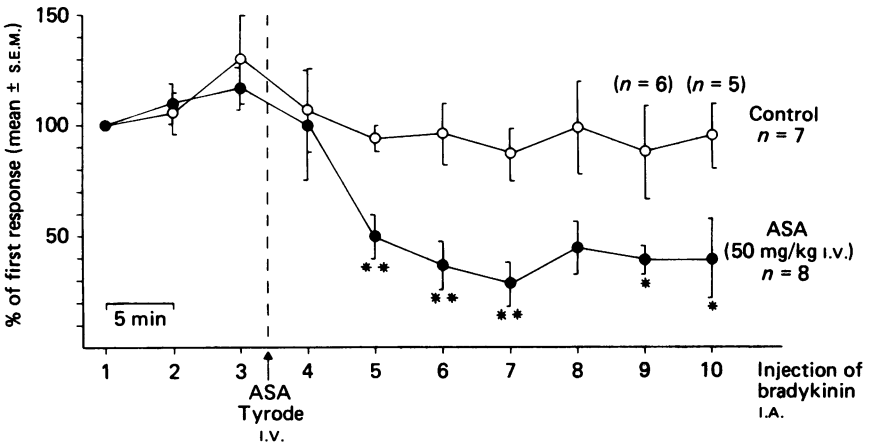

Fig. 5. Quantitative evaluation of injection series as shown in Fig. 4*A*. Ordinate: mean response magnitude (impulses per response minus background activity) in percent of first response. Abscissa: number of injections given at intervals of 5 min. Two minutes after the third injection of bradykinin (mostly 26 μg) ASA (50 mg/kg body weight) was administered intravenously. In the control series instead of ASA an identical volume of Tyrode solution was injected. The statistical significance of differences between the test (●) and control (○) series was calculated using the U test of Wilcoxon, Mann and Whitney. Asterisks indicate level of significance: *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$ (two-tailed). The test sample consisted of six group IV and two group III, the control of four group IV and three group III receptors.

Intramuscular injection of both bradykinin and ASA

Since bradykinin is a vasoactive substance the effects of ASA could be due to a reduction of the vasodilative action of bradykinin (cf. Toda, 1977) and thus to an altered accessibility of the muscle receptors via the blood stream. In order to exclude this possibility attempts were made to reduplicate the ASA effect using the intramuscular route of drug administration. Doses of 0·15–0·6 mg ASA injected into the mechanosensitive receptive field proved to be effective in reducing the bradykinin-induced excitations of the receptors.

Fig. 6 shows the response behaviour of a group III ending which reacted with a short burst of impulses to the introduction of the needle into the receptive field; in the case of bradykinin injections it was followed by a longlasting increase in impulse activity (Fig. 6*A*). After intramuscular injection of 0·6 mg ASA the bradykinin responses were completely abolished for about 70 min. The reactions to the pin pricks were still present and served as an indication that the receptor was not damaged and the needle had hit the receptive field.

Thus, it appears that vascular factors such as the vasodilatation or increased permeability produced by bradykinin are not important for the ASA effects observed. The interference of ASA with the bradykinin responses obviously takes place at or near the receptive ending in the muscle tissue.

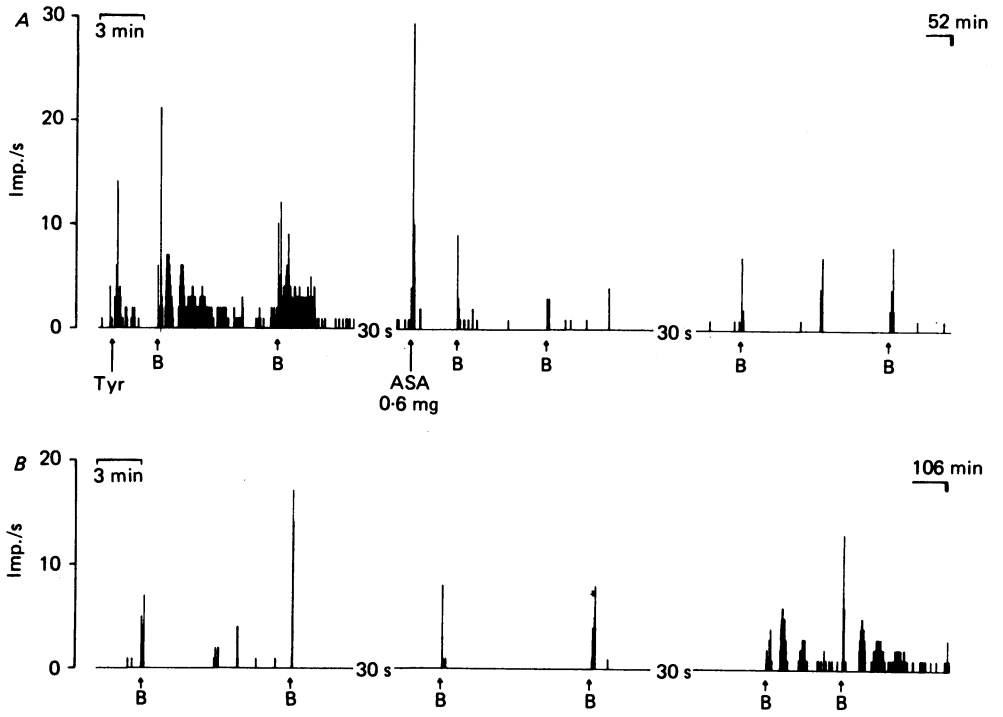

Fig. 6. Reduction of bradykinin responses by ASA. Both substances were injected intramuscularly. Group III receptor, h.t.p., c.v. 6·67 m/s. Arrow heads mark introduction of injecting needle into the receptive field of the unit and injection of Tyrode solution (Tyr), bradykinin 17·3 μg (B) or ASA. The injection volume was 0·2 ml. The rectangular brackets above the right end of the histograms indicate the time from the beginning of panel *A*. Bin width of histograms: 1 s. Note that repeated introduction of the needle into the muscle caused short bursts of impulse activity which – in the case of the bradykinin injections at the beginning of *A* and at the end of *B* – are followed by longer lasting activations.

Differential effect of ASA on bradykinin- and 5-HT-induced activations

In earlier reports from this laboratory evidence was obtained that bradykinin and 5-HT may excite muscular group IV endings via different receptor sites and/or different mechanisms of action (Hiss & Mense, 1976). Therefore, the question arose whether the activations induced by 5-HT could likewise be reduced by ASA.

The responses of group IV muscle receptors to repeated administrations of 5-HT show a marked tachyphylaxis at short injection intervals of 1–3 min (cf. Hiss & Mense, 1976); therefore, attempts were made to obtain better reproducible effects at longer intervals of 10 min. Two units were found which gave responses to repeated intra-arterial injections of 5-HT under these conditions. The one shown in Fig. 7*A* produced responses of a decreasing magnitude; the slope of the decrease appeared not to be steeper after ASA. The apparent lack of a depressing action of ASA on the 5-HT-induced activations is clearer in Fig. 7*B* in which the reactions to 5-HT are increased rather than decreased after administration of ASA. In this case the effects of 5-HT could be abolished by an injection of the serotonin antagonist methysergide (Curran, Hinterberger & Lance, 1967).

Both units of Fig. 7 gave no clear responses to I.A. injection of bradykinin. A search was made for group IV and III receptors responding in a reproducible way to both bradykinin and 5-HT in order to see whether a differential action of ASA on the effects of the two substances could also be demonstrated in one and the same unit. In one out of the three units found the excitatory effects of 5-HT were slightly enhanced

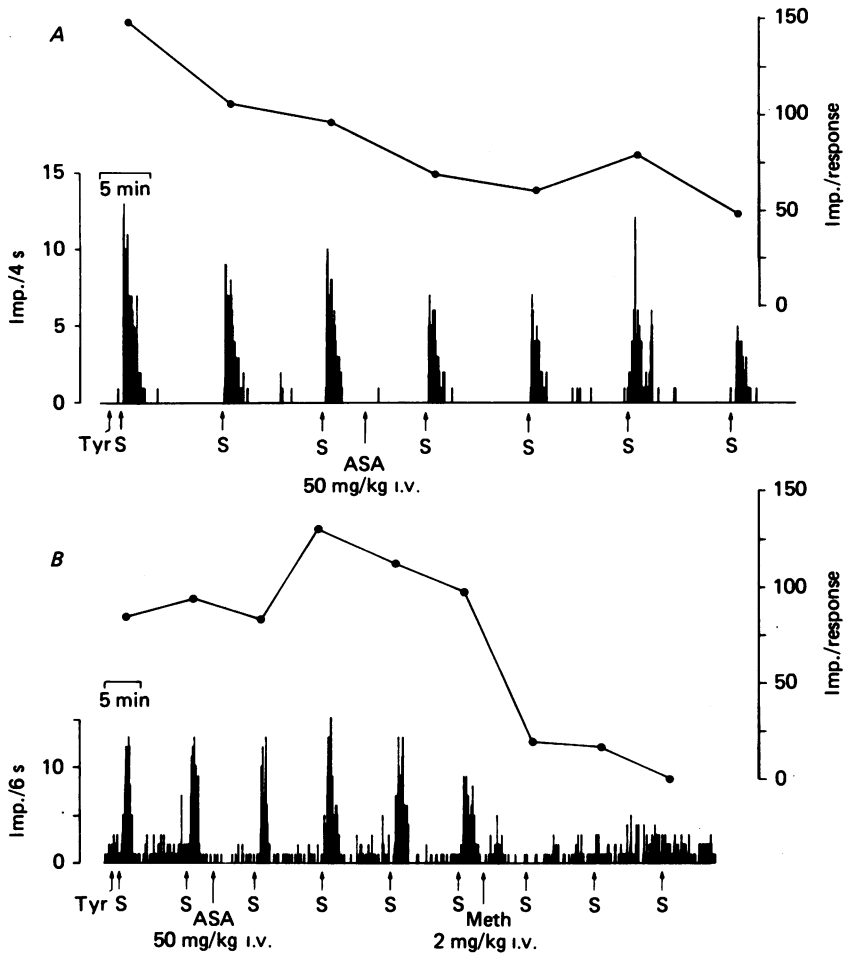

Fig. 7. Lack of effect of ASA on 5-HT-induced activations. 5-HT (S, serotonin) and Tyrode (Tyr) were injected intra-arterially, ASA and methysergide (Meth) intravenously. Above the histograms the response magnitude (impulses per response minus background activity) is plotted. *A*, group IV receptor, not excited by the mechanical stimuli used, c.v. 0·85 m/s. Bin width of histogram: 4 s. *B*, group III receptor, mechanical sensitivity not tested, c.v. 2·50 m/s. Bin width of histogram: 6 s.

after ASA 50 mg/kg I.V. whereas those of bradykinin were reduced for a period of about 100 min. Of the other two units tested with alternate injections of bradykinin and 5-HT, one also showed slightly increased 5-HT effects after ASA, and in the other one no influence of ASA on the chemically induced excitations could be detected. Thus, of five units (three group III and two group IV) responding repeatedly to 5-HT, none showed a reduction of the response magnitude after administration of ASA.

DISCUSSION

The results show that the excitatory action of the algesic substance bradykinin on group III and IV muscle receptors can be depressed by systemic administration of ASA in doses as used in human medicine (Gilman, Goodman & Gilman, 1980). When the analgesic was applied locally (I.A. or I.M.), small amounts in the milligram range proved to be effective. As most of the receptors – in addition to responding to bradykinin – had a high mechanical threshold, they probably were nociceptors mediating painful sensations originating in the muscle tissue. The reduction of their bradykinin-induced activation by ASA is considered to reflect the depression of nociceptive information by an analgesic. Since the recordings were obtained from primary afferent units the results constitute direct evidence for a peripheral site of action of the analgesic ASA.

The time course of the ASA action on bradykinin-induced activations of muscle receptors is similar to that of the pain reduction in human beings, in whom the influence of ASA on painful sensations after intra-arterial (Coffman, 1966) or intra-peritoneal (Lim *et al.* 1967) injection of bradykinin was tested. Pseudaffective responses to bradykinin in animals were likewise abolished by ASA 50–100 mg/kg I.V. for periods of about 30 min (Guzman *et al.* 1964). As to the mechanism of action of ASA it is probable that the analgesic does not interfere with bradykinin directly but acts on a secondary process which is started by the injection of the peptide.

More and more evidence has now accumulated that bradykinin releases PG precursors (Damas & Deby, 1974), PGs themselves (McGiff, Terragno, Malik & Lonigro, 1972; Terragno, Crowshaw, Terragno & McGiff, 1975) and thromboxane A2 (Rossoni, Omini, Vigano, Mandelli, Folco & Berti, 1980). At least some of the actions of the peptide (e.g. those on smooth muscles) are mediated by PGs (Barabé, Park & Regoli, 1975; Needleman, Key, Denny, Isakson & Marshall, 1975; Wong, Terragno, Terragno & McGiff, 1977). A probable mechanism for the PG release by bradykinin is the activation of phospholipase, an enzyme which starts the synthesis of PGs from arachidonic acid (Damas & Deby, 1976; Juan, 1977).

The release of PGs could be of importance for the effects of bradykinin as an algesic agent, since it has been shown that PGs enhance bradykinin-induced pain in humans (Ferreira, 1972), pseudaffective responses in animals (Moncada, Ferreira & Vane, 1975) and the reflexly produced fall in blood pressure in the isolated ear preparation (Lembeck, Popper & Juan, 1976). At the level of single receptors, our own experiments have shown that PGs increase the excitatory action of bradykinin on thin-fibre muscle receptors (Mense, 1981). This action can be interpreted as a sensitization of presumable nociceptors by PGs to bradykinin (Ferreira, 1972).

Reduction of the PG synthesis therefore could relieve bradykinin-induced pain by removing the sensitization of nociceptors. Such a mechanism has been made likely as the basis of the analgesic action of ASA and ASA-like drugs (Vane, 1971; Ferreira, 1972; Ferreira, Moncada & Vane, 1973; Moncada *et al.* 1975). The bulk of the existing evidence indicates that only the synthesis of PGs but not the effects of preformed PGs are blocked by anti-inflammatory drugs (cf. Collier & Schneider, 1972; Messina, Weiner & Kaley, 1975). Yet there are also data which support the assumption that ASA (and indomethacin) block the action of PGs on their receptors (Lembeck & Juan, 1974; Tolman & Partridge, 1975).

In the present study the background activity, which was present in most of the units, was usually not influenced by ASA. This finding is consistent with that of King *et al.* (1976) who did not observe an effect of indomethacin on the background discharge of heat-sensitive units. As the ASA doses applied in this study were probably sufficient to block the PG synthesis, this would mean that formation of PGs is not an important factor for the production of background activity. The two units whose resting discharge could be depressed by ASA had an exceptionally high discharge frequency. It is possible that in these two cases the impulse activity indeed signalled tissue damage with concomitant release of PGs. As has been shown in a recent publication (Mense, 1981), PGs can sensitize muscular group IV endings, a process which may lead to background activity.

ASA possesses many different actions in a living organism so that other mechanisms are possible, too. Among these is an interference with the release of 5-HT. Similar to PGs, 5-HT has been shown to enhance several effects of bradykinin such as vascular pain in humans (Sicuteri, Fanciullacci, Franchi & Del Bianco, 1965; Sicuteri, 1968), vasoconstriction (Starr & West, 1966) and activation of thin-fibre muscle receptors (Mense, 1981). A decrease in the tissue level of 5-HT resulting in a reduced sensitivity of the receptors could be brought about by ASA by inhibition of platelet aggregation (Krane, 1972) possibly via blocking of the thromboxane synthesis (Whiting, Salata & Bailey, 1980). As can be seen from the results obtained with injection of 5-HT, the actions of preformed 5-HT on the receptors appear not to be influenced by ASA.

The finding that ASA reduces the effects of bradykinin but not those of 5-HT is consistent with data on the bronchoconstrictive action of these endogenous substances (Collier & Shorley, 1960); it supports the hypothesis that bradykinin and 5-HT excite thin-fibre muscle receptors via different modes of action or receptor sites (Hiss & Mense, 1976).

A further possible mechanism for the ASA effects observed has been described by Hellekant & Gopal (1975). They assumed that the ASA-induced uncoupling of the oxidative phosphorylation could reduce the metabolism and excitability of receptive nerve endings. This can lead to a conduction block in mammalian nerve fibres at high concentrations of ASA and ASA-like drugs (Schorderet & Straub, 1971; Riccioppo Neto, 1980). For the experiments with systemic administrations of 50 mg ASA/kg such a mechanism appears to be unlikely, yet after local injections (I.A., I.M.) higher concentrations were probably reached at the receptor site. As the background activity of the afferent units was usually not depressed by local administration of the drug, a blocking action of the ASA doses used in the present experiments is questionable.

Since bradykinin and 5-HT are vasoactive substances, and ASA (and indomethacin) have been reported to interfere with this action (Starr & West, 1966; Messina *et al.* 1975; Toda, 1977) a possible interpretation of the results of this study would be that ASA reduces the chemically induced changes in vascular tone which in turn excite the muscle receptors. Besides the fact that other authors did not observe such an action of ASA if given in doses as used in the present study (cf. Coffman, 1966), this assumption appears to be unlikely as most of the receptors studied had a high mechanical threshold and therefore were probably insensitive to changes in the vascular tone: twenty units were h.t.p., eleven l.t.p., two did not react to the

mechanical stimuli used and five were not tested for mechanical sensitivity (cf. Franz & Mense, 1975).

Likewise, the removal of the bradykinin-induced vasodilatation by ASA resulting in an impaired accessibility of the receptors via the blood vessels has to be considered an improbable explanation for the ASA effects because of the results obtained with intramuscular injections of both the analgesic and bradykinin.

Therefore, the most likely interpretation of the present data appears to be that bradykinin activates thin-fibre receptors and simultaneously releases PGs which sensitize the afferent units. ASA could block the bradykinin-activated synthesis of PGs and thus remove the sensitization of the bradykinin-sensitive endings. Such an action could account for the pain relief by ASA in inflammatory states which are associated with release of bradykinin, or other disturbances (e.g. headache) in which changes of the chemical composition of the tissue are considered to be of importance.

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REFERENCES

BARABÉ, J., PARK, W. K. & REGOLI, D. (1975). Application of drug receptor theories to the analysis of the myotropic effects of bradykinin. *Can. J. Physiol. Pharmac.* **53**, 345–353.

BROCKLEHURST, W. E. (1971). Role of kinins and prostaglandins in inflammation. *Proc. R. Soc. Med.* **64**, 4–6.

BURCH, G. E. & DEPASQUALE, N. P. (1962). Bradykinin, digital blood flow, and the arteriovenous anastomoses. *Circulation Res.* **10**, 105–115.

COFFMAN, J. D. (1966). The effect of aspirin on pain and hand blood flow responses to intra-arterial injection of bradykinin in man. *Clin. Pharmac. Ther.* **7**, 26–37.

COLLIER, H. O. J., HAMMOND, A. R., HORWOOD-BARRETT, S. & SCHNEIDER, C. (1964). Rapid induction by acetylcholine, bradykinin and potassium of a nociceptive response in mice and its selective antagonism by aspirin. *Nature, Lond.* **204**, 1316–1318.

COLLIER, H. O. J. & SCHNEIDER, C. (1972). Nociceptive response to prostaglandins and analgesic actions of aspirin and morphine. *Nature, New Biol.* **236**, 141–143.

COLLIER, H. O. J. & SHORLEY, P. G. (1960). Analgesic antipyretic drugs as antagonists of bradykinin. *Brit. J. Pharmac.* **15**, 601–610.

CURRAN, D. A., HINTERBERGER, H. & LANCE, J. W. (1967). Methysergide. *Res. & clin. Stud. Headache* **1**, 74–122.

DAMAS, J. & DEBY, C. (1974). Libération de prostaglandines par la bradykinine chez le rat. *C.r. Séanc. Soc. Biol.* **168**, 375–378.

DAMAS, J. & DEBY, C. (1976). Sur la libération des prostaglandines et de leur précurseurs, par la bradykinine. *Arch. int. Physiol. Biochim.* **84**, 293–304.

EMELE, J. F. & SHANAMAN, J. (1963). Bradykinin writhing: a method for measuring analgesia. *Proc. Soc. exp. Biol. Med.* **114**, 680–682.

FERREIRA, S. H. (1972). Prostaglandins, aspirin-like drugs and analgesia. *Nature, New Biol.* **240**, 200–203.

FERREIRA, S. H., MONCADA, S. & VANE, J. R. (1973). Prostaglandins and the mechanism of analgesia produced by aspirin-like drugs. *Br. J. Pharmac.* **49**, 86–97.

FRANZ, M. & MENSE, S. (1975). Muscle receptors with group IV afferent fibres responding to application of bradykinin. *Brain Res.* **92**, 369–383.

GILMAN, A. G., GOODMAN, L. S. & GILMAN, A. (1980). *The Pharmacological Basis of Therapeutics*, pp. 682–698. New York: Macmillan.

GUZMAN, F., BRAUN, C., LIM, R. K. S., POTTER, G. D. & RODGERS, D. W. (1964). Narcotic and non-narcotic analgesics which block visceral pain evoked by intra-arterial injection of bradykinin and other algesic agents. *Arch int. Pharmacodyn. Thér.* **149**, 571–588.

HELLEKANT, G. & GOPAL, V. (1975). Depression of taste responses by local or intravascular administration of salicylates in the rat. *Acta physiol. scand.* **95**, 286–292.

HISS, E. & MENSE, S. (1976). Evidence for the existence of different receptor sites for algesic agents at the endings of muscular group IV afferent units, *Pflügers Arch.* **362**, 141–146.

JUAN, H. (1977). Mechanism of action of bradykinin-induced release of prostaglandin E. *Naunyn-Schmiedebergs Arch. Pharmac.* **300**, 77–85.

KING, J. S., GALLANT, P., MYERSON, V. & PERL, E. R. (1976). The effects of anti-inflammatory agents on the responses and the sensitization of unmyelinated (C) fiber polymodal nociceptors. In *Wenner-Gren Int. Symp. Series*, vol. 27, ed. ZOTTERMAN, Y., pp. 441–461. Oxford: Pergamon Press.

KRANE, S. M. (1972). Action of salicylates. *New Engl. J. Med.* **286**, 317–318.

KUMAZAWA, T. & MIZUMURA, K. (1980). Chemical responses of polymodal receptors of the scrotal contents in dogs. *J. Physiol.* **299**, 219–231.

LEMBECK, F. & JUAN, H. (1974). Interaction of prostaglandins and indomethacin with algesic substances. *Naunyn-Schmiedebergs Arch. Pharmac.* **285**, 301–313.

LEMBECK, F., POPPER, H. & JUAN, H. (1976). Release of prostaglandins by bradykinin as an intrinsic mechanism of its algesic effect. *Naunyn-Schmiedebergs Arch. Pharmac.* **294**, 69–73.

LIM, R. K. S., GUZMAN, F., RODGERS, D. W., GOTO, K., BRAUN, C., DICKERSON, G. D. & ENGLE, R. J. (1964). Site of action of narcotic and non-narcotic analgesics determined by blocking bradykinin-evoked visceral pain. *Arch int. Pharmacodyn. Thér.* **152**, 25–58.

LIM, R. K. S., MILLER, D. G., GUZMAN, F., RODGERS, D. W., ROGERS, R. W., WANG, S. K., CHAO, P. Y. & SHIH, T. Y. (1967). Pain and analgesia evaluated by the intraperitoneal bradykinin-evoked pain method in man. *Clin. Pharmac. Ther.* **8**, 521–542.

MCGIFF, J. C., TERRAGNO, N. A., MALIK, K. U. & LONIGRO, A. J. (1972). Release of a prostaglandin E-like substance from canine kidney by bradykinin. Comparison with eledoisin. *Circulation Res.* **31**, 36–43.

MENSE, S. (1976). Reduction of the bradykinin-induced activation of muscular nociceptors by acetylsalicylic acid. *Pflügers Arch.* **362**, R 32.

MENSE, S. (1977). Nervous outflow from skeletal muscle following chemical noxious stimulation. *J. Physiol.* **267**, 75–88.

MENSE, S. (1981). Sensitization of group IV muscle receptors to bradykinin by 5-hydroxytryptamine and prostaglandin E_2. *Brain Res.* **225**, 95–105.

MENSE, S. & SCHMIDT, R. F. (1977). Muscle pain: which receptors are responsible for the transmission of noxious stimuli? In *Physiological Aspects of Clinical Neurology*, ed. ROSE, F. C., pp. 265–278. Oxford, London, Edinburgh, Melbourne: Blackwell Scientific Publications.

MESSINA, E. J., WEINER, R. & KALEY, G. (1975). Inhibition of bradykinin vasodilation and potentiation of norepinephrine and angiotensin vasoconstriction by inhibitors of prostaglandin synthesis in skeletal muscle of the rat. *Circulation Res.* **37**, 430–437.

MONCADA, S., FERREIRA, S. H. & VANE, J. R. (1975). Inhibition of prostaglandin biosynthesis as the mechanism of analgesia of aspirin-like drugs in the dog knee joint. *Eur. J. Pharmac.* **31**, 250–260.

NEEDLEMAN, P., KEY, S. L., DENNY, S. E., ISAKSON, P. C. & MARSHALL, G. R. (1975). Mechanism and modification of bradykinin-induced coronary vasodilation. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2060–2063.

PERL, E. R., KUMAZAWA, T., LYNN, B. & KENINS, P. (1976). Sensitization of high threshold receptors with unmyelinated (C) afferent fibers. In *Progress in Brain Research*, vol. 43, ed. IGGO, A. & ILYINSKY, O. B., pp. 263–276. Amsterdam: Elsevier Scientific Publishing Company.

RICCIOPPO NETO, F. (1980). Further studies on the actions of salicylates on nerve membranes. *Eur. J. Pharmac.* **68**, 155–162.

ROCHA E SILVA, M. (1964). Chemical mediators of the acute inflammatory reaction. *Ann. N.Y. Acad. Sci.* **116**, 899–911.

ROCHA E SILVA, M. & ROSENTHAL, S. R. (1961). Release of pharmacologically active substances from the rat skin *in vivo* following thermal injury. *J. Pharmac. exp. Ther.* **132**, 110–116.

ROSSONI, G., OMINI, C., VIGANO, R., MANDELLI, V., FOLCO, G. C. & BERTI, F. (1980). Bronchoconstriction by histamine and bradykinin in guinea pigs: relationship to thromboxane A2 generation and the effect of aspirin. *Prostaglandins* **20**, 547–557.

SCHORDERET, M. & STRAUB, R. W. (1971). Effects of non-narcotic analgesics and nonsteroid anti-inflammatory agents upon inorganic phosphates, intracellular potassium and impulse conduction in mammalian nerve fibers. *Biochem. Pharmac.* **20**, 1355–1361.

SICUTERI, F. (1968). Sensitization of nociceptors by 5-hydroxytryptamine in man. In *Pharmacology of Pain*, ed. LIM, R. K. S., ARMSTRONG, D. & PARDO, E. G., pp. 57–86. Oxford: Pergamon Press.

SICUTERI, F., FANCIULLACCI, M., FRANCHI, G. & DEL BIANCO, P. L. (1965). Serotonin-bradykinin potentiation on the pain receptors in man. *Life Sci., Oxford* **4**, 309–316.

STARR, M. S. & WEST, G. B. (1966). The effect of bradykinin and anti-inflammatory agents on isolated arteries. *J. Pharm. Pharmac.* **18**, 838–841.

TAIRA, N., NAKAYAMA, K. & HASHIMOTO, K. (1968). Vocalization response of puppies to intra-arterial administration of bradykinin and other algesic agents, and mode of actions of blocking agents. *Tohoku J. exp. Med.* **96**, 365–377.

TERRAGNO, D. A., CROWSHAW, K., TERRAGNO, N. A. & MCGIFF, J. C. (1975). Prostaglandin synthesis by bovine mesenteric arteries and veins. *Circulation Res.* **36** and **37** (Suppl. I), I-76–I-80.

TODA, N. (1977). Actions of bradykinin on isolated cerebral and peripheral arteries. *Am. J. Physiol.* **232**, H267–274.

TOLMAN, E. L. & PARTRIDGE, R. (1975). Multiple sites of interaction between prostaglandins and non-steroidal anti-inflammatory agents. *Prostaglandins* **9**, 349–359.

UCHIDA, Y. & MURAO, S. (1974*a*). Potassium-induced excitation of afferent cardiac sympathetic nerve fibers. *Am. J. Physiol.* **226**, 603–607.

UCHIDA, Y. & MURAO, S. (1974*b*). Bradykinin-induced excitation of afferent cardiac sympathetic nerve fibers. *Jap. Heart J.* **15**, 84–91.

VANE, J. R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biol.* **231**, 232–235.

WHITING, J., SALATA, K. & BAILEY, J. M. (1980). Aspirin: an unexpected side effect on prostacyclin synthesis in cultured vascular smooth muscle cells. *Science, N.Y.* **210**, 663–665.

WONG, P. Y.-K., TERRAGNO, D. A., TERRAGNO, N. A. & MCGIFF, J. C. (1977). Dual effects of bradykinin on prostaglandin metabolism: relationship to the dissimilar vascular actions of kinins. *Prostaglandins* **13**, 1113–1125.