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EFFECTS OF ACETYLCHOLINE ON THE ACTIVITY OF THE LUMBOSACRAL CORD OF THE CAT

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Acetylcholine has been known, for many years, to influence the activity of the spinal cord (see review by Feldberg, 1950). Feldberg, Gray & Perry (1953) made close arterial injections of acetylcholine (ACh) into the basilar arteries of cats anaesthetized with chloralose; recording from the first cervical ventral roots they observed increased numbers of impulses and increases in the reflexes and root potentials. Recently Curtis, Eccles & Eccles (1957) have injected ACh into the circulation of the lumbosacral cord of cats anaesthetized with pentobarbital and observed a reduction in the amplitude of certain reflexes. They argued from their results that the known effect of ACh on Renshaw cells (Eccles, Eccles & Fatt, 1956) could account for the over-all action of ACh on the spinal cord, and that the excitatory effects observed by Feldberg *et al.* could be explained by the excitation by ACh of afferent receptors (see review by Gray & Diamond 1957). This paper presents experimental results that indicate that ACh must have actions in the spinal cord in addition to those on the Renshaw cells.

METHODS

Preparations. Most experiments were performed on cats whose cords had been divided in the high cervical region and brains destroyed under ether anaesthesia. Satisfactory preparations showed vigorous tendon and withdrawal reflexes. Chloralose anaesthesia, 100 mg/kg, without section of the cord, was used in a number of preparations. Most animals received 1 mg atropine intravenously.

Recording. A laminectomy was performed to expose the low lumbar and sacral spinal cord and was extended caudally for a sufficient distance to obtain the full length of the first sacral ventral root. Records of activity were obtained from ventral rootlets with platinum wire electrodes and from the dorsal horn with glass capillary electrodes of 1–5μ tip diameter filled with 10% NaCl solution. The potentials were amplified with a system having a high input impedance and variable frequency response.

Stimulation. Dorsal roots, the sciatic nerve in the thigh or the sural nerve in the popliteal space were stimulated with short ($< 0{\cdot}5$ msec) pulses from a low impedance source isolated from earth.

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Injections. A number of techniques were used, all of which were intended to allow the ACh to reach the cord and as few other tissues as possible, as rapidly as possible and with a minimal mixing with blood. Of these, three should be mentioned: (1) retrograde cannulation of one internal iliac with tying or snaring the aorta above the seventh lumbar pair and tying all branches below this except other dorsal pairs, the medial sacral artery and in most experiments on the side used for nerve stimulation the external iliac, which was snared; (2) cannulation of one renal artery with the tying of all branches of the admonal aorta except the dorsal pairs and on one side the external iliac, which was snared; or (3) cannulation of a lumbar artery. In exposing the spinal cord all arteries that were seen to supply muscles were tied. In all experiments the cannula consisted of a short piece of stainless steel tube connected to a polythene tube which was passed out of the abdomen. Heparin was used in the cannula.

Solutions. All injections were made with a solution containing (g/l.) NaCl 9·0, KCl 0·42, $NaHCO_3$ 0·15, $CaCl_2$ 0·24. ACh (chloride) was dissolved in this solution immediately before use. Further details of the techniques and procedures have already been published (Fernandez de Molina & Gray, 1957).

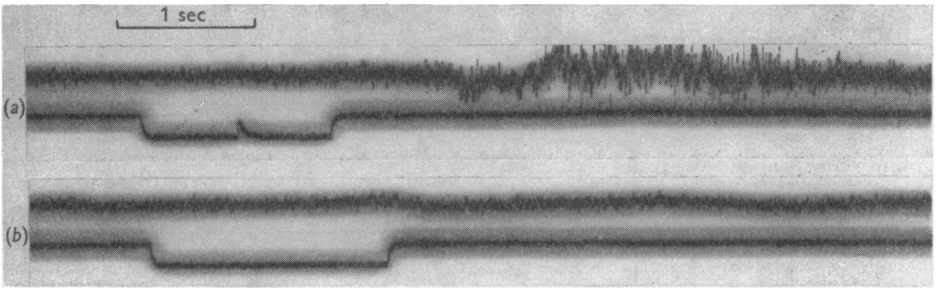

Fig. 1. Activity in dorsal horn and ventral root evoked by ACh. Top traces, records from electrode in dorsal horn of S1. Bottom traces, records from ventral rootlet of S1, and injection signal (approx. only). Time 1 sec. (*a*) injection of ACh, 1 ml. of 10^{-4} mg/ml. (*b*) control, dorsal roots of S1 cut.

RESULTS

Ventral root

Discharge excited by acetylcholine. Spinal preparations that were in good condition responded to injections of ACh (1 ml. of 10^{-4} g/ml.) with discharges of impulses in the ventral roots (Figs. 1–3); this dose was that normally used, but 1 ml. of 10^{-5} g/ml. was shown to be effective. Such discharges were not, however, found regularly in anaesthetized animals; they were only seen in four out of thirteen preparations and these instances were all doubtful. These discharges were seen both in the absence of stimulation (Fig. 1) and when reflexes were excited at regular intervals (Figs. 2, 3). Reflex excitation of this kind sometimes altered the pattern of the discharge. In one experiment ACh caused a discharge which started immediately after each stimulus and then decreased in frequency. Another experiment (Fig. 3) showed the reverse phenomenon; before the injection and at the end of the record the impulses occurred mainly in the early part of the interval, while immediately after the

injection there was a pause that followed the stimulus and the rest of the interval was occupied by a considerable number of impulses.

Ventral root discharges in response to ACh do not appear to be due to an increased afferent activity resulting from excitation of receptors by ACh. The effects of ACh on receptors can be blocked by curarine and by hexamethonium (see Gray & Diamond, 1957), but the discharge still occurred after 22 mg/kg of tubocurarine and after 10 mg/kg of hexamethonium. Such discharges were seen after wide deafferentation (L5 to end) in three experiments. Fig. 2 was recorded after the injection of hexamethonium but before deafferentation, while Fig. 3 was recorded from the same preparation after crushing all dorsal roots below and including L5; there is little difference in the discharges of impulses recorded from the ventral roots.

Reflexes. Our results agree with Curtis *et al.* (1957) in that the usual effect of ACh was to reduce the amplitude of both monosynaptic and polysynaptic reflexes. Increases in reflexes after deafferentation have however been seen twice, one occasion being illustrated in Fig. 3. In this experiment the dorsal root of S1 was stimulated and it can be seen that the injection has caused an increase in the monosynaptic spike while reducing the polysynaptic response. Sometimes an increase in the reflex size has been seen after an initial decrease.

Dorsal horn activity

Discharges elicited by acetylcholine. Increased activity in the dorsal horn was recorded after injections of ACh. No attempt has been made to plot the distribution of this activity, all records having been taken when the electrode had been placed to record activity set up by stimulation of cutaneous nerves (Coombs, Curtis & Landgren, 1956; Fernandez de Molina & Gray, 1957). The activity, which can be seen in Figs. 1–4, consists of spike activity and slower waves. The latter are seen with little spike activity in Fig. 4 and with many spikes superimposed in Fig. 1. This activity follows an injection of ACh after deafferentation or hexamethonium (Figs. 2, 3).

Potentials elicited by stimulation of cutaneous afferents. Fernandez de Molina & Gray (1957) divided the dorsal horn activity that results from cutaneous nerve stimulation into three phases: phase I occurs within 1 msec of the afferent volley and in the region of the internal basilar nucleus; phase II is maximal at 1–3 msec and found in the ventrolateral region of the dorsal horn; phase III is maximal 8–12 msec after the afferent volley. The effect of ACh on all three phases of potential change has been studied. Investigations of phase I did not lead to any definite conclusions, as this response is found in a region in which the potential gradients are large. Control injections, apparently by moving the cord in relation to the electrode, caused changes in the amplitude of this phase. Phase II, which was investigated most frequently, was consistently reduced in amplitude by ACh. This is well shown in Fig. 4. Each

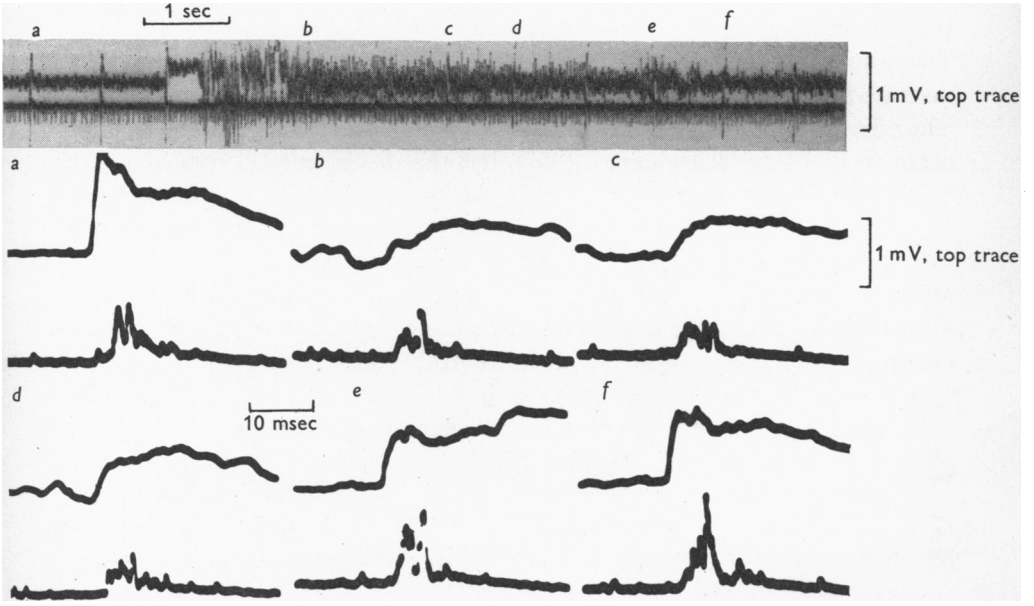

Fig. 2. Effect of ACh, 1 ml. of 10^{-4} g/ml., on activity in dorsal horn and ventral root of a rhythmically stimulated preparation. Top record on slow time scale (1 sec); lower records faster samples (time 10 msec) at times indicated on top record. In all records; top beam from dorsal horn and injection signal (approx. only); bottom beam from ventral root. Animal had received hexamethonium 10 mg/kg. Sural stimulation. (Ventral root spikes retouched in top record.)

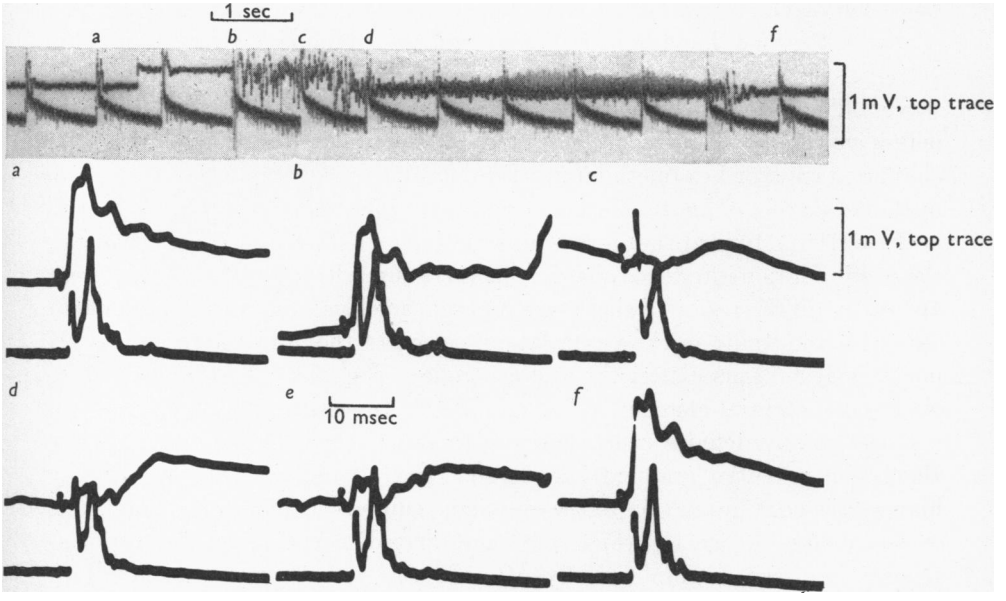

Fig. 3. As fig. 2. Records after dividing all dorsal roots below L5. Dorsal root stimulation. (Ventral root spikes retouched in top record.)

record in the lower part of the figure is a photograph of nine superimposed traces, while the record at the top shows the time course of the same events. The potential recorded is almost entirely a phase II potential, though in this instance it was obtained by stimulation of the dorsal root rather than a pure cutaneous nerve. An injection of ACh reduced phase II to about a third of its original value. Another experiment is illustrated in Fig. 2 but in this the sural

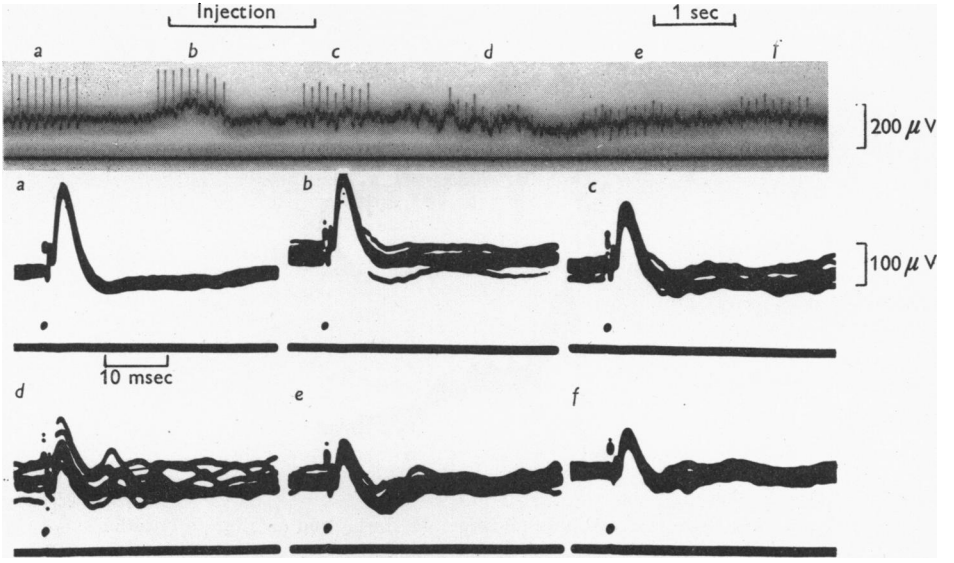

Fig. 4. Effect of ACh, 1 ml. of 10^{-4} g/ml., on phase II of the dorsal horn activity. Top record on slow time scale (1 sec); lower records, photographs of superimposed traces (time 10 msec) of each group of stimuli. In all records; top beam, dorsal horn potential; bottom beam, stimulus. Dorsal roots of S1 cut. Dorsal root stimulation.

nerve was stimulated. The ACh injection virtually abolished phase II. These changes in the activity of the dorsal horn do not appear to be related to changes in afferent inflow. The records shown in Fig. 2 were obtained after the animal had received 10 mg/kg of hexamethonium, which would be expected to block the stimulating action of ACh on receptors. Furthermore, the same results appear in Fig. 3, which was obtained from the same preparation after all dorsal roots caudal to L5 had been divided. In one experiment the changes in phase III were investigated and a considerable reduction in amplitude observed.

Spike amplitude in the records in the lower parts of Figs. 2–4 was considerably attenuated by reducing the high-frequency response of the amplifier. Fig. 5 was recorded with an adequate high-frequency response and it can be seen that there is an increase in the spike activity associated with both phases II and III.

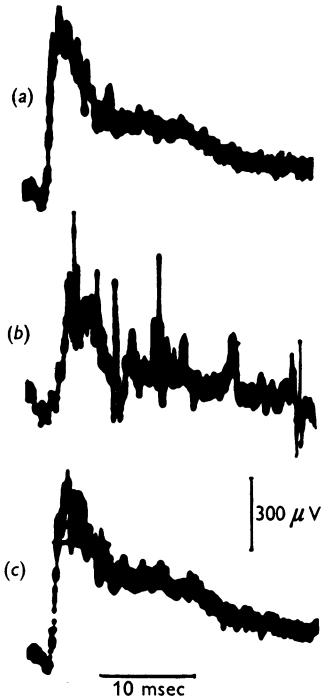

Fig. 5. Records from dorsal horn (*a*) before, (*b*) during and (*c*) after an injection of 1 ml. of 10^{-4} g/ml. ACh.

DISCUSSION

Activity in the dorsal horn and in the ventral root was induced by ACh; that this occurred in preparations in which an increased afferent inflow had been prevented indicates that the activity was set up by a central action of ACh. The behaviour of the lumbosacral cord to injections into the aorta was however markedly different from that of the cervical cord to injections into the basilar artery (reported by Feldberg *et al.* 1953). In the anaesthetized animals of the present series the discharge was usually absent, or if present was very slight; in the experiments of Feldberg *et al.* (1953) on the cervical cord, however, massive discharges were normally observed in animals anaesthetized with chloralose, though such impulse activity could be much reduced or abolished with barbiturates. The explanation of this difference would seem to be that with basilar artery injections the solution goes directly into the vascular supply of the cord; during the injection the segments under consideration become momentarily blood free and in consequence a high concentration gradient between the vascular bed and the tissue is built up very rapidly. It is well known that in order to demonstrate the excitant action of ACh a rapid rise

of concentration may be necessary (Brown, Dale & Feldberg, 1936); it is more likely to be important in the central nervous system where the problem of the 'blood-brain barrier' arises. In the present experiments none of the injection techniques was able to produce more than a local blanching of some vessels in the cord and usually no obvious blanching was seen during an injection. Another important difference is that in the cervical cord preparation only such part of the injection as flowed sufficiently far against the normal direction of blood flow reached other tissues; with the present techniques, the surrounding tissues were injected as efficiently as the cord itself. If we compare the almost complete failure of injections into the anaesthetized animals of this series even when not deafferented, with the success of the experiments described by Feldberg *et al.* (1953) on a preparation which had had the main parts of the first three cervical nerves cut, it seems unreasonable to ascribe the success of the cervical cord experiments to the excitation of peripheral receptors. In the present experiments the low efficiency of the injection technique has been compensated by the ability to use the much more excitable cords of unanaesthetized spinal animals.

Our results agree with those of Curtis *et al.* (1957) that the main effect of ACh on reflex activity in the lumbosacral region is to reduce the reflexes. Contrary to their findings we have on two occasions seen ACh produce an increase in a reflex after deafferentation. The question then is why should ACh have a predominantly depressant effect on reflexes in the lumbosacral preparation and an excitant one in the cervical cord. There is no doubt that inhibitory effects of ACh are to be found (Eccles *et al.* 1956), and the present results confirm the finding of Feldberg *et al.* that excitatory ones also occur; which of the two predominates at any time depends on the organization and state of activity of the particular part of the cord. There are certainly differences between the organization in the lumbo-sacral cord and in the cervical cord; it would be interesting to know if antidromic (Renshaw) inhibition was as marked in the cervical region as in the lumbosacral. An important point to emphasize is the variability of these phenomena; for example, one spinal preparation gave an increase in reflexes with ACh when they were excited by sural stimulation but a decrease when they were excited by dorsal root stimulation; similarly, the activity that follows immediately after a reflex response has been both increased and decreased by ACh.

The potential changes in the dorsal horn that can be elicited by sural stimulation show a decrease in amplitude during an injection of ACh. These changes can hardly be due to activation of Renshaw cells. It is impossible to be certain about the cause of this decline in amplitude. It could be due to the excitation of inhibitory pathways, which converge on those stimulated electrically, but it could also be due to the fact that these artificially stimulated potentials are depressed when they occur at the time of the intense

asynchronous activity that results from the ACh injection. During such intense asynchronous activity many cells are refractory at any one time so that a synchronous response to stimulation becomes smaller.

SUMMARY

1. Techniques for injecting solutions into the circulation of the lumbosacral cord have been used to investigate the action of acetylcholine on its activity.

2. In spinal animals acetylcholine itself excited a discharge of impulses in the ventral root. This is not due to an increased afferent inflow. This response was seldom seen in anaesthetized animals and if it was present it was very small.

3. Reflexes, monosynaptic and polysynaptic, were normally reduced in amplitude (Curtis *et al.* 1957). Increases have, however, been seen in deafferented preparations.

4. Acetylcholine excited activity in the dorsal horn and caused a reduction in amplitude of the potential changes that result from cutaneous nerve stimulation.

5. It is argued that acetylcholine has an excitant action on the spinal cord. Its over-all effects cannot be entirely explained by its known action on the Renshaw cells.

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