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Central effects of picrotoxin when acting from the liquor spaces in anaesthetized cats

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Summary

1. Picrotoxin was perfused through different parts of the cerebral ventricles in cats anaesthetized with chloralose, and applied topically to the upper cervical cord in cats anaesthetized with intraperitoneal pentobarbitone sodium. With both methods, picrotoxin produced effects similar to those produced by tubocurarine; but it was active in weaker concentrations.

2. Perfused through the third ventricle, picrotoxin caused shivering resulting in a rise of rectal temperature, increased motor excitability, muscle jerks, tachypnoea, mydriasis, and withdrawal of the nictitating membranes. The effects were due to an action on structures in the walls of the ventral half of the ventricle, because they occurred only on perfusion of tubocurarine through this half and not on its perfusion through the dorsal half of the third ventricle. Noradrenaline perfused through the third ventricle abolished the shivering and the rise in temperature, but did not affect the motor hyperexcitability or the muscle jerks, whereas pentobarbitone sodium similarly perfused abolished these effects as well.

3. Perfused through the inferior horn of a lateral ventricle, picrotoxin caused excitation of the hippocampus. This resulted in a rhythmic discharge of high voltage negative spikes in the electrocorticogram taken from the occipital cortices. The spikes developed after-positivity with after-discharges and were interrupted from time to time by bursts of fast activity termed episodes. This abnormal discharge was recorded also from the perfusion cannula which was inserted into the inferior horn and acted as a lead from the surface of the hippocampus.

4. Perfused through the anterior horn of a lateral ventricle picrotoxin caused a rhythmic discharge of negative spikes which was recorded from the anterior horn cannula and resulted from excitation of the grey matter in the anterior limbic area which forms part of the medial wall of the horn.

5. Applied to the surface of the upper cervical cord, picrotoxin produced scratching movements.

Introduction

When picrotoxin is applied by microinjection into the hippocampus (Baker, Kratky & Benedict, 1965), or into the grey matter of the cerebral gyri (Banerjee,

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Feldberg & Georgiev, 1970) it acts like tubocurarine. Injected into the hippocampus, both substances cause hippocampal activation, and injected into the cerebral gyri they set up foci of excitation which give rise to a rhythmic discharge of negative spikes in the lead from the injection site.

In the present experiments it is shown that picrotoxin produces the same effects as tubocurarine also when acting from the liquor spaces—that is, when it is perfused through the third ventricle, the inferior or anterior horn of a lateral ventricle, or when it is injected into the cisterna magna or applied topically to the upper cervical cord. With all these methods, picrotoxin was found to be more potent than tubocurarine.

Methods

Cats of both sexes weighing between 2·8 and 3·5 kg were anaesthetized with either chloralose or pentobarbitone sodium. The trachea was cannulated and with the cat lying on its belly, the head was fixed to the ear bars and mouthpiece of a Moruzzi-Dell stereotaxic instrument.

Perfusion of cerebral ventricles

For these experiments, the cats were anaesthetized with chloralose (60 mg/kg) injected into the left femoral vein which was cannulated in anaesthesia induced by ethyl chloride and ether. Picrotoxin was perfused either through the third ventricle, or its dorsal or ventral part, or through the inferior or through the anterior horn of a lateral ventricle. The methods of perfusion and the perfusion cannulae used were those described previously (Carmichael, Feldberg & Fleischhauer, 1964). The perfusion fluid was the artificial cerebrospinal fluid of Merlis (1940).

Perfusion of picrotoxin through the third ventricle. The picrotoxin was perfused from a cannula inserted into the third ventricle to the cannulated aqueduct and its entrance into the lateral ventricles was prevented by their perfusion with artificial cerebrospinal fluid. The third ventricle cannula was inserted 18 to 20 mm deep in the midline and 12·5 mm anterior to the interaural line. The cannulae (Collison cannulae) in the lateral ventricles were inserted 9 mm deep, 7 mm lateral from the midline and 3 mm anterior to the interaural line. The rate of perfusion was 0·1 ml/min through the third ventricle, and 0·5 ml/min through each lateral ventricle cannula. The concentration of picrotoxin in the third ventricle was thus diluted by half.

When either the dorsal or ventral part of the third ventricle was perfused with picrotoxin a double bore cannula was used and inserted 22 mm deep, so that the opening of its protruding inner tube was below that of its outer tube above the massa intermedia (see inset of Fig. 1). The rate of perfusion was 0·1 ml/min through each tube and 0·05 ml through each lateral ventricle cannula. The concentration of picrotoxin in the dorsal or ventral part of the third ventricle was thus diluted by one-third.

During these perfusions rectal temperature was measured at room temperature (19°–22·5° C) by a thermistor probe inserted about 10 cm into the rectum and held in position by gently wrapping the protruding end of the probe to the root of the tail with adhesive tape. Temperature was monitored continuously by a Kent multichannel recorder. Figures 1 and 2 are plotted directly from the tracings obtained in this way.

Perfusion of picrotoxin through an inferior horn. The picrotoxin was perfused from a cannula inserted into the left inferior horn to the cannulated aqueduct, and its entrance into the left anterior horn and into the right lateral ventricle was prevented by their perfusion with artificial cerebrospinal fluid. With this arrangement the picrotoxin passed not only through the inferior horn but also through the third ventricle and the rostral end of the aqueduct. The insertion of the inferior horn cannula was 14–17 mm deep, 13·5 mm lateral from the midline and 5 mm anterior to the interaural line; that of the left anterior horn cannula, 14–15 mm deep, 1·5–3 mm lateral from the midline and 2 mm posterior to the coronal suture; that of the cannula (Collison cannula) in the right lateral ventricle, 11 mm deep, 2 mm lateral from the midline and 8 mm anterior to the interaural line. The rate of perfusion through the left inferior horn cannula and through the right lateral ventricle cannula was 0·05 ml/min and through the left anterior horn cannula 0·1 ml/min.

Perfusion of picrotoxin through an anterior horn. Cannulation was the same as for perfusion of picrotoxin through the inferior horn but the picrotoxin was perfused through the anterior horn cannula and the rate of perfusion through this cannula and that in the right lateral ventricle was 0·05 ml/min, and that through the inferior horn cannula was 0·1 ml/min. Again the third ventricle and the rostral end of the aqueduct were perfused with picrotoxin in addition to the left anterior horn.

At the end of each perfusion experiment, the correct position of the cannula perfusing the picrotoxin and the areas perfused by the drug were ascertained by substituting the dye bromophenol blue for the picrotoxin and continuing perfusion for 10 min. The brain was then fixed by perfusing the head first with 0·9% NaCl solution and then with 10% formalin through the cannulated aorta after clamping the heart and cutting the jugular veins. Following exposure of the brain and dissection of the ventricular cavities, the areas stained by the dye were confirmed by inspection.

During perfusion of the inferior or anterior horn with picrotoxin the electrical activity from various sites in the brain was recorded simultaneously with monopolar electrodes connected to an Offner pen recorder. The right metal ear bar of the stereotaxic instrument was earthed and served as indifferent electrode. The time constant of the Offner pen recorder was set at 0·3 s and the high frequency limit at 500 Hz. The perfusion cannulae inserted into the inferior and anterior horns were insulated except at the tip and served as recording electrodes as well; they acted as leads from the walls of the horn. In addition, the electrical activity was recorded from the occipital cortex on each side with epidural electrodes consisting of platinum wire held in a nylon screw and inserted through burr holes over the middle suprasylvian gyrus. When the anterior horn was perfused with picrotoxin the electrical activity was further recorded from an insulated steel needle electrode with a free tip of about 0·5 mm inserted stereotaxically 15 mm deep into the left anterior limbic area at a point 1 mm lateral from the midline and 2 mm posterior to the coronal suture. The location of the tip was ascertained histologically at the end of the experiment after the brain was fixed with formalin, and sections of the brain cut on the freezing microtome and stained by the method of Klüver & Barrera (1953). The diagram in the inset of Fig. 4 showing the needle tract was from such a section.

Cisternal injections and topical application of picrotoxin to the upper cervical cord

Picrotoxin was applied by these methods to find out if it would produce scratching movements, and since those produced by tubocurarine similarly applied were obtained only in pentobarbitone sodium but not in chloralose anaesthesia (Feldberg & Fleischhauer, 1960) the experiments were done in cats anaesthetized with intraperitoneal pentobarbitone sodium (35 mg/kg).

For the injections of picrotoxin into the cisterna magna the atlanto-occipital membrane was exposed by dissecting away the muscles covering the membrane. For the topical application, small pledgets of cotton wool soaked in a picrotoxin solution were applied for at least 15 min to the dorsal surface of the upper cervical cord which was exposed as described by Feldberg & Fleischhauer (1960). They also give the method for recording the scratching movements in the tibialis anterior muscle. To record these movements the leg was fixed in a flexed position by a drill bored through the condyle of the femur, and another through the lower end of the tibia ; the freed tendon was then attached to a spring lever, the movements of which were transmitted to a strain gauge coupled to an Offner dynograph.

Drugs. Picrotoxin (Ralph N. Emanuel Ltd., Alperton, Middlesex) ; nikethamide, which was kindly given to us by Dr. Graham Lewis of Ciba Laboratories, Horsham, Sussex ; noradrenaline acid tartrate (Hoechst), amphetamine sulphate, cocaine hydrochloride, nicotine hydrogen tartrate and prostigmine methyl sulphate (B.D.H.) and Flaxedil (gallamine triethiodide, May & Baker). The amounts given in the text of the last six drugs refer to the salts. All drugs were freshly dissolved in artificial cerebrospinal fluid.

Results

Picrotoxin

Perfusion of picrotoxin through the third ventricle cannula

When perfused from third ventricle to aqueduct, picrotoxin, like tubocurarine, caused shivering which resulted in a rise of rectal temperature, increased motor excitability, spontaneous muscle jerks, tachypnoea, mydriasis, withdrawal of the nictitating membranes and sometimes salivation. Shivering was always the first effect to be observed, and it occurred with concentrations too weak to elicit the other effects. To a certain extent the sensitivity was dependent on the depth of anaesthesia. In deep anaesthesia, stronger concentrations of picrotoxin were required to produce shivering as well as the other effects.

On perfusion of picrotoxin 1/5,000 to 1/20,000 through the third ventricle cannula, shivering began often within 1 to 2 min, appeared first in the flanks, spread quickly to the trunk, shoulder girdle, neck and head, so that within a few minutes the cat was shivering vigorously all over its body. Shivering persisted not only throughout the period of picrotoxin perfusion, which in the present experiment varied between 10 and 60 min, but continued sometimes for over 2 h whilst the third ventricle was perfused with artificial cerebrospinal fluid. The shivering led to a rise in rectal temperature. Experiment 1 of Fig. 1 illustrates a strong and long-lasting rise produced by a 10 min perfusion of picrotoxin 1/20,000 through the third ventricle cannula.

With weaker concentrations of picrotoxin, shivering occurred later, was not so vigorous, stopped earlier after discontinuation of the picrotoxin perfusion and the effect on rectal temperature sometimes consisted solely of an interruption of the fall produced by the chloralose anaesthesia. Such an effect is illustrated in experiment 2 obtained from an experiment in which picrotoxin 1/100,000 was perfused through the third ventricle cannula for 20 min.

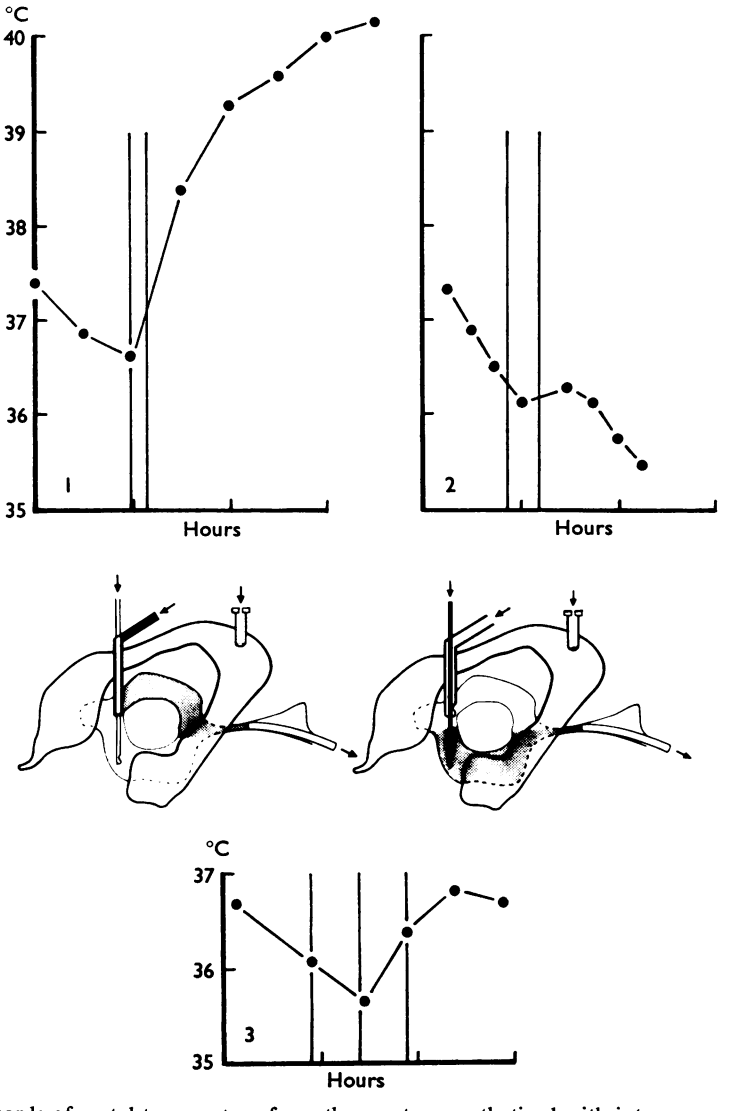

FIG. 1. Records of rectal temperature from three cats anaesthetized with intravenous chloralose during perfusion of the cerebral ventricles. Records begin 46 to 60 min after the chloralose injection. Artificial cerebrospinal fluid delivered at a rate of 0·05 ml/min into each lateral ventricle and of 0·1 ml/min either into the third ventricle (experiments 1 and 2) or into each half, dorsal and ventral, of this ventricle (experiment 3). Outflow from cannulated aqueduct. The two vertical lines indicate, in experiment 1, 10 min perfusion with picrotoxin 1/20,000, and in experiment 2, 20 min perfusion with picrotoxin 1/100,000 delivered through the third ventricle cannula. The three vertical lines in experiment 3 indicate two consecutive 30 min perfusions of picrotoxin 1/25,000 delivered for the first 30 min into the dorsal and for the second 30 min into the ventral half of the third ventricle through a double bore cannula, as shown diagrammatically in the inset.

Shivering and the other effects were due to an action on structures in the walls of the ventral half of the third ventricle because they did not occur when the picrotoxin passed through only the dorsal half of the ventricle. This is illustrated for the effect on temperature in experiment 3 of Fig. 1. A 30 min perfusion of picrotoxin 1/25,000 through the dorsal half produced no hyperexcitability, no shivering, and temperature which had been falling as a result of the chloralose anaesthesia continued to fall unchanged, but when the picrotoxin solution was then perfused, for 30 min, through the ventral half, vigorous shivering occurred within 2 min, temperature rose, and 10 min later hyperexcitability developed.

Hyperexcitability was characterized by muscle jerks in response to auditory or tactile stimuli. A loud clap or tapping the spine with a finger caused a forceful propulsion of the trunk of the cat, due to a sudden extension of the limbs, which lifted the body several millimetres from the surface of the table. The condition resembled the hyperexcitability of the early stage of chloralose anaesthesia. On continued picrotoxin perfusion the jerks in response to auditory or tactile stimuli became more violent and finally jerks occurred apparently spontaneously, without the application of external stimuli. The jerks were mainly in the forelegs, occurred at irregular intervals varying between 2 and 12/min, and in some instances were followed by short myoclonus. When perfusion with picrotoxin was then discontinued, and the third ventricle was perfused with artificial cerebrospinal fluid, it took 1 to 2 h before these effects disappeared.

Tachypnoea was not pronounced ; the rate of respiration usually did not increase more than 30%. However, when spontaneous muscle jerking developed in the course of the perfusion it became difficult to assess the rate of respiration by simple observation.

The pupils, which were usually slit-like before the picrotoxin perfusion, dilated gradually and became maximally dilated within 60 min. At that time the previously protruded nictitating membranes were withdrawn.

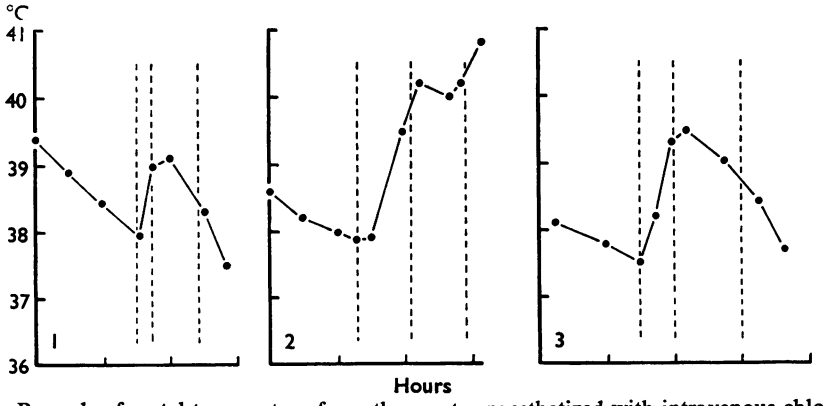

FIG. 2. Records of rectal temperature from three cats anaesthetized with intravenous chloralose during perfusion of the cerebral ventricles. Records begin 20 to 40 min after the chloralose injection. Artificial cerebrospinal fluid delivered into each lateral ventricle at a rate of 0·05 ml/min and into the third ventricle at a rate of 0·1 ml/min. Outflow from cannulated aqueduct. Between the first and second dotted vertical line, perfusion through third ventricle cannula of picrotoxin 1/10,000 in experiment 1 for 12 min, and 1/20,000 in experiment 2 for 42 min, and in experiment 3 for 32 min. Between the second and third dotted vertical line perfusion through third ventricle cannula of noradrenaline 1/10,000 in experiment 1 for 53 min, of noradrenaline 1/20,000 in experiment 2 for 40 min, and of pentobarbitone sodium 1/250 in experiment 3 for 60 min.

Effect of noradrenaline. On perfusion of noradrenaline 1/10,000 or 1/20,000 through the third ventricle, the shivering produced by picrotoxin was abolished and temperature stopped rising, or fell. The motor hyperexcitability and muscle jerks, however, were not affected. Following a short period of picrotoxin perfusion, the inhibitory effect of noradrenaline on shivering was maintained, but following a longer period of picrotoxin perfusion, shivering recurred after some time and temperature rose again, although the noradrenaline perfusion continued. This is illustrated by experiments 1 and 2 of Fig. 2.

In experiment 1, picrotoxin 1/10,000 had been perfused for 12 min before noradrenaline, also in a concentration of 1/10,000, was delivered through the third ventricle cannula. The shivering and the rise in temperature produced by the picrotoxin were abolished and when perfusion was then continued, without noradrenaline, shivering did not recur and temperature continued to fall. In experiment 2, picrotoxin 1/20,000 had been perfused for 42 min before the noradrenaline 1/20,000 was delivered through the third ventricle cannula. Again, shivering stopped and temperature began to fall, but after about half an hour shivering recurred and as it became stronger, temperature began to rise although perfusion with noradrenaline had not been discontinued.

Effect of pentobarbitone sodium. Injected intravenously in a dose of 18 mg or perfused through the third ventricle cannula in a concentration of 3–4 mg/ml, pentobarbitone sodium abolished not only shivering and the rise in temperature, but also the hyperexcitability and the muscle jerks. Experiment 3 of Fig. 2 shows the effect of pentobarbitone sodium (4 mg/ml) perfused through the third ventricle cannula on the rise in temperature produced by picrotoxin 1/20,000 similarly perfused. Once shivering, muscle jerks and motor hyperexcitability were abolished by the pentobarbitone sodium perfusion, they did not reappear even when perfusion was continued without pentobarbitone sodium, and temperature continued to fall.

Perfusion of picrotoxin through an inferior horn cannula

On perfusion of picrotoxin through an inferior horn of a lateral ventricle, an abnormal discharge of surface negative high voltage spikes appeared in the electrocorticogram taken from the occipital cortices. The spikes appeared first in the lead from the ipsilateral and then synchronously in that from the contralateral cortex; they developed a pronounced after-discharge and were interrupted from time to time by a burst of fast activity, termed "episode", followed by a short period of electrical silence during which no abnormal spikes were recorded. When the electrical activity was recorded also from the inferior horn cannula which acted as a lead from the surface of the hippocampus, negative spikes appeared in this lead a few seconds earlier than in the lead from the ipsilateral occipital cortex, and they were of higher voltage. Later spikes with after-discharges, episodes and periods of electrical silence occurred synchronously in all leads.

The abnormal discharge resembled that produced by tubocurarine, but on perfusion with picrotoxin the threshold concentration was weaker, about 1/200,000 as compared with about 1/20,000 on perfusion with tubocurarine, the incidence of episodes was greater, but on terminating the perfusion with picrotoxin the discharge did not persist as long as after a tubocurarine perfusion.

The various features of the abnormal discharge are illustrated in Fig. 3. Experiment 1 shows the discharge during a perfusion of picrotoxin 1/200,000. Negative spikes appeared 30 min after the onset of the picrotoxin perfusion, and (a) was taken 10 min later when the discharge had appeared in all three leads. The spikes recorded from the inferior horn cannula had a voltage of about 4 mV, as compared with less than 1 mV and to about 0·2 mV for the spikes recorded in the leads from the ipsilateral and contralateral occipital cortex. One minute after (a), when (b) was taken, an episode occurred ; it was followed by a period of electrical silence which lasted for over a minute. The reappearance of the discharge is shown at (c), taken 2 min after (b). Perfusion with picrotoxin was then terminated and

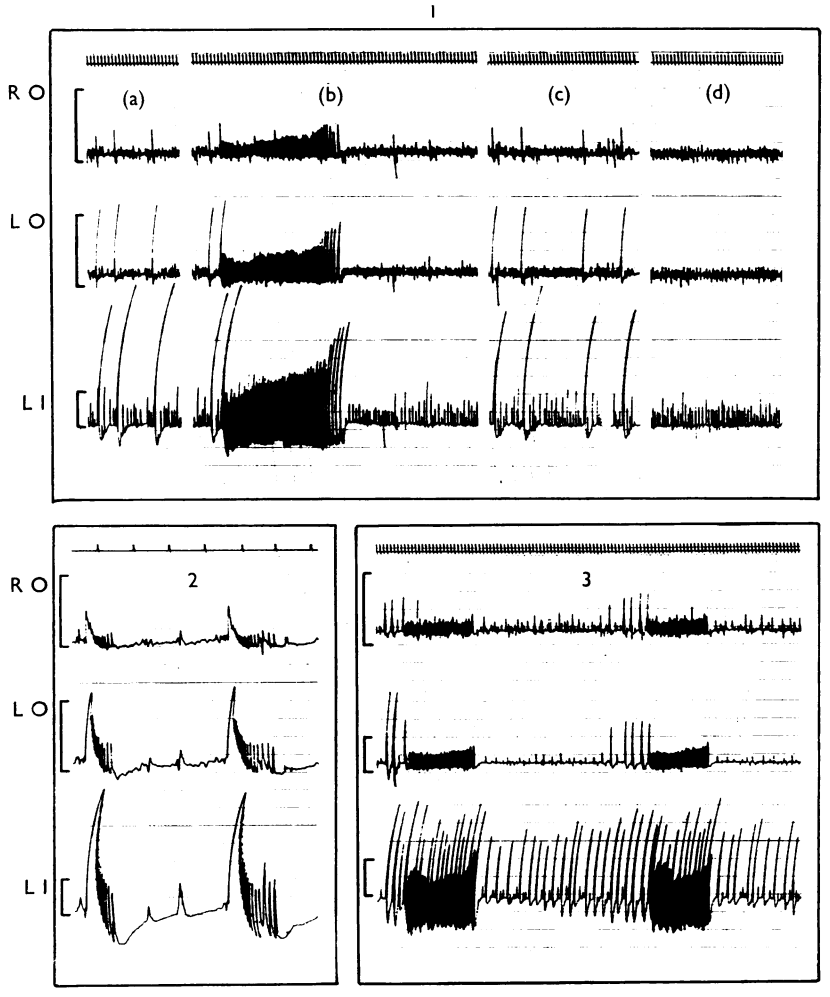

FIG. 3. Monopolar records of electrical activity from the surface of the right and left occipital cortex (RO and LO) as well as from the cannula inserted into the left inferior horn (LI) during its perfusion with picrotoxin in three cats anaesthetized with intravenous chloralose. Experiment 1, perfusion of picrotoxin 1/200,000 for 45 min through inferior horn followed by its perfusion with artificial cerebrospinal fluid ; (a), (b) and (c) obtained 40, 41 and 43 min after beginning, and (d) 30 min after termination of the picrotoxin perfusion. Experiments 2 and 3, perfusion of picrotoxin 1/50,000 through inferior horn. Calibration 1 mV, negativity upwards. Time marker in seconds.

perfusion was continued with artificial cerebrospinal fluid. The spike discharge had disappeared 30 min later, when (d) was taken. After a perfusion with a threshold concentration of tubocurarine—1/20,000—for a similar time, the abnormal discharge would have continued for about 2 hours. In one experiment in which picrotoxin 1/5,000 was perfused through an inferior horn for 20 min the spikes appeared within 5 min and continued for about 2 h, whereas after a 20 min perfusion with tubocurarine 1/500 they would have continued for over 3 hours.

When recording at slow speed the after-discharge was revealed merely by a thickening of the spikes. To bring out the after-discharge more clearly recording had to be at a faster speed. This is shown in experiment 2 of Fig. 3 which illustrates the pronounced after-discharge of two spikes obtained during a perfusion with picrotoxin 1/50,000.

The perfusion cannula inserted into the inferior horn acted as a lead not only from the surface of the hippocampus, but also from those areas of cerebral cortex which lie ventral to the hippocampus, that is, the Area entorhinica, which forms part of the pyriform cortex, and the adjacent more laterally situated Area perirhinica. In previous experiments (Feldberg & Lotti, 1970) in which tubocurarine was perfused through an inferior horn, it was found that when the tip of the cannula had penetrated one of these areas, some of the tubocurarine diffused into them and there set up foci of excitation which gave rise to a discharge of negative spikes in the record from the perfusion cannula. These spikes which could activate the hippocampus differed from those of hippocampal origin in that they occurred at a greater frequency, developed no after discharges, continued during an episode and the subsequent period of electrical silence and were not conducted to the occipital cortices. The same effect was sometimes produced on perfusion of picrotoxin through an inferior horn. Experiment 3 of Fig. 3 was obtained from such an experiment. It illustrates the continuation of abnormal negative spikes, recorded from the inferior horn cannula, during an episode and its subsequent period of electrical silence ; this did not happen in (b) of experiment 1 in which the picrotoxin had apparently not diffused into the cerebral cortex lying ventral to the hippocampus.

Since the picrotoxin perfused through the inferior horn cannula passed through the third ventricle on its way to the cannulated aqueduct the effects described in the previous section on perfusion of picrotoxin through this ventricle alone were also observed. Of these effects only the muscle jerks interfered with the electrical recording of the hippocampal discharge ; when they occurred they were abolished by intravenous injection of 4–6 mg/kg of Flaxedil, and perfusion was continued under artificial ventilation.

Perfusion of picrotoxin through an anterior horn cannula

When picrotoxin was perfused through the anterior horn of a lateral ventricle and the anterior horn cannula served as an electrode it recorded a rhythmic discharge of negative spikes. Such a discharge was also obtained when tubocurarine was perfused through an anterior horn and was shown to result from an excitatory action on the grey matter of the anterior limbic area lying rostral to the septum pellucidum (Feldberg & Fleischhauer, 1967).

Figure 4 illustrates the effect of picrotoxin 1/20,000, perfused through the left anterior horn cannula, on the electrical activity recorded from this cannula and from a needle electrode inserted into the left anterior limbic area. The spike discharge began 12 min after the onset of the picrotoxin perfusion (at (b)) and was fully developed 10 min later (at (c)). Negative spikes were then recorded synchronously in both leads but they were of higher voltage in the lead from the anterior limbic area. At the beginning, at (b) only those spikes which had attained a high voltage in this lead were also recorded from the anterior horn cannula. With the others, no synchronous spikes were clearly distinguishable from the background activity in the record from the anterior horn cannula. The differences in the two leads are explained by the fact that the discharge originates in the anterior limbic area because the tip of the perfusion cannula is not in so close proximity to this area as the needle electrode. No abnormal spikes were recorded in the lead from the occipital cortex.

As the picrotoxin perfused through the anterior horn cannula passes through the third ventricle it produced also those effects obtained on perfusion through this ventricle alone. Therefore 4–6 mg/kg of Flaxedil was injected intravenously when muscle jerks developed, and perfusion was continued under artificial ventilation.

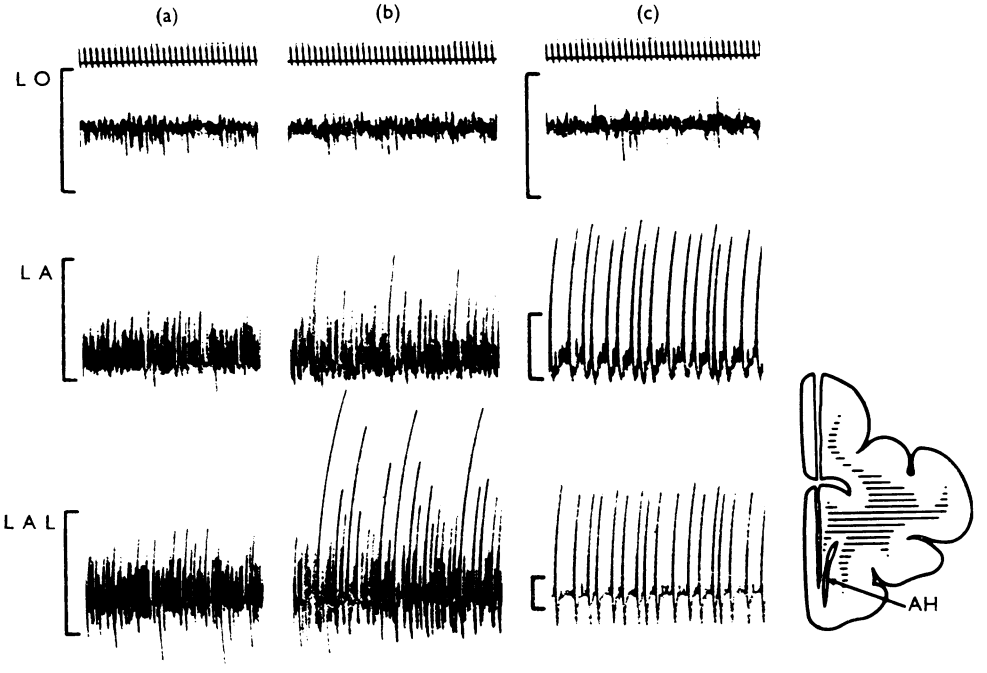

FIG. 4. Monopolar records of electrical activity from surface of the left occipital cortex (LO), from cannula inserted into the left anterior horn (LA) and from needle electrode inserted into the left anterior limbic area (LAL) during perfusion of the cerebral ventricles. Records (a) before, (b) and (c) 12 and 22 min after beginning of perfusion with picrotoxin 1/20,000 through left anterior horn cannula. Calibration mV; negativity upwards. Time marker in seconds. Inset on the right, drawing made from brain section showing the tract of the needle electrode in anterior limbic area; AH, anterior horn.

Cisternal injections and topical application of picrotoxin to the upper cervical cord

In preliminary experiments picrotoxin (2 mg in 0·2 ml) injected into the cisterna magna (or into a lateral cerebral ventricle) was found to produce bursts of strong scratching movements which began about 10 min after the injections and recurred at irregular intervals for over half an hour. It has been shown that scratching movements when produced by tubocurarine, bromophenol blue (Feldberg & Fleischhauer, 1960 ; Domer & Feldberg, 1960), or morphine (Mehes, 1938 ; Königstein, 1939 ; Banerjee, Feldberg & Lotti, 1968) injected in this way result from an action of the drugs on superficial structures of the upper cervical cord. The same site was found to be responsible for picrotoxin because scratching movements were elicited by applying a pledget of cotton wool soaked in a solution of picrotoxin 1/1,000 or 1/2,000 to the dorsal surface of the cervical cord at the region of C_1 and C_2.

When picrotoxin was applied to one side of the cord, bursts of scratching movements occurred on that side only ; they were accompanied by strong extension and abduction of the foreleg of the other side. The first effect, however, was fasciculation in the muscles of the thigh, of the lower part of the back and the leg, but again on the ipsilateral side only. At this stage, before the scratching movements occurred, it was often possible to elicit a scratch reflex on the side of picrotoxin application, by rubbing the pinna or the skin at the base of the ear, or by blowing air into the ear. Later a scratch reflex could be elicited in the intervals between the bursts of scratching movements, and for about one hour when these had disappeared after removing the pledget soaked in picrotoxin solution and instead applying several times one soaked in saline solution.

The scratching movements, like those of the scratch reflex, began with a strong sustained flexion at the hip, so that the foot was brought forward along the side of the body. From this position, strong myoclonic bursts of movements of the leg were set off by rhythmic alternate flexion and extension at the hip, knee and ankle. In the experiment of Fig. 5, scratching movements were recorded from the tibialis anterior muscle and the rhythmic contractions are seen to be set off by a strong, more sustained initial contraction. The upper record shows a strong burst of scratching movements followed by two weaker bursts and interspersed by some irregular contractions. The lower record shows a scratch reflex elicited by rubbing the skin of the ear at its base. The scratching movements came to an end after about 5 s, although rubbing of the ear continued for another 6 seconds. Such " exhaustion " was always obtained.

When picrotoxin was applied to both sides of the dorsal surface of the upper cervical cord, bursts of scratching movements occurred alternately in both hind legs.

Other drugs

Nicotine, perfused from an inferior horn to aqueduct in a concentration of 1/100, did not produce shivering and did not activate the hippocampus. The only effect observed was a reduction in background activity recorded from the inferior horn cannula. The nicotine used for these perfusions was the hydrogen tartrate, but the

reduction in background activity was not due to tartaric acid since perfused through the inferior horn in a concentration of 1/100 it did not produce this effect.

The perfusion with nicotine 1/100 also did not render the hippocampus insensitive to the action of picrotoxin but it delayed the onset of the hippocampal spike discharge produced by picrotoxin, attenuated the after-discharge of the spikes and reduced the incidence of episodes. For instance, on perfusion of picrotoxin 1/20,000 through an inferior horn the abnormal high voltage negative spikes recorded in the leads from the occipital cortices and from the inferior horn appeared usually within 10 to 15 min and rapidly developed pronounced after-discharges which

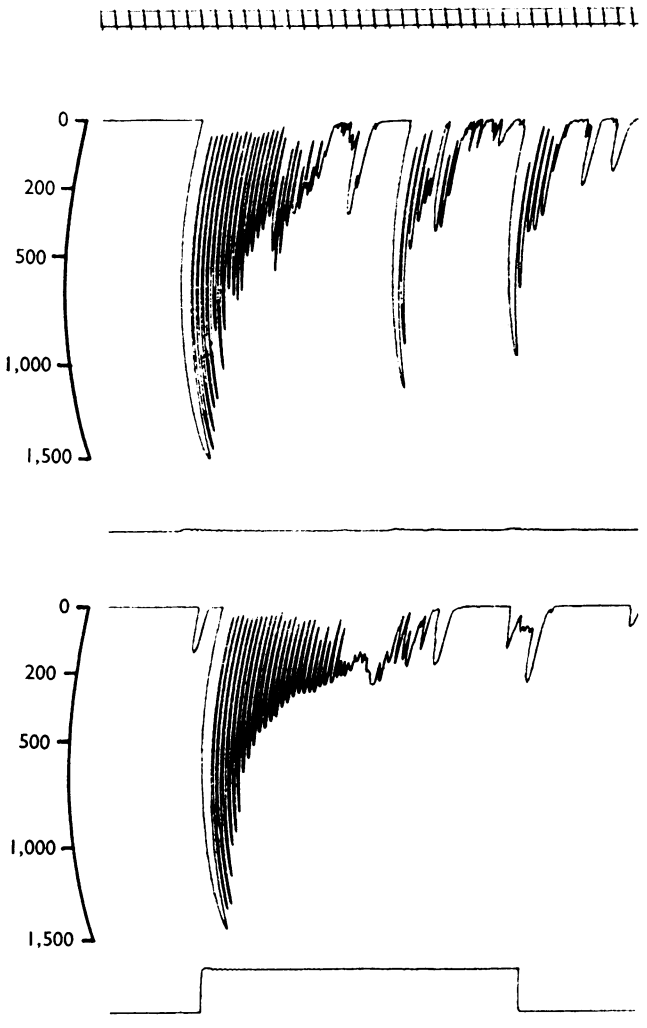

FIG. 5. Scratching movements recorded from right anterior tibialis muscle of a cat anaesthetized with intraperitoneal pentobarbitone sodium during topical application of picrotoxin 1/1,000 to the right side of the dorsal surface of the spinal cord at the region of C_1. Upper record: three bursts of scratching movements. Lower record: scratching reflex elicited during an interval between bursts of scratching movements by rubbing the base of the right ear for 11 s as indicated by the signal at the bottom of the record. On the left, tension in grammes. Time marker in seconds.

frequently led to episodes. On the other hand, after a previous perfusion, for 1 h, of nicotine 1/100 through the inferior horn, the abnormal spikes on subsequent perfusion with picrotoxin appeared only after a delay of 30 to 60 min, their after-discharges were not pronounced and rarely led to episodes. The same result was obtained when nicotine was perfused together with picrotoxin.

Cocaine (1/10,000 and 1/100), amphetamine (1/1,000 and 1/500), nikethamide (1/100 and 1/20), and prostigmine (1/10,000 and 1/1,000), did not activate the hippocampus when perfused through the inferior horn.

Discussion

The present experiments show that picrotoxin applied to the central nervous system from the liquor spaces—either perfused through the cerebral ventricles or applied to the surface of the upper cervical cord from the opened cisterna magna—produces the same effect as tubocurarine. The only difference found was that picrotoxin was more potent and that the spike discharge of hippocampal origin produced by picrotoxin perfused through the inferior horn of a lateral ventricle was more frequently interrupted by bursts of fast activity than that produced by tubocurarine. Similar differences have been found with microinjections of the two substances into the cerebral gyri and into the hippocampus. On injection into the cerebral gyri both substances produced a spike discharge, but the threshold dose for tubocurarine was about 0·2 μg as compared with 0·04 μg for picrotoxin, and with picrotoxin in large doses, but not with tubocurarine, the spikes developed after-discharges (Banerjee *et al.*, 1970). On injection into the hippocampus Baker & Benedict (1967) produced local discharges with 4 μg tubocurarine, whereas Baker, Kratky & Benedict (1965) had found that non-discharging foci were produced with as little as 0·5 to 0·1 μg of picrotoxin.

The problem of whether the different central effects of picrotoxin as well as those of tubocurarine can all be explained by " disinhibition " has been fully discussed in the preceding paper by Banerjee *et al.*, and although this possibility cannot be excluded it is anything but proved.

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