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EFFECTS OF CLOSE ARTERIAL INJECTIONS OF ACETYLCHOLINE ON THE ACTIVITY OF THE CERVICAL SPINAL CORD OF THE CAT

By W. FELDBERG, J. A. B. GRAY and W. L. M. PERRY

From the National Institute for Medical Research, Mill Hill, London, N.W. 7

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The role of acetylcholine in central synaptic transmission has for long been a vexed question, and one reason for the difficulty in settling it is the relative inaccessibility of the spinal cord to drugs. In the present experiments an analysis was made of the effects of acetylcholine given by close arterial injection, whereby momentarily the injected solution completely fills the vascular bed of the region being studied. Hitherto acetylcholine has either been injected into the blood stream at a more distant point, applied locally to the surface of the central nervous system, or injected intraventricularly (for references see Feldberg, 1945, 1950), or into the substance of the grey matter (Kennard, 1951).

In order to study some excitatory effects of drugs given intravascularly, for example acetylcholine at the neuromuscular junction, it is important to obtain a rapid rise in the concentration in the intercellular space. To obtain such a rapid rise it is necessary to establish a high concentration gradient across the vascular membrane. For a given dose of drug this is most easily done by close arterial injection. In the spinal cord, where the 'blood-brain barrier' may accentuate the difficulty of building up the necessary intercellular concentration, there may be a special need for this technique. Further, when using acetylcholine, which is quickly hydrolysed enzymically in the blood, close arterial injections permit the study of its effects without previous administration of an anticholinesterase which may modify the response.

The arterial supply of the spinal cord runs on its ventral surface. This makes close arterial injections technically difficult, because for most of the length of the cord the bodies of the vertebrae present a formidable obstacle to a ventral approach. This difficulty was avoided by exposing the first cervical segment of the cord which, however, has very short rootlets from which we could obtain neither monosynaptic nor even monosegmental reflexes.

METHODS

The experiments were performed on cats anaesthetized with ethyl chloride and ether, followed by intravenous chloralose (80 mg/kg) or dial (Dial liquid 'Ciba' 0·4–0·8 ml./kg). A short description of the exposure of the cord and the cannulation of the basilar artery has been given elsewhere (Feldberg, Gray & Perry, 1952).

The head and neck of the cat were held rigidly by transfixing the muscles and ligaments of the neck and jaw with knitting needles and clamping the needles in a rigid framework. The upper parts of the trachea and oesophagus were dissected out and removed, the tracheal cannula being inserted low. The prevertebral muscles were removed and the fine twigs of the first cervical nerves which supply them were carefully dissected out. Lengths of the second cervical nerves on both sides were prepared and cut peripherally. The third cervical nerves were divided to prevent neck movements during stimulation.

The atlanto-occipital membrane was opened by dividing it strand by strand with a curved triangular cutting needle. If bleeding occurred, the head was raised and this was usually sufficient to stop it; occasionally bleeding was severe and the carotid arteries were then clamped. This was avoided if possible, as the preparation was not so healthy if the carotids were clamped at this stage. After opening the membrane, the occipital bone up to the bullae osseae, the arch of the atlas and the odontoid peg and upper part of the body of the axis were removed.

The edges of the wound were then stitched to a ring to form a paraffin bath, and the dura mater was opened and stitched back to expose the cord. A diagram of the exposed cord has been published (Feldberg *et al.* 1952). Under a dissecting microscope the basilar artery was freed from the arachnoid and pia mater. Fine silk threads for tying in the cannula were passed beneath it and the larger branches tied.

By gentle retraction on a dentate ligament, the root of the first cervical nerve could be pulled upwards into view; it was then divided as far from the cord as possible. The rootlets, fanning out towards their origins from the cord, could then be brushed backwards and small strands prepared for recording.

The animal was then given 2 ml. of a 10% (w/v) heparin solution intravenously. The basilar artery was tied at the cephalic end and temporarily clamped with a fine pair of screw forceps at its caudal end, just at the origin of the vertebral arteries. A 26-gauge stainless steel cannula was used; to facilitate its insertion it was mounted on a long Perspex rod which acted as a light but rigid handle which could be manipulated from a distance while the cannula itself was being rigidly fixed to the frame. The blood supply to the cord does not always have the pattern shown in the diagram in our previous paper. When, for example, the basilar artery was a double trunk for part of its length, an effort was made to cannulate in such a way that the injection reached the anterior spinal artery via both vertebral arteries: e.g. by cannulating the united basilar artery at a higher level.

The animal was transferred to an electrically screened, heated box. One of the ventral rootlets was placed on two recording electrodes which consisted of horse hairs mounted in agar-saline in which was embedded a chlorided silver wire. Potentials were amplified with an amplifier having a high impedance input and a frequency response which could be varied up to a maximum range of 0–25 kc/s, and were recorded by photographing two cathode-ray oscillographs. These were double-beam oscillographs and the potentials were recorded on one beam of each, the Y-plates of these two beams being connected in parallel. One oscillograph was used without a time base and was photographed on moving paper; a time mark, recorded on the moving paper, triggered the time base of the other oscillograph. In this way it was possible to synchronize the records obtained by the two methods and thus simultaneously to record spontaneous activity and the reflex response to stimulation. This stimulus was a short shock from a low impedance source applied through bright platinum electrodes to the trunk of C2.

During recording, the constantly collecting cerebrospinal fluid was removed by continuous suction and the paraffin in the bath was replenished by a slow drip.

When chloralose was used, the reflex spike was sometimes abolished by the intravenous injection of appropriate doses of pentobarbitone Na; whenever this was done it has been stated specifically in the text.

Control injections into the basilar artery were made with fresh Locke's solution and the acetylcholine and other drugs were dissolved in the same solution. If not otherwise stated, the volume injected was 0·2 ml. and concentrations are given as g/ml. Doses of acetylcholine are given in terms of the hydrochloride, doses of eserine and atropine in terms of the sulphates.

RESULTS

Effects of acetylcholine

Close arterial injections of acetylcholine into the basilar artery produced regular changes in the spontaneous activity in the cord and in the polysynaptic reflex set up in the first cervical ventral root by stimulation of either the ipselateral or contralateral second cervical nerve. The reflex changes were accompanied by changes in the ventral root potential, of which only the early phases have been studied. The dose of acetylcholine normally used was 0·2 ml. of a 10^{-4} solution, but the changes were also observed with 0·2 ml. of 10^{-5} acetylcholine.

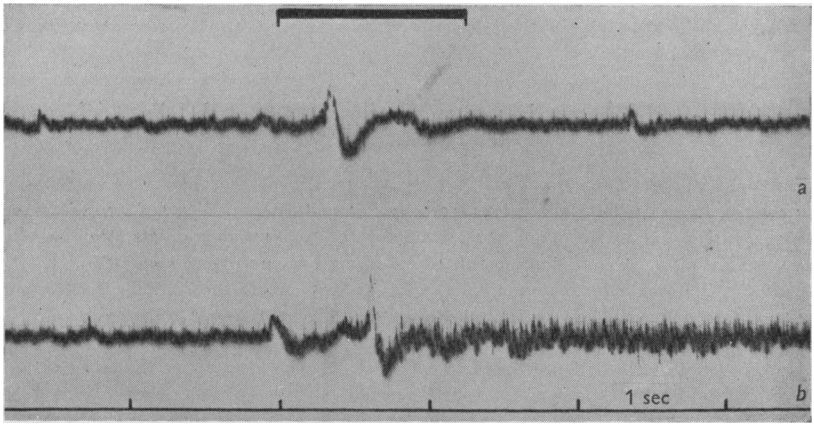

Fig. 1. Cat, chloralose. Records from ventral rootlet of C1. At signal, close arterial injections of (*a*) 0·2 ml. Locke's solution and (*b*) 0·2 ml. acetylcholine 10^{-4}. Time, seconds. Amplifier frequency response 4 c/s to 2 kc/s.

Spontaneous activity. An injection of acetylcholine produced in the first or second cervical nerve an outburst of motor impulses which began within 0·5 sec of the beginning of the injection and lasted, with steadily declining frequency, for periods varying from 2 to more than 40 sec (Fig. 1). If the recording electrodes were on a ventral rootlet, the proximal electrode being on or near the cord surface, a fluctuation of the base-line was also seen to start about 0·5 sec after the beginning of the injection (Fig. 1). The period of the more obvious fluctuations was of the order of 20–40 msec. The impulse dis-

charge was most intense on the negative peaks (cord electrode negative) of the fluctuation. When a barbiturate was given in order to diminish or abolish the impulse discharges, an injection of acetylcholine was still followed by the

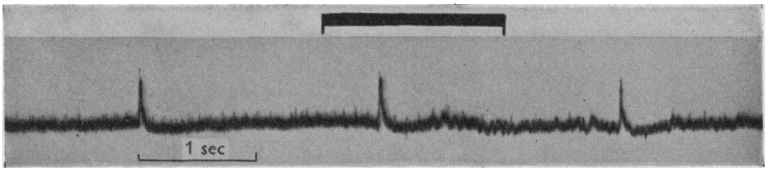

Fig. 2. Cat, chloralose, followed by pentobarbitone. Records from ventral rootlet of C1. At signal, close arterial injection of 0·2 ml. acetylcholine 10^{-4}. Time, seconds. Amplifier frequency response 0·4 c/s to 2 kc/s.

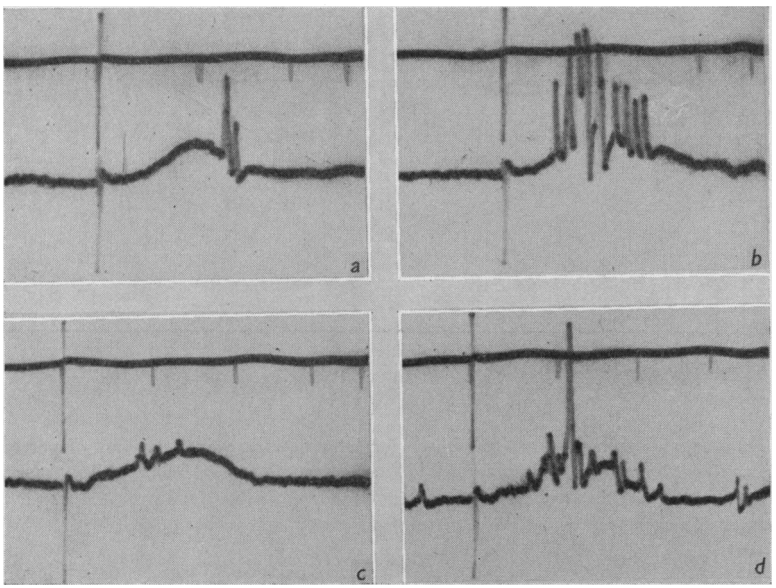

Fig. 3. Cat, dial. Records of polysynaptic reflex from rootlet C1. (*a*) and (*c*) before, (*b*) and (*d*) 3 and 5 sec respectively after close arterial injection of 0·2 ml. acetylcholine 10^{-4}. Between (*b*) and (*c*) the rootlet used for recording was changed and atropine (80 μg in 0·2 ml.) given arterially. Time, 10 msec. Amplifier response at (*a*) and (*b*) 0–2 kc/s, at (*c*) and (*d*) 4 c/s to 2 kc/s.

base-line fluctuations. In the experiment illustrated in Fig. 2 this change is clearly seen. In this particular experiment, such spontaneous discharge as remained after pentobarbitone was diminished by the injection of acetylcholine.

The ipselateral polysynaptic reflex. When the main part of the second cervical nerve was stimulated with short shocks every 2 sec and the polysynaptic reflex recorded from the ipselateral first cervical nerve or ventral root, an injection of acetylcholine caused an increase in the area of the reflex. This is illustrated in Fig. 3, in which the reflex resulting from a supramaximal stimulus is seen on top of the ventral root potential recorded from a fine rootlet. In this experiment 80 μg atropine in 0·2 ml. was given by close arterial injection; it did not abolish the effect of acetylcholine (Fig. 3*d*).

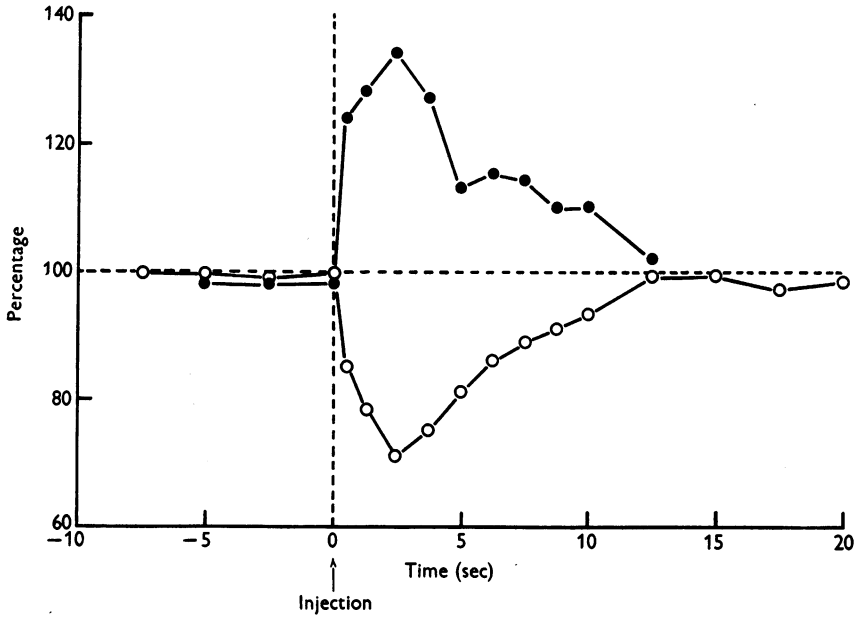

Fig. 4. Time course of changes produced by acetylcholine in area and latency of polysynaptic reflex. Records from small branch of C1. (Same experiment as Figs. 8 and 9.) Ordinates: percentage change in area (●—●) and in latency (○—○) of reflex. Abscissae: time in seconds. At zero time close arterial injection of 0·2 ml. acetylcholine 10^{-4}. (For details see text.)

The time course of the change can be seen in Fig. 4, in which the area of the reflex, given as a percentage of the mean pre-injection value, is plotted against time. The curve is the mean of three separate injections; the individual results were plotted and adjusted so that the beginnings of the injections were aligned and means were then taken at regular intervals along the abscissa. In this particular experiment the reflex was recorded diphasically, and in consequence there may be some distortion due to changes in synchronization. The change in reflex size was visible in records taken less than 0·5 sec after the beginning of the injection, and reached a maximum in about 3 sec. The changes in area were larger with submaximal stimuli than with supramaximal ones.

It can also be seen from Figs. 3 and 4 that the interval between the stimulus and the beginning of the reflex discharge is shortened after the injection of acetylcholine and that the general time course of this change is similar to that of the change in area. The reduction in time was seen even in records taken within 0·5 sec of the injection. The time was reduced to about 70% of its pre-injection value and this minimum occurred between 2·5 and 6 sec after the injection began. Thus the interval which was originally about 6 msec

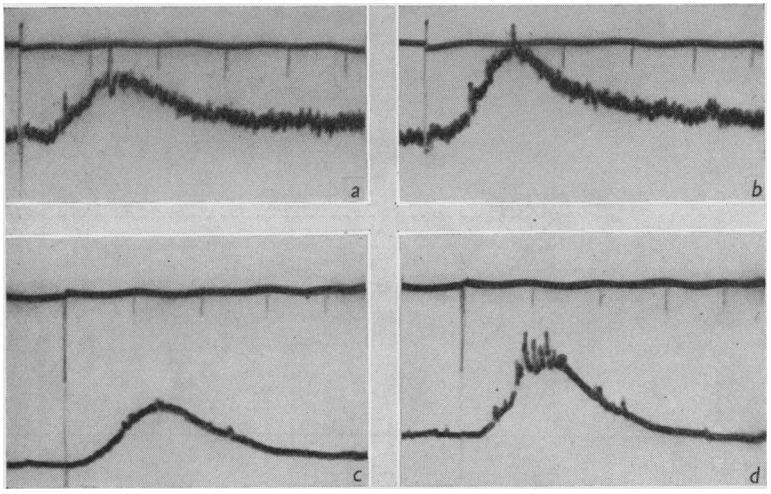

Fig. 5. Records from ventral rootlet of C1. Two experiments: (*a*) and (*c*) before, (*b*) and (*d*) 6 and 8 sec respectively after close arterial injection of 0·2 ml. acetylcholine 10^{-4}. Records (*a*) and (*b*) cat, chloralose followed by pentobarbitone; amplifier frequency response 0·4 c/s to 2 kc/s. Records (*c*) and (*d*) cat, dial; amplifier frequency response 0–6 kc/s. Time, 10 msec.

was reduced to about 4 msec when the effect was at a maximum. It is unlikely that the extra-cordal conduction time of the sensory fibres responsible for the bulk of the reflex was more than 0·5 msec, since the length of the second cervical nerve lying between the stimulating electrodes and the cord was about 15 mm.

Ventral root potential produced by ipselateral stimulation. When the first phase of the ventral root potential was recorded from rootlets of the first cervical segment during stimulation of the ipselateral second cervical nerve, an injection of acetylcholine was followed in most preparations by an increase in the amplitude of the ventral root potential; in one preparation, however, there was a decrease and, in another, a transient decrease preceded the usual increase. Examples of ventral root potentials before and after an injection of acetylcholine are shown in Fig. 5. The time course of the change in amplitude is shown in Fig. 6, which also illustrates the time course of the change in the

interval between stimulus and peak of the root potential. The curves in Fig. 6 are the means of seven separate injections. The interval was reduced after the injection of acetylcholine to about 80% of its pre-injection level. In any given experiment, the time course of this change was practically identical with that of the change in reflex latency. The absolute value of the time interval between the stimulus and the peak of the root potential was of the order of 11–13 msec and the reduction in the interval from 2 to 4 msec.

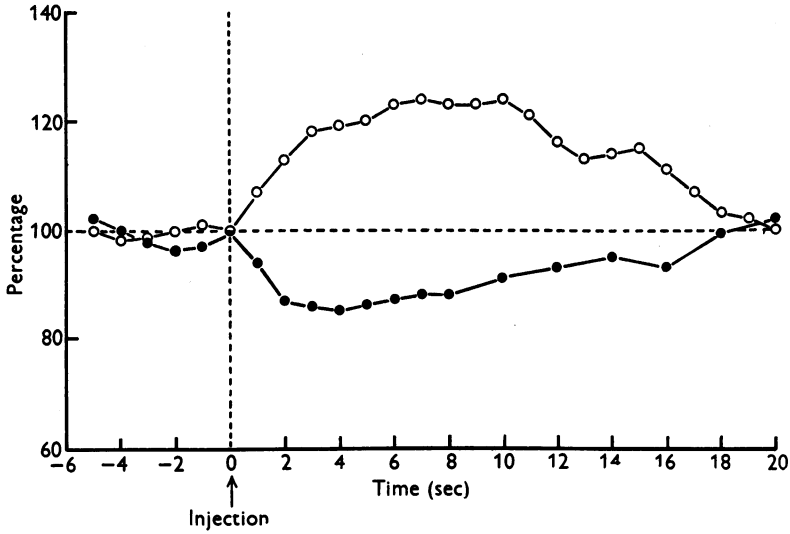

Fig. 6. Time course of changes produced by acetylcholine in amplitude and interval between stimulus and peak of root potential (polysynaptic reflex). Records from ventral rootlet C1. Ordinates: percentage change in amplitude (○—○) and interval (●—●). Abscissae: time in seconds. At zero time close arterial injection of 0·2 ml. acetylcholine 10^{-4}. (For details see text.)

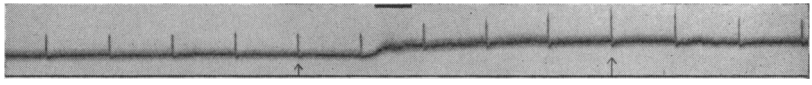

Fig. 7. Cat, dial. Record from ventral rootlet C1. Effect of close arterial injection (at signal) of 0·2 ml. acetylcholine 10^{-4} on resting potential. Root potentials elicited every 2 sec. Two of these (marked by arrows) are reproduced in Fig. 5*c* and *d*. Amplifier frequency response 0–6 kc/s.

Excitability and resting potential of the motor neurone. The excitability of the motor neurones can, to some extent, be gauged by the potential at which the reflex discharge begins. During recording from a large number of fibres, changes in synchronization can produce apparent changes in threshold, but it is fair to say that if acetylcholine increased the excitability of the motor neurones by reducing the membrane potential in the junctional region, there

should be a systematic reduction in the level at which the root potential excites its first impulse and also an increase in the reflex discharge for a given size of root potential. The latter is difficult to measure, particularly as the occurrence of an impulse discharge may reduce the root potential (Brock, Coombs & Eccles, 1952).

The potential at which the first impulse arose was measured for several runs in each of four experiments, using ipselateral stimulation. Acetylcholine always increased the variability of this threshold, and in most experiments there was a tendency for the threshold to be lowered. However, the variability after acetylcholine was so great that the reduction was not significant in any experiment.

Changes of membrane potential in the junctional region of the motor neurone can be recorded from electrodes on the motor root. In two experiments records were taken with the amplifier direct coupled. In one of these each injection of acetylcholine caused a potential change, the electrode nearer the cord going negative with an amplitude approximately one third of that of the root potential (Fig. 7). In the other experiment no change was detectable.

Effects of eserine and prostigmine

In a few experiments eserine or prostigmine (0·2–0·5 ml. of a 10^{-4} solution) was injected into the basilar artery. No effects were seen unless the cord was stimulated either reflexly or by acetylcholine; in one experiment a spontaneous discharge began about 15 min after the injection of eserine, but this may not have been due to the eserine.

In two experiments in which the reflex was recorded, eserine caused an increase in the area of the reflex which, unlike the increase produced by acetylcholine, was mainly due to a prolongation of the discharge (Fig. 8). In this experiment bursts of impulses occasionally followed the reflex discharge after a short interval (Fig. 9); this delayed response probably coincides with the peak of the second negative phase of the ventral root potential (Barron & Matthews, 1938).

Eserine and prostigmine prolonged the effects of acetylcholine. In two experiments, after eserine and prostigmine, the stimulating effects of acetylcholine were unusually short, but following the outburst there was a long period during which the background discharge was abolished. In another experiment the area of the reflex increased for only a short period after the acetylcholine injection and thereafter it decreased to below pre-injection level.

DISCUSSION

Our results show that, in the spinal cord, effects are produced by acetylcholine in concentrations which are of the same order of magnitude as those required to excite motor end-plates, post-ganglionic neurones and sensory pathways in

the skin. Although the possibility that the observed effects are due to vascular changes has not been excluded, there is no evidence suggesting that this is so, and it seems more reasonable to attribute them to a direct action of acetylcholine on the nervous tissue.

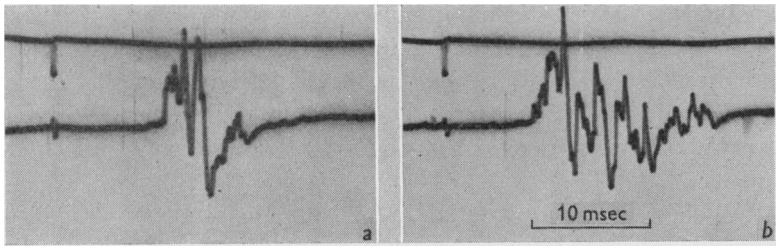

Fig. 8. Cat, chloralose. Records of polysynaptic reflex from ventral rootlet C1. (*a*) before and (*b*) 2 min after close arterial injection of 0·5 ml. eserine 10^{-4}. Amplifier frequency response 0·4 c/s to 2 kc/s. Time, 10 msec.

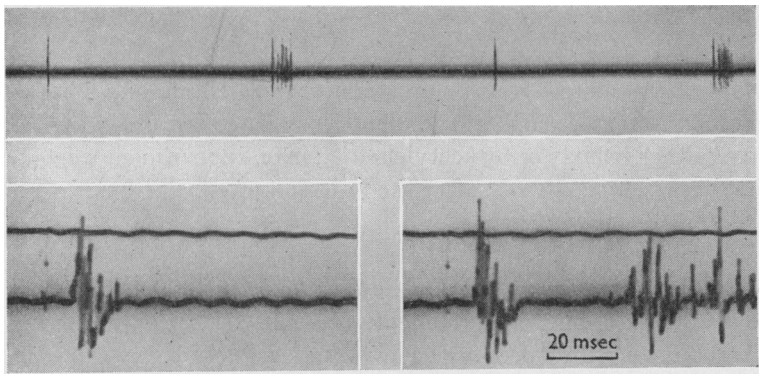

Fig. 9. Same experiment as Fig. 8. Upper record taken 2 min after eserine, showing reflex elicited every 2 sec followed twice by spontaneous outburst of activity. The first two reflexes are shown on lower record with a faster time scale (time, 20 msec).

Further, our results on the cervical cord suggest that acetylcholine acts predominantly on interneurones, although a direct effect on the motor neurones cannot be excluded. The changes produced in the polysynaptic reflex can all be interpreted on this basis. The decrease in the latency of the reflex is of the order of 2 msec; changes in conduction velocity could hardly account for this large reduction, which must therefore occur at synapses, perhaps by the by-passing of one or more of them. Facilitation at these synapses could result from direct depolarization of the post-synaptic region or from bombardment of the synapse by impulses excited by the acetylcholine. Furthermore, the peak of the ventral root potential occurs some 2–4 msec

earlier after acetylcholine; since Brock *et al.* (1952) have shown with an internal electrode that the rising time of the synaptic potential in response to a single presynaptic impulse is less than 1 msec, increasing the rate of rise of this potential would save little time. Changes in the cable properties of the motor neurone would also be insignificant. The decrease in time from the stimulus to the peak of the root potential can only be accounted for satisfactorily by assuming that impulses reach the motor neurone earlier. The main saving in time must therefore be at the synapses of interneurones.

The changes in the slow potentials occurring during a spontaneous discharge initiated by acetylcholine also support the view that acetylcholine acts predominantly on interneurones. These changes consisted of a regular increase in the random fluctuations of the base-line and, in one experiment, of a steady negativity of the cord with respect to the ventral root. If the effect of acetylcholine were predominantly on the motor neurone, one would expect this steady negativity to occur regularly and to be proportional to the intensity of the spontaneous discharge. The most striking non-propagated change that began after acetylcholine was the base-line irregularities on the negative peaks of which the impulses appeared. Such a pattern would result if a population of motor neurones were subjected to a random bombardment of presynaptic impulses. The mean potential would probably tend to be negative to that in the quiescent state, but it would depend on the duration and intensity of the slow positive and negative components of the root potential. Similar changes in the excitability of the motor neurone would be expected; thus, the excitability after an injection of acetylcholine should fluctuate more than before, and the mean level of excitability might be expected to rise, depending on the duration and intensity of the two phases of the change in excitability that follows a reflex discharge (Bernhard & Therman, 1947). When we use the level of the root potential at which the first impulse appears as an index of the excitability of the motor neurone, our results are in accord with these expectations.

All the evidence, therefore, suggests that the predominant effect of acetylcholine injected into the spinal cord is to excite some part of some interneurones, either by synaptic depolarization which could account directly for the synaptic facilitation and the spontaneous discharge, or by excitation of impulses at a non-synaptic point which would facilitate the interneuronal synapses by impulse bombardment.

SUMMARY

1. A method is described for close arterial injections into the cervical spinal cord through the basilar artery. The effects of acetylcholine, injected by this route, on the activity of the cord were recorded from ventral rootlets of C1.

2. Acetylcholine caused an outburst of impulses lasting for 2–40 sec. This was regularly accompanied by an increase in the base-line fluctuations which represent changes in the potential of the cord relative to the rootlet. Most of the impulses arose from the negative peaks of these fluctuations.

3. Acetylcholine increased the area of the electrically excited ipselateral reflex and decreased its latency by about 2 msec.

4. Acetylcholine usually increased the amplitude of the ventral root potential and always decreased the interval between the stimulus and the peak of the root potential by 2–4 msec.

5. Acetylcholine increased the variability of the level of the root potential at which the first impulse arose.

6. Eserine and prostigmine prolonged the effects of acetylcholine and occasionally modified them.

7. The effects of acetylcholine are all compatible with the view that its action is predominantly on interneurones.

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