Br. J. Pharmac. (1973), **49**, 192–204.

A study of compounds which initiate and block nerve impulses in the perfused rabbit liver

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Summary

1. A technique for the perfusion of the rabbit liver with Krebs solution is described: the hepatic nerves were monitored for centripetal action potentials. Spontaneous afferent activity, and a good response to stimulating agents persisted for 7 h or longer.

2. Action potentials were elicited by injections or infusions of acetylcholine, 5-hydroxytryptamine, bradykinin, phenyldiguanide, adrenaline and various other compounds. From comparison with experiments carried out *in vivo* by other authors, it would appear that most of the nerves stimulated were afferent.

3. Inhibition of action potentials, either those occurring spontaneously or those elicited by injections, was produced by aspirin, paracetamol, mepyramine, chlorpheniramine, procaine and lignocaine but not by morphine or atropine. Pentolinium produced some inhibition, as did hexamethonium in concentrations of about 7·5 μg/ml.

4. Three methods for using the preparation for the assessment of local anaesthetic action are described.

5. Lobeline, potassium cyanide and 2,4-dinitrophenol stimulated nerve bundles which were not stimulated by anoxia or hypoxia.

Introduction

In the study reported here, various substances were injected into the portal vein while the hepatic nerves were monitored for centripetal action potentials. The object of the experiments was to obtain some indication of how many different types of nerve fibre are present in the rabbit liver and also to try to determine whether the elicited action potentials were in afferent nerves or whether they were antidromic and originated from stimulation of motor nerve terminals. Although the work was begun as an investigation of hepatic nerves, it soon became apparent that the perfused liver is a useful preparation for the study of actions and interactions of various substances on afferent nerve endings, and can also be used to test whether a compound possesses a local anaesthetic action. The innervation of the liver is profuse (Sutherland, 1964) and several different types of nerve receptors have been described on anatomical (Tsai, 1958) and physiological evidence (Andrews & Palmer, 1967; Niijima, 1969a, b). The organ may be perfused for long periods at low pressures, and does not readily become oedematous. Many of the hepatic nerves are long, accessible and easily split into small bundles. The environment of a perfused organ may be controlled within small limits and, without recirculation of perfusion fluid, there is no accumulation of injected substances. There are,

however, technical difficulties in keeping the preparation stable and the method is therefore given in detail.

Methods

Apparatus

The liver was perfused with Krebs solution via the portal vein. In earlier experiments the flow was gravitational from a reservoir placed about 1 m above the table and the perfusion pressure reduced by means of a screw clamp. Later experiments were carried out with a constant-flow pump placed outside the Faraday cage. A Watson-Marlow 'Flow-inducer' was used and the pulsatile flow was damped with an air cushion. The perfusion fluid passed through a heating coil situated close to the liver, a perspex bubble-trap, which also incorporated a manometer for measuring portal pressure, and a small chamber for injections. There was an arrangement of Y-pieces and tubing placed between the inflow and outflow cannulae so that the direction of flow through the liver could be reversed. Apart from the reversals of flow, the system could be kept constant and on several occasions a single nerve bundle was studied unchanged over a period of 60–80 min without apparent alteration in sensitivity or response to administered substances. The portal pressure was usually about 60 mm saline (0·9% w/v NaCl solution), and a rise of 20–30 mm was taken as evidence that the portal venous flow was being obstructed by bubbles. The temperature of the preparation was maintained constant in any one perfusion, usually at 37° C.

Experiments were carried out in a Faraday cage. Wick electrodes were used and a fine, stainless steel wire was placed in the centre of the wick to reduce impedance. The amplification and monitoring systems were conventional and action potentials were recorded on magnetic tape.

Operative procedures

The operation and perfusion were carried out in a shallow perspex tray so that transudate or leaking perfusate could be drained away. In a few experiments, the rabbit was killed by a blow on the back of the neck but most of the experiments were begun under ether anaesthesia after ethyl chloride induction, the animal being allowed to die once the portal vein had been cannulated. It was considered that volatile anaesthetic agents would be unlikely to have persistent effects on nerves; no difference was detected between the experiments in which anaesthetics were used and those in which the animal was killed by trauma.

The abdomen was opened with a mid-line incision, a polythene cannula inserted into the portal vein and perfusion begun with a slow flow of Krebs solution at about 17° C. The thorax was opened immediately afterwards, the right atrium incised and the thoracic vena cava cannulated through this incision. The flow was now increased to (1·5 ml/min)/g wet weight of liver tissue and the temperature raised to 37° C. The duodenum was divided about 80 mm below the pylorus and the upper part allowed to drain outside the abdominal cavity in order to prevent bile from coming into contact with the nerves. The remaining intestines were removed. A ligature was tied around the antral part of the stomach and the part of the stomach between this ligature and the oesophagus was also removed. The abdominal vena cava was ligated immediately caudal to the liver. The

hepatic nerves were traced from the liver to the coeliac ganglion where they and the accompanying artery were divided and mobilized. In many experiments most of the rib cage was removed and the liver laid flat so that the whole of the hilum of the liver became readily accessible; cotton wool pads placed in the thorax prevented the thoracic vena cava from being obstructed. In some experiments the base of the omental lobe (Cameron & Mayes, 1930) was ligated and the lobe removed to increase the exposure of the hilum. The liver was covered with polythene sheeting and then a layer of cotton wool to keep it at a constant temperature and the experiment started. It was found that, in spite of the presence of a bubble trap, small bubbles of gas collected within the inflow cannula and the portal venous tree, restricting inflow to some parts of the liver and sometimes giving rise to action potentials. It proved virtually impossible to prevent the formation of these bubbles but it was found that if the flow was reversed periodically, and the liver gently massaged, the bubbles could be flushed out.

Administration of test substances was made in two different ways. In the first, referred to in the text as ' bolus injections ', the substance under test was injected rapidly (0·1 ml in 0·3 s) into the special injection chamber. The second method of administration was by dissolving the substance in Krebs solution which was perfused at the same rate of flow, pH etc. as the control fluid. Bolus injections were compared to control injections of Krebs fluid or physiological saline.

Nerve bundles of various sizes were examined. The nerve was divided and the distal, i.e. the hepatic portion, tested. If possible, the sheath was removed and the nerve, if large, was placed in a pool of saline on a platform of black perspex and teased into small bundles with needles. Small bundles, such as those dissected from the wall of the portal vein in the hilum, were used whole.

Solutions

The composition of the Krebs solution was, (mM): NaCl, 118·5; $NaHCO_3$, 24·9; KCl, 4·74; KH_2PO_4, 1·18; $MgSO_4$, 1·18; $CaCl_2$, 2·54. Glucose, 5·5 mM, was usually added. In many experiments polyethyleneglycol (mol. wt. 6,000) was added as a plasma expander; in some experiments polyvinylpyrrolidone (mol.wt. 44,000) was used instead of polyethyleneglycol, but the presence or absence of these substances of large molecular weight did not affect the results obtained in this study. The perfusing solutions were gassed throughout the experiments with 95% oxygen and 5% carbon dioxide, except for brief periods in certain experiments when nitrogen was substituted for oxygen. With O_2-CO_2 mixture the pH of the fluid was $7{\cdot}4 \pm 0{\cdot}05$, but the reactions of nerves did not appear to be affected within the range of pH 7·2–7·5.

Drugs

The drugs used were acetylcholine chloride (Hopkin & Williams), adrenaline (MacArthys), γ-amino-butyric acid (B.D.H.), aspirin (Boots), atropine sulphate (B.D.H.), bradykinin (BRS 640) (Sandoz), chlorpheniramine maleate (Allen & Hanbury), L-glutamic acid (B.D.H.),hexamethonium bromide (May & Baker), histamine acid phosphate (B.D.H.), 5-hydroxytryptamine creatine sulphate (B.D.H.), lignocaine hydrochloride (Pharmaceutical Manufacturing Co.), lobeline hydrochloride (Boehringer), mepyramine maleate (May & Baker), morphine sulphate

(May & Baker), 2,4,dinitro-phenol (B.D.H.), (−)-noradrenaline acid tartrate (Bayer), paracetamol (W. B. Pharmaceuticals), L-phenyldiguanide hydrochloride (Koch-Light), polyethyleneglycol (mol.wt. 6,000) (B.D.H.), polyvinylpyrrolidone (mol.wt. 44,000) (B.D.H.), polyvinylpyrrolidone (mol. wt. 700,000) (B.D.H.), potassium cyanide (B.D.H.), procaine hydrochloride (MacArthys), suberyl-dicholine-diiodide (courtesy of Dr. Milton). Solutions were prepared freshly each day and weights are expressed in terms of the compounds used.

Results

The nerve supply to the liver is so profuse that only a small proportion of nerve bundles could be investigated in any one experiment; it is therefore not possible to estimate what percentage of the nerves studied reacted to any one substance. Some of the nerves from the coeliac ganglion ran with the hepatic nerves for a great part of their course, but turned caudally on the portal vein and terminated in duodenal or pancreatic areas. Other nerves, although entering the liver, appeared to be insensitive to injected substances, except for hypertonic NaCl solution, and it is thought that they may have been motor nerves. In order to test the viability and suitability of a nerve bundle for study, acetylcholine, 2–15 μg, was injected into the injection chamber while the bundle was monitored for action potentials. It was noted that the most reactive nerves were often fine, transparent bundles which ran in the walls of the portal vein. No attempt was made to measure the velocity of the action potentials. With the small bundles, found in the venous wall, the length of nerve available was often so short that the procedure would have been difficult; since wick electrodes could not have been employed, an investigation of the types of fibre involved would have necessitated a separate study. In the figures, scales for voltage and frequency are given in order to provide some indication of the magnitude of the responses. It is recognized, however, that the voltages of action potentials were affected greatly by electrical shorting by the sheath and fibrous tissues and that the ratemeter reading depended upon the degree of discrimination used before impulses were counted; moreover, the number of active fibres in a bundle was not known. The difficulty of deciding at what level the discrimination should be set is shown in Fig. 1 where the effect of cooling the liver on spontaneous afferent impulses is illustrated. The recorded voltage of some impulses was almost indistinguishable from background 'noise'.

In this study, as in previous similar studies (Andrews & Stratmann, 1968; Niijima, 1969a, b) action potentials occurred spontaneously throughout the duration of experiments, i.e. up to 7 hours. Under the conditions of the experiment, alteration of the intra-hepatic vascular pressures by varying portal flow or by altering the height of the tip of the hepatic venous cannula, did not produce changes of impulse frequency. Also, perfusion with Krebs solution containing 25 mg/ml of polyvinylpyrrolidone (mol.wt. 700,000) did not affect the nerve potentials; the solution is viscous but has only a negligible oncotic pressure.

Niijima (1969b) reported that, when in livers of guinea-pigs the perfusion fluid was changed from one containing glucose to one without, or *vice versa*, there was a change in the pattern of nerve impulses which he ascribed to the action of glucose receptors. This finding was not confirmed in rabbit livers perfused by our method.

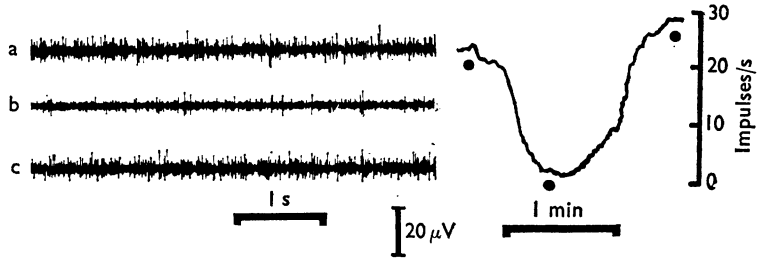

FIG. 1. Effect of cooling on spontaneous discharge in the hepatic nerve. On the left, oscilloscope records taken at (a) 37° C, (b) 18° C and (c) 37° C. On the right, a ratemeter trace; the three dots indicate the points at which the oscilloscope tracings were taken.

Acetylcholine

The sensitivity of nerves to stimulation by bolus injections of acetylcholine varied greatly. The smallest effective amount was about 1 μg but 100 μg did not stimulate all the fibres; hypertonic sodium chloride solution elicited many more action potentials, a large proportion of which had a greater voltage than could be produced by acetylcholine. Although injections of large amounts of acetylcholine and hypertonic saline did not appear to damage the preparation, they were not used in routine experiments. The response to bolus injections of acetylcholine was usually a single burst of firing lasting from 0·1 to about 5 s, according to the amount given, the rate of perfusion and the nature of the nerve bundle. Sometimes the firing was followed by a second, more prolonged burst of activity. It was originally thought that the second burst was due to stimulation of nerves around the hepatic venous tree but, when the direction of perfusion was reversed, 0·5 to 1·0 mg of acetylcholine was required to produce action potentials (Figure 2). A single injection of acetylcholine frequently induced activity in a nerve which had previously been quiescent, and atropine, given either as a single rapid injection of 100 μg or as an infusion of 1·0 μg/ml, inhibited the secondary burst of firing, the induced activity; the first burst of firing remained unchanged.

When injections of acetylcholine were repeated at regular intervals of 20, 30 or 60 s, there was a progressive increase in the nervous response for the first 3–6 injections, after which the response remained constant for 15 min or longer (Figure 3). The response to such spaced injections was unaffected by atropine, but was diminished by pentolinium given as a bolus injection of 0·5 mg or as an infusion of 1·0 μg/ml Krebs solution. The effect of hexamethonium on these

FIG. 2. Effect of the direction of perfusion flow on the response of nerve terminals to injection (●) of acetylcholine and phenyldiguanide. A1, flow forward, 5 μg acetylcholine; A2, flow reversed, 35 μg acetylcholine; B1, flow forward, 5 μg phenyldiguanide; B2, flow reversed, 20 μg phenyldiguanide; there is some spontaneous nervous activity.

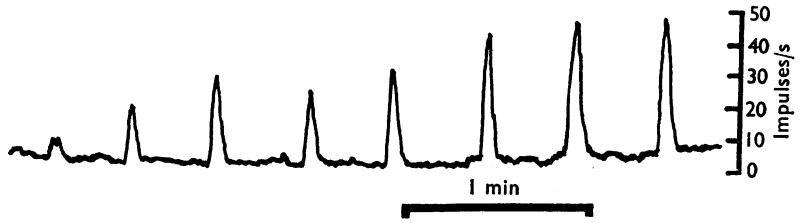

FIG. 3. Effects of repeated injections of acetylcholine. Ratemeter record. Injections of 25 μg were given every 30 seconds. The response was constant after the sixth injection. In this preparation the progressive increase in the responses was unusually great.

spaced injections depended greatly on the concentration at which it was administered. At 1·0 μg/ml it was without effect, but at 7·5 μg/ml most impulses were blocked and at 15 μg/ml all impulses due to injection of acetylcholine were inhibited. The blocking action is complex: acetylcholine was shown to enhance the effect of spaced phenyldiguanide injections, and this enhancement was not affected by 15 μg/ml of hexamethonium. Therefore, although sufficient hexamethonium was present to inhibit stimulation of action potentials by acetylcholine, the response to phenyldiguanide was greatly increased if the acetylcholine was injected shortly beforehand. Subliminal amounts of acetylcholine also increased the response to injections of 5-hydroxytryptamine.

When given as a continuous infusion, acetylcholine stimulated most nerve bundles at a concentration of 0·1 μg/ml. The infusion could be continued over a period of minutes without the occurrence of tachyphylaxis and the elicited action potentials were depressed by local anaesthetics and the antihistaminics tested. Every nerve bundle which responded to one of the pharmacologically active substances used in this study, also responded to acetylcholine.

5-Hydroxytryptamine

Injections of 0·1–0·5 μg of 5-hydroxytryptamine elicited action potentials in less than a quarter of the bundles stimulated by acetylcholine. Tachyphylaxis to further injections of 5-hydroxytryptamine but not other drugs was marked and the effect of a single injection lasted for up to 3 minutes. When a continued infusion of 5-hydroxytryptamine was given, there was an initial burst of firing, lasting for 3–4 s, after which the response almost ceased and the nerve became refractory to further injections of 5-hydroxytryptamine but sensitivity was regained a few minutes after the infusion had been discontinued (Figure 4). Because of tachyphylaxis, it was not possible to test accurately the interaction of 5-hydroxytryptamine with other substances, but hexamethonium and atropine had no discernible effect on its action. Injection of 50 μg or more did not stimulate

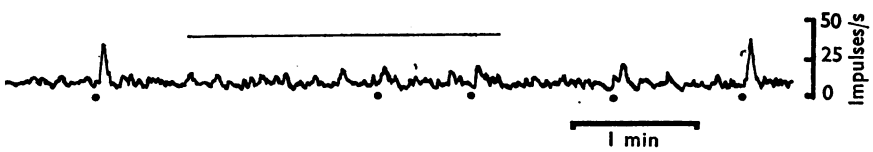

FIG. 4. Effect of 5-hydroxytryptamine. Ratemeter record. The filled circles denote bolus injections of 5 μg of 5-hydroxytryptamine and the line above the recording denotes an infusion at a concentration of 0·1 μg/ml. There was a lack of sensitivity to injections during the infusion.

nerves when the circulation was reversed, unless the liver had been perfused for several hours.

Phenyldiguanide

About one-fifth of the nerve bundles which reacted to acetylcholine also responded to injections of phenyldiguanide, 0·1–5·0 μg. Sensitive receptors were stimulated by infusions of 0·1 μg/ml (Fig. 6B), and the rate of discharge remained constant during the period of infusion, 5 min being the longest period tested. On return to control perfusion, the frequency of action potentials fell rapidly to its resting level. Phenyldiguanide was ineffective when the circulation was reversed, except when very large amounts were used. As there was no tachyphylaxis, it was a useful substance for determining the 'local anaesthetic' effect of various drugs.

Bradykinin

Injections of 1–5 μg bradykinin stimulated about one-sixth of the nerve bundles stimulated by acetylcholine. The initial response began within 2 s of injection and there was maximal activity from 12 to 35 s with some increase of activity continuing for up to 8 minutes. If, however, an injection of bradykinin had been made within the previous 20 to 30 min, there was a longer latent period of about 8 s before potentials were elicited, although a few sporadic impulses sometimes began earlier (Figure 5).

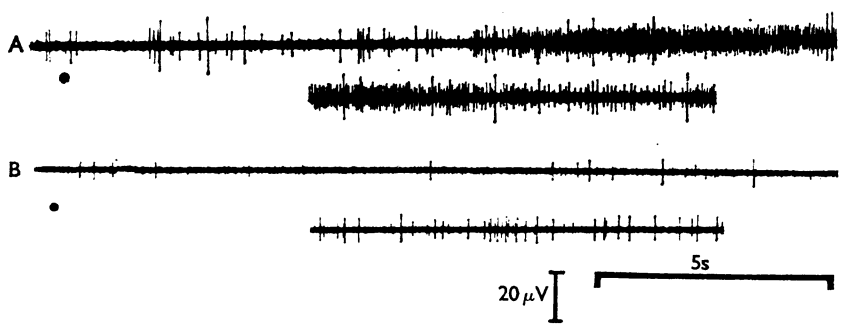

FIG. 5. Response to injections of bradykinin and histamine. Oscilloscope record. Substances injected at the filled circles. A, bradykinin, 5 μg; bradykinin had been injected 10 min previously. B, histamine, 50 μg in a different nerve bundle. There was some background activity in tracing A. The upper and lower parts of each tracing are continuous.

A continued infusion of 0·2 μg/ml produced a slow increase in the rate of nervous discharge over about 20 s, after which the activity became fairly constant. The activity caused by infusions of bradykinin was easily shown to be reduced by aspirin, paracetamol and substances with a local anaesthetic effect. Atropine did not affect the response to bradykinin.

Histamine

Histamine was injected on about 50 occasions into 8 livers. The amount necessary to elicit action potentials was 20–50 μg, and only a few fibres responded. There was a delay of 10–20 s before activity began which then continued for

about 90 seconds. There was some tachyphylaxis to repeated injections. In view of the large amounts needed, and the long latent period, it was considered that the action of histamine was unlikely to be directed on nerve endings or afferent organs, and the substance was not studied further.

Catecholamines

The effects of adrenaline and noradrenaline were studied in about 7 livers, more than 60 bundles being tested. The substances produced an increase in portal pressure, but it seemed that the vasoconstrictor action was not related to any stimulatory effect on nerves. Amounts of 0·1–2·0 μg occasionally produced a short, well-defined burst of nerve impulses, beginning within 2–3 s and lasting for 1 s or less whereas any vascular response had a much slower onset and longer duration. A second injection, given whilst the vascular reaction was at its height, produced the same nervous response. Only a small proportion of nerves responded to injections of adrenaline and noradrenaline, amounts of up to 10 μg usually being without effect.

Pharmacological stimulation of chemoreceptors

Potassium cyanide and 2,4-dinitrophenol, given separately as infusions of 50 μg/ml, elicited action potentials in a small proportion of nerve bundles, as did bolus injections of 5 μg of lobeline. The three substances appeared to activate the same nerve bundles, although the lobeline stimulated more endings than did the other two substances. On no occasion did Krebs solution, which had been equilibrated with 95% nttrogen and 5% carbon dioxide, stimulate nerve bundles, even those which had reacted to cyanide. It appeared that chemoreceptors, if present, did not function under the conditions of our experiments.

Suberyl dicholine has been used to stimulate arterial chemoreceptors (McQueen & Ungar, 1971) although it also has other actions (Milton, 1968). It stimulated the same nerve bundles as did cyanide, but the nature of the discharge differed. With injections of cyanide, 50–100 μg, and lobeline, 5 μg, the monitored response showed a rapid rise of nervous discharge with a maximum in about 2 s, and a cessation in about 6 seconds. With 5–10 μg of suberyl dicholine the maximum was not reached for 10 s or more, and the full response lasted for over 30 seconds. As an intusion, suberyl dicholine was effective at a concentration of 1·0 μg/ml, there being no evidence of tachyphylaxis. Lobeline acted on the same nerve bundles at the same concentration. After an injection of cyanide or lobeline a second injection of the same substance, if given within 3–5 min, produced a much smaller response than did the first but no such inhibition was seen with suberyl dicholine.

Local anaesthetics and antihistaminics

The effects of substances with a local anaesthetic action on nerve activity was shown in three ways. Firstly, when there was a high level of spontaneous activity, such as is shown in Fig. 1, an injection or infusion of the substance diminished the activity reversibly. In the second method, repeated injections of acetylcholine, bradykinin or phenyldiguanide were given at regular intervals and when the response was constant as measured on a ratemeter, the substance under test was

infused without interrupting the repeated injections. Thirdly, a constant infusion of acetylcholine, bradykinin or phenyldiguanide was given and when the rate of nervous discharge was constant, bolus injections of the substance under test were made.

The three methods gave similar results. No substance was considered to have an anaesthetic effect unless its effect was completely reversible.

The following substances were shown to have a local anaesthetic effect: procaine, lignocaine and chlorpheniramine, 2·0 μg/ml, and mepyramine, 10 μg/ml. These substances given as bolus injections during infusion of acetylcholine, bradykinin or phenyldiguanide (Fig. 6) were effective in the following quantities: procaine, lignocaine and chlorpheniramine, 10 μg, and mepyramine, 50 μg; γ-amino butyric acid and L-glutamic acid had no effect, even in amounts of 100 mg, or in concentrations of 10 mg/ml. Ethanol in a concentration of 1 mg/ml occasionally appeared to have a small inhibitory effect which was statistically not significant.

Analgesics

The methods of testing local anaesthetics were applied to analgesics, and it was shown that aspirin and paracetamol inhibited the production of action potentials (Figure 7). Small action potentials were more affected than large. The inhibitory effect of aspirin infusions lasted for about 4 min and that of paracetamol about 3–4 min longer. Morphine, 10 μg/ml, had no inhibitory effect; 1·0 mg, given as a bolus injection, stimulated some nerve bundles and caused some temporary

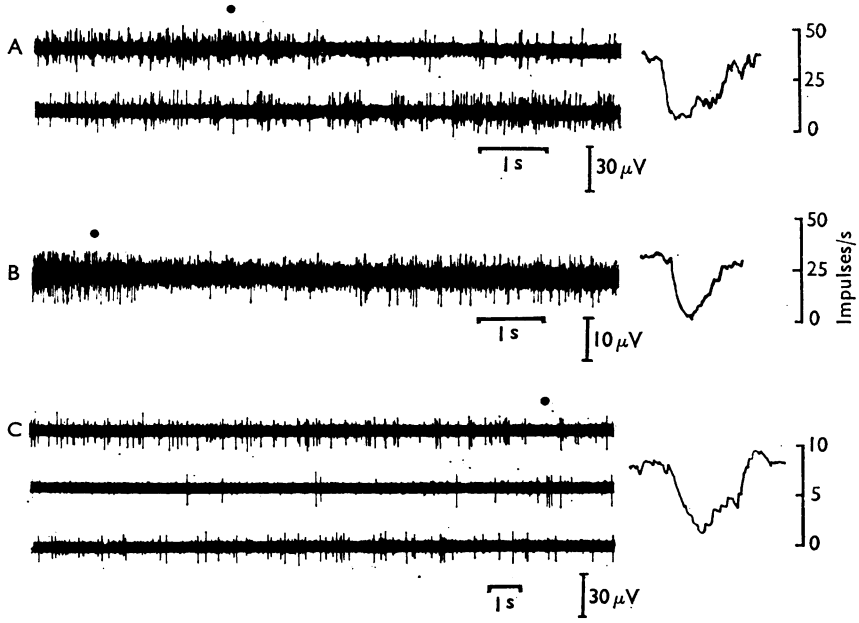

FIG. 6. Inhibition of nerve stimulation by substances with a local anaesthetic action. Oscilloscope and ratemeter recordings. A, injection of 100 μg mepyramine during infusion of acetylcholine, 0·1 μg/ml; the two records are continuous. B, injection of 25 μg chlorpheniramine during infusion of phenyldiguanide, 0·1 μg/ml. C, injection of 10 μg lignocaine during infusion of acetylcholine, 1·0 μg/ml. The three records are continuous. In each case, the ratemeter recordings cover the period of diminished nervous activity.

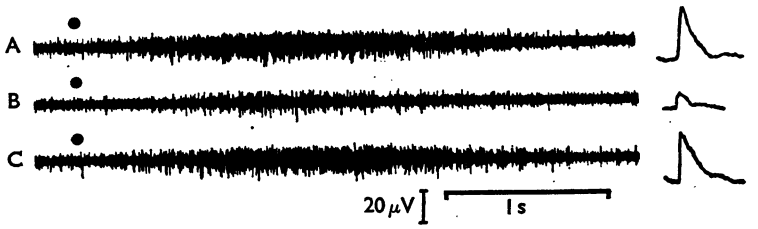

FIG. 7. The effect of aspirin on the stimulating action of acetylcholine. Oscilloscope and ratemeter records. Acetylcholine, 2·0 μg, was injected three times. A, during perfusion with control Krebs solution; B, during perfusion with Krebs solution which contained aspirin, 1 mg/ml; C, 5 min after return to control.

inhibition of acetylcholine and bradykinin responses on the few occasions that they were given.

In some experiments aspirin was added to the saline of the wick electrodes and although the nerve bundle was bathed in the fluid for 10 min or more, the aspirin did not have any effect on the responses to bradykinin and other stimulating substances.

Specificity of stimulants

It was not possible to test the reaction of every nerve bundle to all the stimulating compounds, but it was shown that some nerve bundles were stimulated by the administration of only one of the following compounds: 5-hydroxytryptamine, bradykinin, phenyldiguanide and cyanide. Bundles stimulated by catecholamines did not respond to histamine or bradykinin.

Site of stimulating effects

Preliminary experiments were carried out on anaesthetized dogs, cats and rabbits to investigate whether some of the substances used in this study could elicit action potentials in afferent fibres *in vivo*. The compound was injected into either the portal or the femoral vein, and the efferent nerves to the spleen, the kidney and the intestines were monitored. Injections into the portal vein of acetylcholine, phenyldiguanide, bradykinin, potassium cyanide and 5-hydroxytryptamine all elicited reflex discharge in these efferent nerves. This latter discharge, however, differed from the efferent discharge which resulted from injection into the femoral vein, in its time course or its nature, or both. The results would appear to indicate that these substances stimulated afferent hepatic nerves but simultaneous antidromic stimulation of motor nerves may also have taken place. These experiments will be published later in detail.

Discussion

The action of many pharmacologically active substances on afferent nerve endings has been reviewed by Paintal (1964, 1971, 1972). There is, however, almost no published information on the stimulation of motor nerve endings with consequent antidromic impulses. This problem is being investigated in our laboratory and, until definitive information is available, any conclusion reached must be regarded as tentative.

Acetylcholine does not stimulate axons directly (Brown & MacIntosh, 1939; Lorente de Nó, 1944; Hodgkin, 1947; Diamond, 1955); since none of the substances used in this study stimulated nerve bundles which were not also stimulated by acetylcholine, it is unlikely that they stimulated the axons directly. Also, aspirin, which does not act directly on axons (Lim, Guzman, Rodgers, Goto, Braun, Dickerson & Engle, 1964), inhibited stimulation by acetylcholine and bradykinin.

Apart from the preliminary experiments described above on reflex efferent discharge following pharmacological stimulation of hepatic nerves, there is firm evidence that chemical agents can induce action potentials in afferent nerves *in vivo*. This has been shown for acetylcholine (Coon & Rothman, 1940; Brown & Gray, 1948; Riker, 1958; Fjällbrant & Iggo, 1961; Lim, Guzman & Rodgers, 1961; Keele & Armstrong, 1964), for 5-hydroxytryptamine (Paintal, 1954; McCubbin, Green, Salmoiraghi & Page, 1956; Fjällbrant & Iggo, 1961; Skinner & Whelan, 1962; Keele & Armstrong, 1964), for bradykinin (Fjällbrant & Iggo, 1961; Lim *et al.*, 1961; Keele & Armstrong, 1964; Blane, 1967), for phenyldiguanide (Paintal, 1954; Karczewski & Widdicombe, 1969) and for histamine (Fjällbrant & Iggo, 1961; Keele & Armstrong, 1964; Karczewski & Widdicombe, 1969).

Cyanide has been shown by many authors to stimulate chemoreceptors, and to induce action potentials in other afferent nerves (Paintal, 1971; Andrews, Andrews & Orbach, 1972). The effect of adrenaline is less well established and may be indirect, for instance by sensitizing receptors to the action of other agents (Aars, 1971), although a direct action may exist (Paintal, 1964). In our experiments, the shortness of the latent period between injection and appearance of impulses favours a direct action, especially as the occurrence of action potentials and the changes in portal pressure appeared to be unrelated in their time courses.

It is not certain whether motor fibres are excited by the chemical agents used in our study. Acetylcholine probably induced some antidromic impulses, as has been described by Ferry (1963) although most efferent fibres may be insensitive (Diamond, 1959). In the perfused liver, acetylcholine appeared to stimulate a large number of different fibres but not as many as hypertonic saline. High concentrations of phenyldiguanide, or of other stimulant compounds, also elicited impulses in a variety of fibres but, with the exception of acetylcholine, each substance appeared to stimulate only a single population of fibres. It would seem, therefore, that if efferent fibres also responded to chemical stimulation, their responses were similar and possibly identical to those of efferent fibres.

Many of the data presented in this study are in accordance with experiments carried out *in vivo*, although mostly on organs other than the liver. For instance γ-aminobutyric acid has no inhibitory action in the periphery and the action of histamine has a long latent period both *in vitro* and *in vivo* (Keele & Armstrong, 1964). Some other findings were at variance, such as the long period over which bradykinin was active; this difference may be due to the use of an artificial perfusing medium. Although cyanide in low concentrations is usually considered to be a selective stimulator of chemoreceptors, it appears that it may also stimulate other nerve terminals *in vivo* (Paintal, 1972; Andrews *et al.*, 1972).

In conclusion, the perfused liver appears to be a useful preparation for the study of the pharmacological responses of nerve endings of afferent fibres.

Although the primary purpose of the study was not a quantitative assessment of local anaesthetics and analgesics, their action may be studied quantitatively in the perfused liver.

This work was supported by the Medical Research Council.

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(*Received March* 22, 1973)