THE ACTION OF MORPHINE-LIKE DRUGS ON IMPULSE TRANSMISSION IN MAMMALIAN NERVE FIBRES

BY

H. W. KOSTERLITZ AND D. I. WALLIS

From the Department of Physiology, University of Aberdeen

Experiments on nerves *in situ* and on isolated nerves provide no evidence that morphine interferes with impulse transmission in myelinated or nonmyelinated nerve fibres. The concentrations used in experiments on isolated nerves were 10- to 100-times as high as those required to depress transmission at autonomic nerve-effector cell junctions. Examination of the resting membrane potential, the action potential and the positive after-potential, the conduction velocity, the time courses of the recovery of the size of the action potential and of the excitability after a conditioning stimulus, the ability of the axons to sustain repetitive activity and the posttetanic hyperpolarization gave no indication that morphine affects either the mechanisms involved in the initiation of the propagated impulse or those leading to restoration of the resting state after activity. Analgesic drugs, such as pethidine and methadone which have a local anaesthetic action, may cause a reversible decrease in the size of the compound action potential and in the conduction velocity of A-B and C fibres.

In recent years evidence has accumulated which shows that morphine and morphine-like drugs depress the responses of certain autonomic effector cells to pre- or postganglionic stimulation (Kosterlitz & Robinson, 1955 ; Schaumann, 1955 ; Paton, 1956, 1957 ; Trendelenburg, 1957 ; Kosterlitz & Taylor, 1959 ; Cairnie, Kosterlitz & Taylor, 1961 ; Szerb, 1961). It was of particular interest to ascertain whether these drugs affected impulse transmission in nerve axons, a field in which experimental results have been almost as various as the investigators (Krueger, Eddy & Sumwalt, 1941). We decided therefore to re-examine the action of these drugs on mammalian nerve fibres using modern methods.

This paper is concerned, first, with extending the findings of Cairnie & Kosterlitz (1962), who were unable to demonstrate any effect of morphine on parameters such as the conduction velocity, the threshold of excitability and the recovery of excitability of some fibre groups in the cat saphenous and the rabbit vagus nerves examined *in situ*. Secondly, the action of morphine and morphine-like drugs was examined on isolated preparations of the rabbit vagus and cat hypogastric nerves by means of the double sucrose-gap method.

It has been shown (Kosterlitz & Taylor, 1959 ; Cairnie *et al.*, 1961) that the depressant action of morphine on the responses of the rabbit sinoatrial node to vagal stimulation and on the response of the cat nictitating membrane to post-ganglionic sympathetic stimulation is greater at low than at high stimulus frequen-

cies. We were therefore particularly interested in the possible action of morphine on action potentials evoked by single stimuli, but included also experiments with repetitive stimulation.

METHODS

Anaesthesia. Cats were anaesthetized by intraperitoneal injection of 5 ml./kg of a solution of 1% (w/v) chloralose and 10% (w/v) urethane, and rabbits by intravenous injection of 5 to 6 ml./kg of a 25% (w/v) solution of urethane.

Experiments on nerves in situ. The nerve to the medial muscle of the cat nictitating membrane was examined in the orbit after removal of the eyeball (Kosterlitz, Thompson & Wallis, 1964). The hypogastric nerve of the cat was dissected after removal of the intestines. In order to disturb the blood supply as little as possible the dissection of the nerves was restricted to a minimum.

The nerves were stimulated with rectangular pulses, having a small biphasic component, from a Grass stimulator. The stimuli, 0.05 to 0.5 msec in duration, were isolated from earth by means of a RF transformer unit. The recording system consisted of a low grid-current cathode follower and a conventional RC-coupled amplifier with a time constant of 0.5 sec; the amplified action potential was displayed on an oscilloscope. Both stimulating and recording electrodes were of platinum. A pool of liquid paraffin covered the nerve.

With the hypogastric nerve preparation two parameters of impulse transmission in C fibres were examined. One was the time course of the recovery of " responsiveness " of the axons (Gasser, Richards & Grundfest, 1938) after a supramaximal conditioning stimulus, measured by the size of the compound action potential caused by a test stimulus 1.5- to 2-times the strength of a maximal stimulus; the recovery of the conduction velocity was determined simultaneously. The second parameter was the recovery of excitability of the fastest C fibres after a supramaximal conditioning stimulus, measured as the minimum current required to produce the smallest detectable response. The voltage drop across a resistor, monitored on an oscilloscope, was used to measure the current. In these experiments, stimuli were applied at intervals of not less than 30 sec.

For two reasons, both the conditioning and the test stimuli were applied through the same electrodes in preference to separate pairs of electrodes at different points on the nerve. First, since the conduction velocities of the fibres were low, the action potential resulting from the conditioning stimulus would be temporally dispersed by the time it reached the site of the second stimulating electrodes. Secondly, preparing two regions of nerve for the application of stimulating electrodes would have resulted in a considerable disruption of the blood supply as only a relatively short length of nerve was available. Moreover, the use of a stimulus with a small biphasic component reduced the extent of local electrotonic changes.

The arterial blood pressure in the left femoral artery was recorded by means of a condenser manometer. Drugs were injected into the right femoral vein. Since morphine depresses the respiratory centre, artificial ventilation was applied throughout the experiments.

Experiments on isolated nerves. Changes in membrane potential were measured by means of the double sucrose-gap apparatus described by König (1962). The nerve was suspended between five fluid-tight chambers through which various solutions flowed (Fig. 1). The central chamber (3), 3 mm wide, was connected to a multi-way tap so that solutions of different composition could be passed through it. The two outer chambers (1 and 5), 3 mm wide, contained Ringer-Locke or Krebs solution, while the two inner chambers (2 and 4), 4 mm wide, on either side of the central chamber contained isotonic sucrose solution to minimize current shunt along the outside of the nerve. The chambers were separated by stretched condom-rubber partitions. Holes for the nerve were punched in the rubber membrane with sawn-off, hand-ground hypodermic needles, a gauge 26 needle being suitable for the vagus and a gauge 30 needle for the hypogastric nerve. One of the sucrose chambers (2) separated the recording and the other (4) the stimulating electrodes; the central chamber
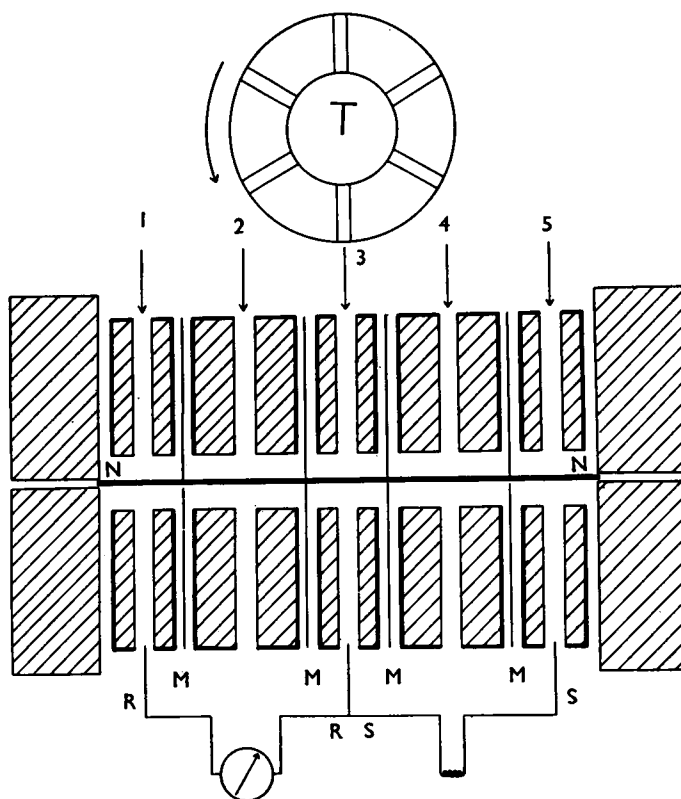
Fig. 1. Arrangement of double sucrose-gap. 1 and 5: Ringer–Locke or Krebs solution. 2 and 4: isotonic sucrose solution. 3: test solution. M: rubber membrane. R: recording electrodes. S: stimulating electrodes. T: tap. N–N: desheathed nerve.

was earthed. The action potential and changes in the resting membrane potential were led off by silver-silver chloride cotton wick electrodes and fed into a low grid-current cathode follower and then into a DC amplifier. The nerve was stimulated by rectangular pulses of 0.01 to 0.5 msec duration through silver-silver chloride electrodes, or in a few experiments through platinum electrodes, dipping in the solutions in chambers 3 and 5.

The nerve was excised from the anaesthetized rabbit or cat and its sheath was removed under a microscope. The effectiveness of sheath removal was tested by observing the depolarizing effect of a Ringer-Locke or Krebs solution containing 20 mM-potassium, potassium chloride replacing an equivalent amount of sodium chloride. Preparations showing a depolarization of less than 10 mV in 90 sec were not used. For the vagus nerve, the bathing solution was a modified Ringer-Locke solution with a sodium phosphate buffer ($pH=7.1$), bubbled with oxygen (Armett & Ritchie, 1960); in the experiments on the hypogastric nerve, Krebs solution, bubbled with 5% carbon dioxide in oxygen, appeared to cause the minimum deterioration of the preparation. The temperature was 29 to 32° C. The hypogastric nerve was stimulated peripherally to the recording electrodes (antidromically for motor fibres) to eliminate impulses transmitted through the ganglion cells present in the cat hypogastric nerve (Langley & Anderson, 1895–6).

The factor limiting long-term stability of the recording system was the rate of flow through the chambers. It was not possible to detect potential changes of less than ±1 mV/min, even though the stability of the equipment was about 50 μV/min.

The experimental procedure had to take into account the spontaneous deterioration of the preparation, which was negligible for vagal A-B fibres, small for vagal C fibres but often quite considerable for hypogastric C fibres. About 15 min after setting up the preparation the depolarizing effect of 20 mM-potassium was tested. If this was satisfactory, records were taken after a recovery period of 4 to 5 min, during which normal Ringer-Locke or Krebs solution flowed through the central chamber. Action potentials were evoked by single stimuli supramaximal for A-B or C fibre groups, and by trains of stimuli supramaximal for C fibres. This procedure was repeated once or twice at intervals of 4 to 5 min. Then the solution flowing through the central chamber was changed to Ringer-Locke or Krebs solution containing the drug being tested, the resting membrane potential was observed for 3 min and further records were taken. This cycle was repeated after the drug-containing solutions had been replaced by drug-free solutions. The action potentials were photographed and enlarged. The height of the action potential and positive after-potential, the size of the posttetanic hyperpolarization and the conduction velocity were plotted against time. To demonstrate that a drug had no effect it was necessary to show that it caused no change in the slope of the plots. A depressant effect was unequivocal only when there was an increase in the slope after the addition of the drug and a reversal after washing it out. If there was a change in slope only after adding or after withdrawing the drug, the effect was considered doubtful.

The action potentials obtained by our method (Fig. 2a, b and c) were similar in size and shape to those reported by Ritchie & Straub (1956) and Armett & Ritchie (1960, 1963) with the single sucrose-gap method. The after-potentials were variable and often small. In some experiments in which the positive after-potential was more distinct, it was photographed at higher amplification (Fig. 2e). The ability of the nerve to sustain repetitive activity was tested by trains of stimuli at 15 shocks/sec for 10 sec (Fig. 2d). These records also gave
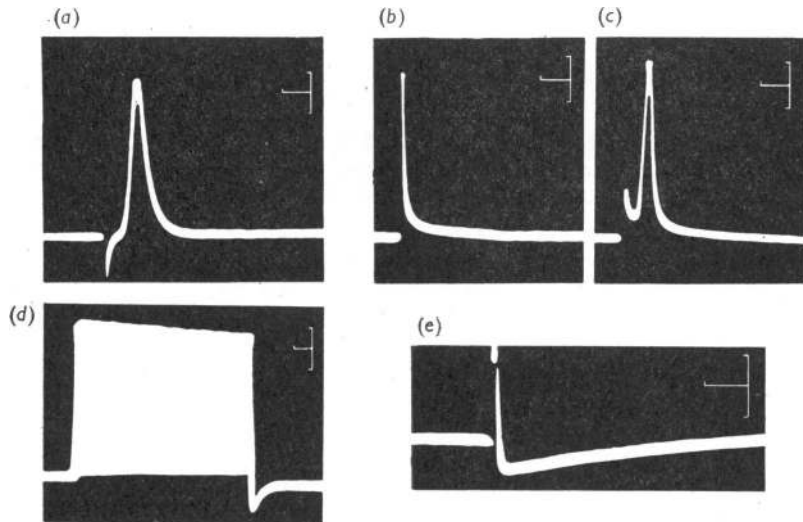

Fig. 2. Action potentials, positive after-potential and posttetanic hyperpolarization. Double sucrose-gap. Stimuli supramaximal for the various fibre groups. (a), action potential of C fibres, cat hypogastric nerve; (b), action potential of A–B fibres, rabbit vagus nerve; (c), action potential of C fibres, the action potential of the A–B fibres being lost in the artifact, rabbit vagus nerve; (d), action potentials and posttetanic hyperpolarization of C fibres with a train of stimuli at 15 [shocks/sec for 10 sec (cat hypogastric nerve); (e), positive after-potential of C fibres, in response to a single supramaximal stimulus, in a rabbit vagus nerve. Calibrations: 5 msec and 5 mV in (a), (b) and (c); 1 sec and 5 mV in (d); 50 msec and 1 mV in (e).

information on posttetanic hyperpolarization which was larger in the hypogastric nerve than in the vagus.

Drugs. Doses and concentrations of drugs refer to the salts. The drugs used were morphine hydrochloride, pethidine hydrochloride, methadone hydrochloride, nalorphine hydrobromide and Ba-20227 (1-diethylaminoethyl-2-*p*-methoxybenzyl-5-nitrobenzimidazole hydrochloride, Ciba). The analgesic drugs were always used in doses which were considerably larger than those required to depress the responses of the effector cells to stimulation of their nerves (Gyang, Kosterlitz & Lees, 1964).

<div align="center">RESULTS</div>

The action of morphine on the sympathetic nerve supplying the medial smooth muscle of the cat nictitating membrane

Experiments on the nerve in situ. It has been shown (Kosterlitz *et al.*, 1964) that the compound action potential recorded from the nerve to the medial smooth muscle has several deflexions ; the postganglionic myelinated fibres which innervate the muscle conduct at a velocity of 1.7 to 3.8 m/sec when measured from the stimulus artifact to the peak of action potential.

It was difficult to obtain a preparation in which the deflexions of the compound action potential persisted unaltered for the time required to test the action of morphine and nalorphine. There was always, independent of the injection of
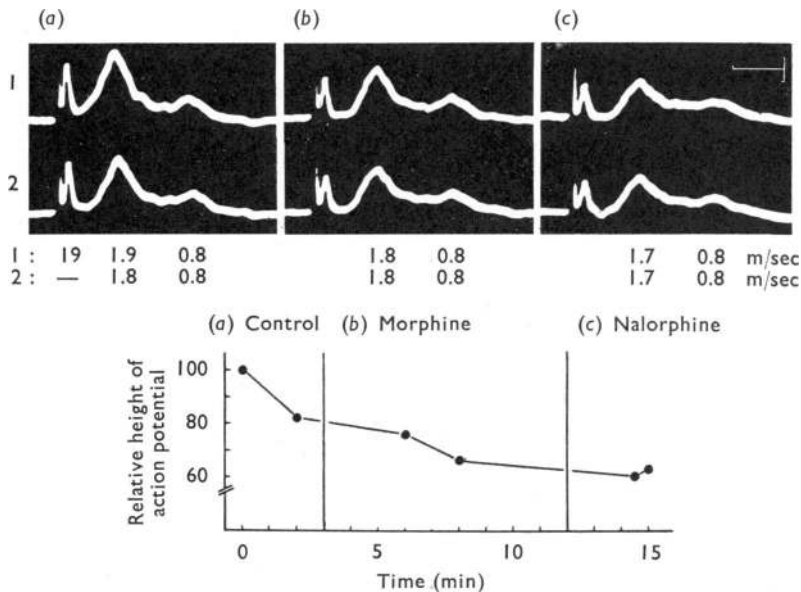

Fig. 3. Effect of morphine on the compound action potential of the nerve supplying the medial smooth muscle of the cat nictitating membrane. Chloralose-urethane anaesthesia. The numerals below the deflexions give the conduction velocities in m/sec, measured from artifact to peak, for each of the two rows of records. The conduction velocity of the fastest group of fibres was measured on a record obtained with a faster sweep. Fibres to the muscle conduct at 1.9 m/sec. (*a*, 1 and 2), control; (*b*, 1 and 2), 3 and 5 min after injection of morphine (3 mg/kg); (*c*, 1 and 2), 2.5 and 3 min after injection of nalorphine (1.5 mg/kg). Calibrations, 5 m/sec and 100 μV. The relative heights of the action potential (1.9 m/sec) are plotted against time in the graph below the records.

R

morphine, a slow decrease in the size of the action potential and in the rate at which the impulse was conducted.

In six experiments we found no evidence which suggested that morphine, in doses large enough to depress the responses of the nictitating membrane (Cairnie *et al.,* 1961), interfered with impulse transmission. In the example shown in Fig. 3, the nerve fibres to the medial.smooth muscle conducted at 1.9 m/sec. During the course of the experiment the height of their action potential decreased slowly but morphine did not accelerate this decline. Further, nalorphine, which Cairnie *et al.* (1961) showed to antagonize the depressant action of morphine on the response of the nictitating membrane to postganglionic stimulation, did not change the slope of the decline. Thus the effect of morphine on the response of the nictitating membrane is unlikely to be caused by its interfering with transmission in the axons.

The action of morphine on the cat hypogastric nerve

Experiments on the nerve in situ. The major deflexion in the compound action potential in this nerve is due to postganglionic sympathetic C fibres conducting at
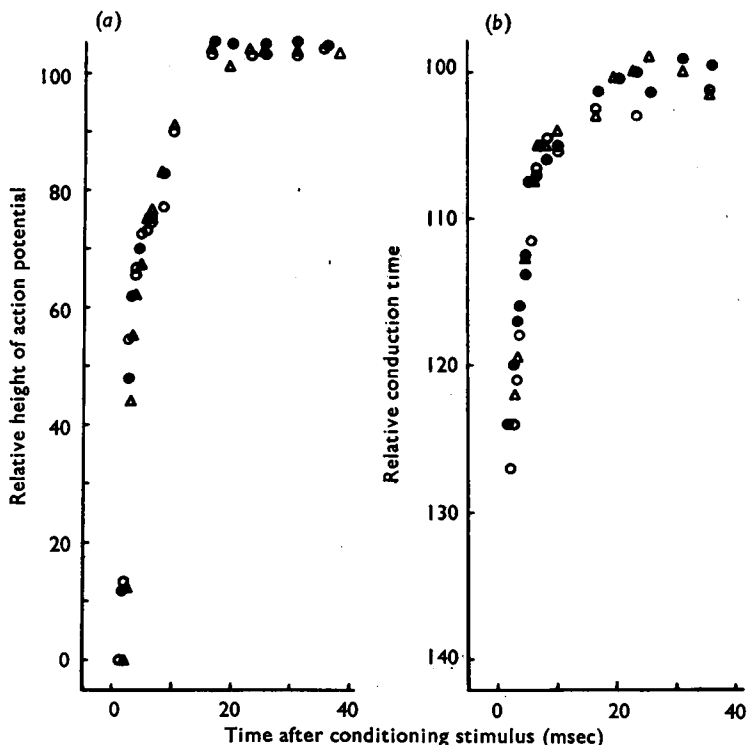

Fig. 4. Effect of morphine on the recovery of the height of the action potential (*a*) and conduction time (*b*) of the C fibres of the cat hypogastric nerve after a single conditioning stimulus. Chloralose–urethane anaesthesia. Abscissae: interval between conditioning and test stimuli, both supramaximal. Ordinate: relative height of action potential (*a*) and conduction time (*b*) expressed as a percentage of the control response to the test stimulus by itself. Closed circles: control. Open circles: 5 min after injection of morphine (3 mg/kg). Triangles: 37 min after injection of morphine and 5 min after injection of nalorphine (1.5 mg/kg).

0.9 to 1.6 m/sec (Lloyd, 1937). In five experiments we found a conduction velocity, measured from artifact to peak, of 0.9 m/sec with a range of 0.6 to 1.1 m/sec. There was no decrease in conduction velocity after injection of morphine (3 mg/kg.).

Morphine had no effect on either the time course of the recovery of the " responsiveness " of the axons after a supramaximal conditioning stimulus or the recovery of the conduction velocity (Fig. 4). Further, it did not affect the recovery of excitability after a conditioning stimulus (Fig. 5). These findings were supported by the absence of any effect of nalorphine.
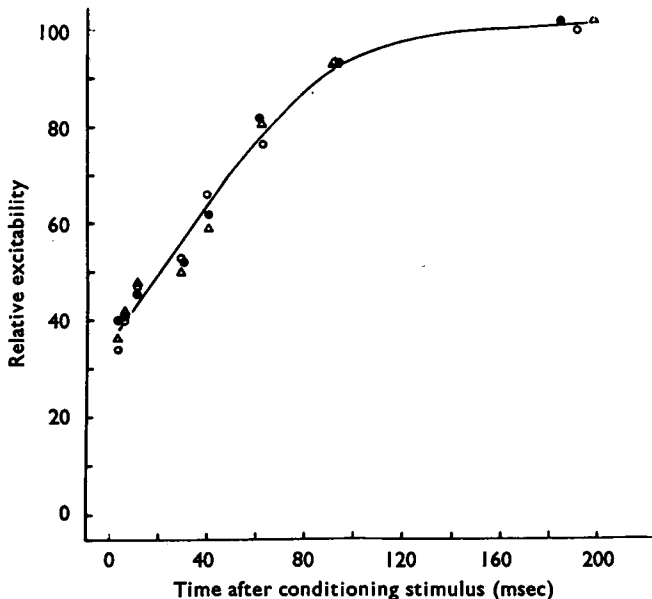

Fig. 5. Effect of morphine on the recovery of excitability of C fibres of the cat hypogastric nerve after a single supramaximal conditioning stimulus. Chloralose–urethane anaesthesia. Abscissa: interval between conditioning and test stimuli. Ordinate: relative excitability, expressed as threshold current without conditioning stimulus ×100/threshold current after conditioning stimulus. Closed circles: control. Open circles: 6 min after injection of morphine (3 mg/kg). Triangles: 35 min after injection of morphine and 6 min after injection of nalorphine (1.5 mg/kg).

Experiments on the isolated nerve. In ten experiments there was no indication that morphine had a depressant effect, although spontaneous deterioration made it difficult to exclude such an effect with certainty. In the example shown in Fig. 6 there appears to be no effect of morphine (10 μg/ml.) or a mixture of morphine (10 μg/ml.) and nalorphine (5 μg/ml.) on the decline in size of the action potential, of the conduction velocity or of the posttetanic hyperpolarization. Further, morphine did not affect the ability of the nerve fibres to maintain the height of the action potential during a train of impulses nor the depolarization during such a train. If morphine caused a change in the resting membrane potential, it was smaller than could be measured (± 1 mV/min).
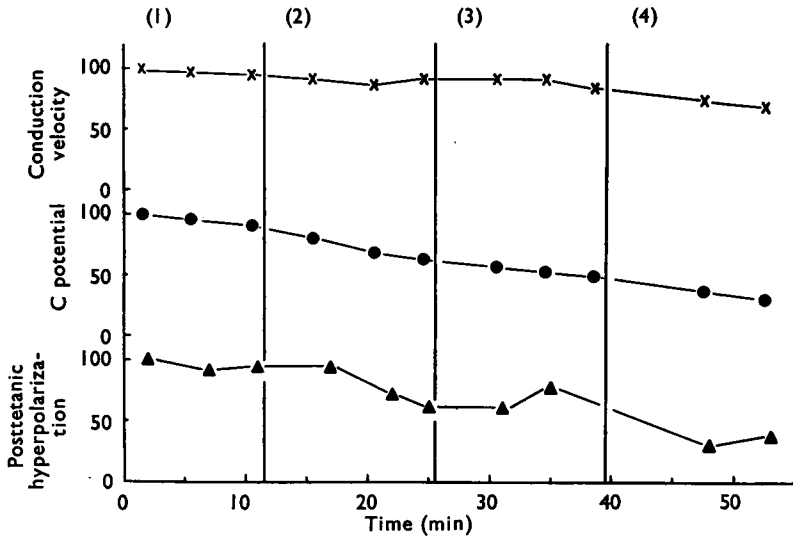
Fig. 6. Effect of morphine on the isolated hypogastric nerve of the cat. Double sucrose-gap. Abscissa: time (min). Ordinates: relative values of conduction velocity, action potential and posttetanic hyperpolarization. Stimuli supramaximal for C fibres. ×——× : conduction velocity; ●——● : heights of action potential of C fibres (100=19.5 mV); ▲——▲ : size of posttetanic hyperpolarization (100=3.5 mV). (1) and (4): Krebs solution alone; (2): Krebs solution containing morphine (10 μg/ml.); (3): Krebs solution containing morphine (10 μg/ml.) and nalorphine (5 μg/ml.).
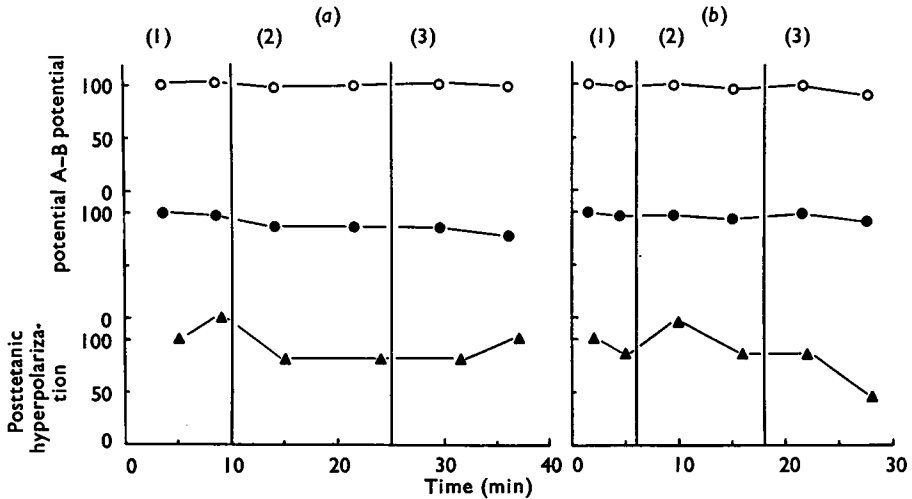

Fig. 7 Effect of morphine on the isolated vagus nerve of the rabbit, two experiments (a and b). Double sucrose-gap. Abscissa: time (min). Ordinates: relative values of action potentials of A–B and C fibres, and of posttetanic hyperpolarization. Stimuli supramaximal for A–B or C fibres. ○——○ : height of action potential of A–B fibres (100=19 mV in a, and 15 mV in b); ●——● : height of action potential of C fibres (100=19.5 mV in a and 15.5 mV n b); ▲——▲ : size of posttetanic hyperpolarization of C fibres (100=1.3 mV in a, and 0.9 mV in b). 1 and 3: Ringer–Locke solution; 2: Ringer–Locke solution containing morphine (10 μg/ml.).

The action of morphine and morphine-like drugs on the rabbit vagus nerve

Cairnie & Kosterlitz (1962) found that morphine caused no change in the threshold of excitability of the A-B and C fibres of the rabbit vagus nerve and in the conduction velocity of the C fibres, most of which are sensory (Evans & Murray, 1954).

Experiments on the isolated nerve. The spontaneous decrease in the height of the action potential was much less than in the hypogastric nerve. In no experiment did morphine (10 μg/ml.) show any detectable depressant action. In the two examples shown in Fig. 7a and b, the height of the action potential of the A-B and C fibres was unaffected by the drug. Posttetanic hyperpolarization was not affected by morphine in one experiment (Fig. 7b), while in the other (Fig. 7a) such an effect could not definitely be excluded. Morphine did not affect the resting membrane potential, the conduction velocity of the C fibres or their ability to maintain the height of the action potential during a train of impulses.

The drug Ba-20227 has an analgesic potency about seventy-times that of morphine (Gross & Turrian, 1957) and is much more potent than morphine in depressing the peristaltic reflex and the response of the nictitating membrane to postganglionic stimulation (Gyang *et al.*, 1964). In a concentration of 1 μg/ml., the drug had no
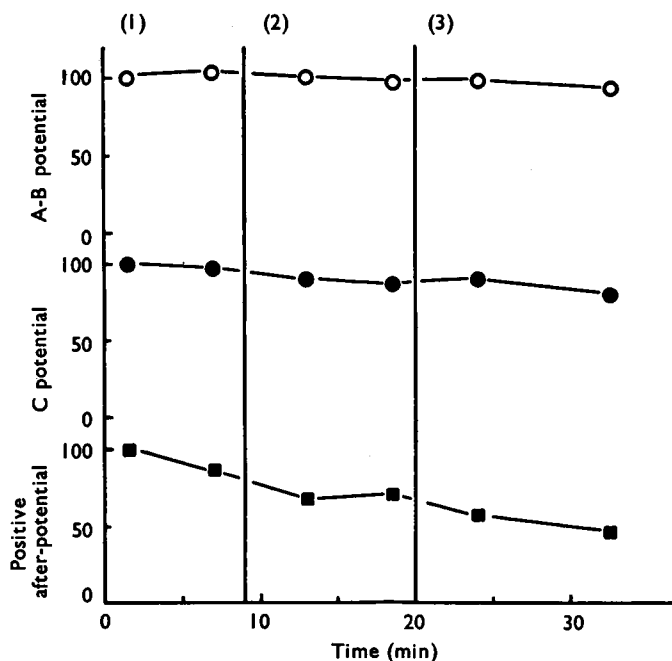

Fig. 8. Effect of the analgesic compound Ba-20227 on the isolated vagus nerve of the rabbit. Double sucrose-gap. Abscissa: time (min). Ordinates: relative values of action potentials of A-B and C fibres and of positive after-potential of C fibres. Stimuli supramaximal for A-B or C fibres. O —— O: height of action potential of A-B fibres (100=11 mV); ● —— ●: height of action potential of C fibres (100=17.5 mV); ■ —— ■: size of positive after-potential (100=0.6 mV). 1 and 3: Ringer-Locke solution; 2: Ringer-Locke solution containing Ba-20227 (1 μg/ml.).

effect on the height of the action potential of the A-B and C fibres or the size of the positive after-potential of the C fibres (Fig. 8). There was no change in the resting membrane potential or the conduction velocity.

In view of the negative results of the experiments with morphine and Ba-20227, it seemed worth while to examine the effects of two analgesic drugs known to have a local anaesthetic action, pethidine (Dawes, 1946) and methadone (Thorp, 1949).
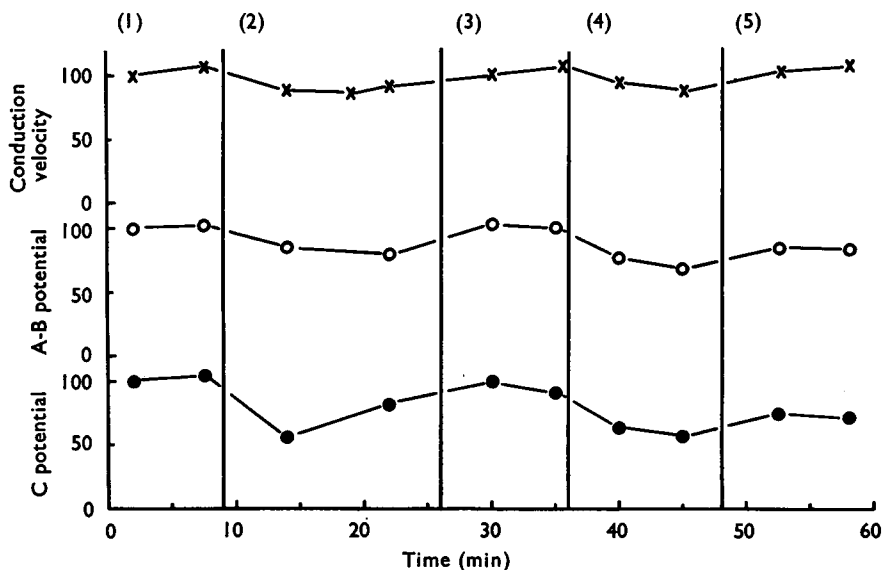

Fig. 9. Effect of pethidine on the isolated vagus nerve of the rabbit. Double sucrose gap. Abscissa: time (min). Ordinates: relative values of conduction velocity, action potentials of A-B and C fibres. Stimuli supramaximal for A-B and C fibres. ×——×: conduction velocity of C fibres; O——O: height of action potential of A-B fibres (100=9 mV); ●——●: height of action potential of C fibres (100=15 mV). 1, 3 and 5: Ringer-Locke solution; 2 and 4: Ringer-Locke solution containing pethidine (100 μg/ml.).

Pethidine (100 μg/ml.) caused a decrease in the size of the action potentials of the A-B (20 to 30%) and particularly the C fibres (20 to 40%), an effect which was reversed by washing out the drug. Pethidine also reduced the conduction velocity of the C fibres by 10 to 15% (Fig. 9). However, the ability to sustain a train of impulses was unimpaired. Pethidine did not cause a change in the resting membrane potential of ±1 mV/min or more. Methadone (100 μg/ml.) had an action similar to that of pethidine.

DISCUSSION

The technical difficulties encountered in this investigation make some of the results less reliable than others. The experiments on the cat hypogastric nerve *in situ* and on the rabbit isolated vagus nerve have yielded clear results, while the experiments on the nerve supplying the medial smooth muscle of the cat nictitating membrane *in situ* and on the cat isolated hypogastric nerve suffered from a spon-

taneous decline in the size of the action potential. However, there is no evidence that morphine, in a concentration of 10 μg/ml., affects the mechanisms involved in the initiation of the propagated impulse or those concerned in the restoration of the resting state after activity.

J. W. Thompson (personal communication) has shown that morphine, in concentrations of 0.1 to 1 μg/ml., depresses the response of the isolated nerve-smooth muscle preparation of the cat nictitating membrane. Moreover, in the guinea-pig isolated ileum preparation, the peristaltic reflex and the graded reflex response of the longitudinal muscle are inhibited by morphine in concentrations of 0.05 to 0.1 μg./ml. (Gyang *et al.*, 1964). The experiments reported in this paper, which show that morphine in a concentration of 10 μg/ml. has no effect on impulse transmission in isolated nerve preparations, preclude the possibility that the effect of morphine on the responses of autonomic effector cells is caused by an action on nerve axons. In this connexion, it should be noted that, even in the very high concentration of 1.4 mg/ml., morphine reduces the action potential in the rabbit vagus nerve by only 10% (Ritchie & Armett, 1963).

It is of interest that pethidine and methadone, two drugs which have a local anaesthetic action, decrease the conduction velocity and the height of the action potential in isolated nerves. These effects are reversed by washing out the drugs. They cause no detectable effect on the resting membrane potential, and this finding accords with those of Shanes, Freygang, Grundfest & Amatniek (1959), who showed that a higher concentration of cocaine or procaine is required to produce an effect on the resting membrane potential than on the action potential.

REFERENCES

ARMETT, C. J. & RITCHIE, J. M. (1960). The action of acetylcholine on conduction in mammalian non-myelinated fibres and its prevention by an anticholinesterase. *J. Physiol. (Lond.)*, **152**, 141–158.

ARMETT, C. J. & RITCHIE, J. M. (1963). Permeability of mammalian non-myelinated fibres to sodium and lithium ions. *J. Physiol. (Lond.)*, **165**, 130–140.

CAIRNIE, A. B. & KOSTERLITZ, H. W. (1962). The action of morphine on impulse propagation in nerve fibres. *Int. J. Neuropharmacol.*, **1**, 133–136.

CAIRNIE, A. B., KOSTERLITZ, H. W. & TAYLOR, D. W. (1961). Effect of morphine on some sympathetically innervated effectors. *Brit. J. Pharmacol.*, **17**, 539–551.

DAWES, G. S. (1946). Synthetic substitutes for quinidine. *Brit. J. Pharmacol.*, **1**, 90–112.

EVANS, D. H. L. & MURRAY, J. G. (1954). Histological and functional studies on the fibre composition of the vagus nerve of the rabbit. *J. Anat. (Lond.)*, **88**, 320–337.

GASSER, H. S., RICHARDS, C. H. & GRUNDFEST, H. (1938). Properties of the nerve fibres of slowest conduction in the frog. *Amer. J. Physiol.*, **123**, 299–306.

GROSS, F. & TURRIAN, H. (1957). Über Benzimidazolderivative mit starker analgetischer Wirkung. *Experientia (Basel)*, **13**, 401–402.

GYANG, E. A., KOSTERLITZ, H. W. & LEES, G. M. (1964). The inhibition of autonomic neuro-effector transmission by morphine-like drugs and its use as a screening test for narcotic analgesic drugs. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **248**, 231–246.

KÖNIG, K. (1962). Membranpotentialmessungen am Skeletmuskel mit der " Saccharose-Trennwand " Methode. *Pflügers. Arch. ges. Physiol.*, **275**, 452–460.

KOSTERLITZ, H. W. & ROBINSON, J. A. (1955). Mechanism of the contraction of the longitudinal muscle of the isolated guinea-pig ileum, caused by raising the pressure in the lumen. *J. Physiol., (Lond.)*, **129**, 18–19P.

KOSTERLITZ, H. W. & TAYLOR, D. W. (1959). The effect of morphine on vagal inhibition of the heart. *Brit. J. Pharmacol.*, **14**, 209–214.

KOSTERLITZ, H. W., THOMPSON, J. W. & WALLIS, D. I. (1964). The compound action potential in the nerve supplying the medial smooth muscle of the nictitating membrane of the cat. *J. Physiol. (Lond.)*, in the press.

KRUEGER, H., EDDY, N. B. & SUMWALT, M. (1941). *The Pharmacology of the Opium Alkaloids*, part 1, p. 63. Suppl. No. 165 to the Public Health Reports. Washington: United States Government Printing Office.

LANGLEY, J. N. & ANDERSON, H. K. (1895–96). The innervation of the pelvic and adjoining viscera. *J. Physiol. (Lond.)*, **19**, 71–139.

LLOYD, D. P. C. (1937). The transmission of impulses through the inferior mesenteric ganglia. *J. Physiol. (Lond.)*, **91**, 296–313.

PATON, W. D. M. (1956). The response of and release of acetylcholine by guinea-pig small intestine in response to coaxial stimulation. *Abstr. 20th Int. Physiol. Congr., Bruxelles*, 708–709.

PATON, W. D. M. (1957). The action of morphine and related substances on acetylcholine output of coaxially stimulated guinea-pig ileum. *Brit. J. Pharmacol.*, **12**, 119–127.

RITCHIE, J. M. & ARMETT, C. J. (1963). On the role of acetylcholine in conduction in mammalian non-myelinated nerve fibres. *J. Pharmacol. exp. Ther.*, **139**, 201–207.

RITCHIE, J. M. & STRAUB, R. W. (1956). After-effects of repetitive stimulation on mammalian non-medullated fibres. *J. Physiol. (Lond.)*, **134**, 698–711.

SCHAUMANN, W. (1955). The paralysing action of morphine on the guinea-pig ileum. *Brit. J. Pharmacol.*, **10**, 456–461.

SHANES, A. M., FREYGANG, W. H., GRUNDFEST, H. & AMATNIEK, K. E. (1959). Anesthetic and calcium action in the voltage clamped squid giant axon. *J. gen. Physiol.*, **42**, 793–802.

SZERB, J. C. (1961). The effect of morphine on the adrenergic nerves of the isolated guinea-pig ileum. *Brit. J. Pharmacol.*, **16**, 23–31.

THORP, R. H. (1949). The pharmacology of the optical isomers of amidone (2-dimethylamino-4:4-diphenylheptan-5-one). *Brit. J. Pharmacol.*, **4**, 98–104.

TRENDELENBURG, U. (1957). The action of morphine on the superior cervical ganglion and on the nictitating membrane. *Brit. J. Pharmacol.*, **12**, 79–85.