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EFFECT OF TOPICAL DRUG APPLICATION TO THE SPINAL CORD ON THE SCRATCHING MOVEMENTS IN CATS

BY

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Feldberg and his co-workers (Feldberg & Fleischhauer, 1960 ; Domer & Feldberg, 1960) have shown that topical application of tubocurarine to the upper cervical cord of anaesthetized and decerebrate cats facilitated the scratch reflex and induced spontaneous scratching movements of the hind limbs. Domer and Dyas (1965) studied the effects of other neuromuscular blocking and anticholinesterase agents on the scratching movements of cats. They found that topical application of hexafluorenium or edrophonium facilitated the scratch reflex and induced spontaneous scratching movements whereas neostigmine, gallamine, suxamethonium and decamethonium had no such effect.

Thus cholinergic synapses of the spinal cord may be possible sites of tubocurarine action in facilitating the scratch reflex. However, other mechanisms may also be considered. These would include liberation of histamine from local storage sites or inhibition of an inhibitory transmitter.

The present investigation was undertaken to explore these possibilities by observing the effect of topical application to the spinal cord of three drugs: hexamethonium, a ganglion blocking agent ; histamine, known to be liberated by tubocurarine (Alam, Anrep, Barsoum, Talat & Weinberger, 1939 ; Mongar, 1956) and gamma-aminobutyric acid (GABA), a substance normally found in the central nervous system where it has been reported to exert an inhibitory action (Eidelberg, Baxter, Rabels & Saldias, 1960 ; Hayashi, 1960 ; Jasper, 1960).

METHODS

Sixty-six cats of either sex, weighing 2 to 4 kg, were used in the present studies. Twenty-eight were anaesthetized with pentobarbitone sodium, 35 mg/kg intraperitoneally and thirty-eight were decerebrated by removing the brain above the midcollicular level under ethyl chloride-ether anaesthesia.

The tibialis anterior tendon of each hind limb was separated from its insertion and connected to a lever which wrote on a kymograph. A 100 g weight was suspended from the lever at an equal distance from the fulcrum in order to maintain a constant tension on the muscle.

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Drills were inserted into the lateral protuberances at each end of both fibulae. The body of the cat was supported on a platform and the head and hind limbs were immobilized.

The dorsal surface of the upper cervical cord was exposed by dissection of the overlying neck muscles, the first and second cervical vertebrae and the meninges.

In approximately 10% of the experiments, normal respiration failed and artificial respiration was then begun and continued for the duration of the experiments.

An artificial cerebrospinal fluid (c.s.f.) (Merlis, 1940) was prepared and gassed with an oxygen-carbon dioxide mixture (O_2 95% + CO_2 5%) for 15 min (resultant *p*H 7.35). The drugs were dissolved in this c.s.f. Both the drug solutions and the artificial c.s.f. were kept in a constant temperature water bath at 37° C throughout the experiment.

Small cotton-wool pledgets soaked in the appropriate solution were placed gently at the desired level of the exposed spinal cord. These were replaced every 5 min until a positive response was obtained, or for a maximum period of 35 min if there was no effect. The spinal cord was washed with cotton-wool pledgets soaked in artificial c.s.f. and replaced every 5 min for a minimum period of 30 min or until the effect of the drug had totally disappeared.

In the experiments involving a study of drug interactions with tubocurarine, the second drug was applied either before, in the same solution or after the application of the tubocurarine. In some experiments the second drugs were administered intravenously. Each sequence was repeated in at least three animals.

Both the spontaneous and evoked contractions of the tibialis anterior muscles were recorded. The scratch reflex was evoked by rubbing the pinna of each ear every 5 or 10 min during the application of the drug. The time of onset of evoked and spontaneous contractions of each tibialis anterior muscle after the initiation of drug application was noted.

The drugs used in the present investigation were D-tubocurarine chloride, hexamethonium bromide, histamine phosphate, gamma aminobutyric acid and diphenhydramine hydrochloride. The concentrations refer to the salts.

RESULTS

Hexamethonium

In cats anaesthetized with pentobarbitone topical application of hexamethonium at C-1 in concentrations of 0.2% to 2% and intravenous injection of 2 mg/kg caused no spontaneous contractions and did not facilitate the evoked movements of the tibialis anterior muscles. These concentrations did not antagonize the effects of tubocurarine. In fact, the effects of tubocurarine application after, or in combination with, hexamethonium appeared earlier, lasted longer and produced a greater height of muscular contractions.

In decerebrate cats topical application of hexamethonium (2%) prior to tubocurarine application resulted in respiratory failure and death in 6 of 8 animals. When the hexamethonium was applied after the tubocurarine none of the cats died and they did not develop respiratory difficulty. Topical application of 1% to 2% hexamethonium at C-1 to animals which had recovered from the effects of tubocurarine application, resulted in both evoked and spontaneous contractions of the tibialis anterior muscles (Fig. 1).

Histamine

In cats anaesthetized with pentobarbitone topical application of histamine in a concentration of 0.1% or slow intravenous injection of 500 μg/kg neither produced spontaneous

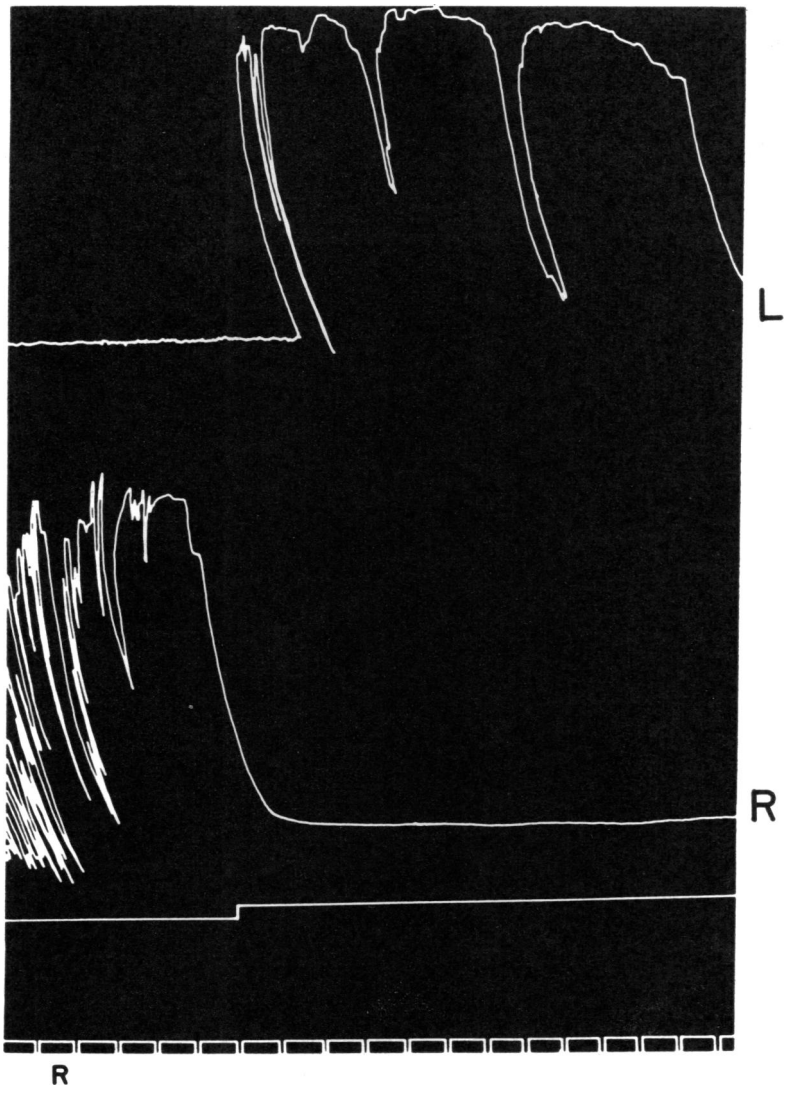

Fig. 1 Records of left (L) and right (R) tibialis anterior muscles of a decerebrated cat. Hexamethonium 2% was applied topically at C-1 after tubocurarine had been washed off. An evoked response of the right and spontaneous contraction of the left tibialis anterior during the 10 min of hexamethonium application. Signal indicates period of rubbing the pinna of the right (R) ear. Time marker, 5 sec.

nor facilitated evoked contractions of the tibialis anterior muscles. There was no alteration in the response to topically applied tubocurarine.

Topical application of histamine in concentrations of 0.02% to 0.1% resulted in a series of powerful contractions of the tibialis anterior muscles in 5 or 6 decerebrate cats (Fig. 2). In four of these experiments the spinal cord subsequently was completely insensitive to all forms of stimuli, e.g. tubocurarine application or tactile stimuli. Although the histamine

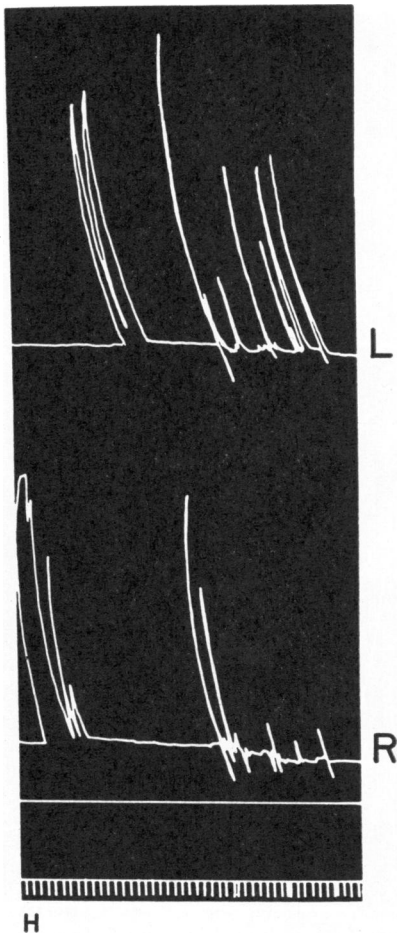

Fig. 2. Records of left (L) and right (R) tibialis anterior muscles of a decerebrated cat showing spontaneous contractions of both muscles during topical application of 0.1% histamine at C-1. Time marker, 5 sec.

caused spontaneous contractions, it did not facilitate the scratch reflex elicited by rubbing the pinna of the ear. Respiration failed after 10 to 15 min in these experiments and, although the animal was kept alive by artificial respiration for 3 to 4 hr, the sensitivity of the spinal cord never returned. Premedication with diphenhydramine 4 to 8 mg/kg intravenously did not block the effect of tubocurarine in 7 decerebrate cats, but blocked the effect of topically applied histamine (0.1%) in 3 cats.

Gamma-aminobutyric Acid

Topical application of GABA in a concentration of 1% to cats anaesthetized with pentobarbitone did not facilitate evoked or induced spontaneous contractions of the tibialis anterior muscles. However, when applied in a 5% concentration for half an hr prior to tubocurarine (0.1%), GABA delayed the appearance and decreased the height

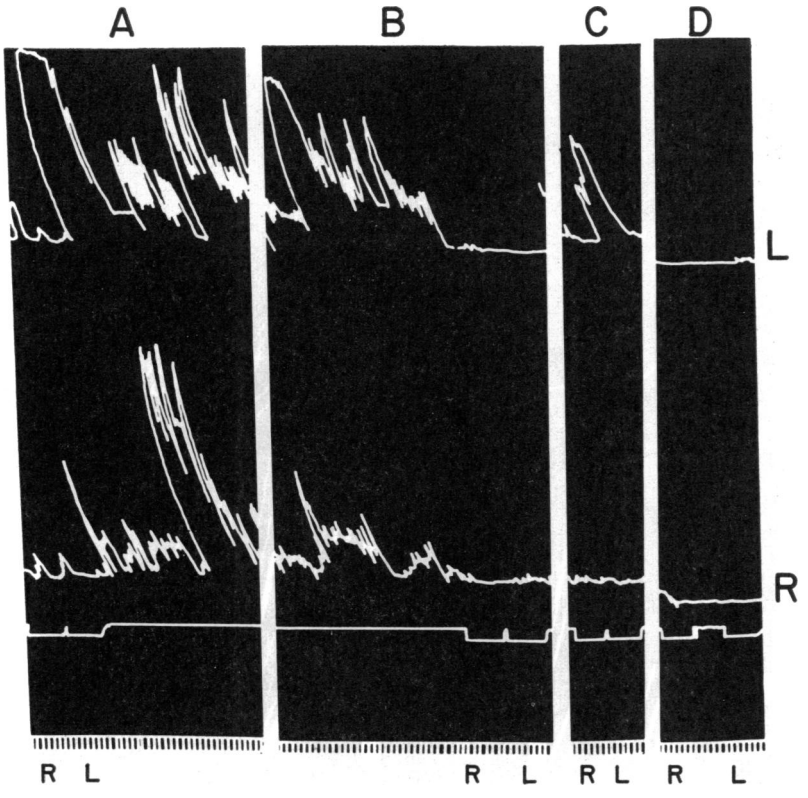

Fig. 3. Records of left (L) and right (R) tibialis anterior muscles of a pentobarbitone-anaesthetized cat. Drugs were applied topically at C-1. (A) Evoked and spontaneous contractions of both muscles during 5 min application of 0.1% tubocurarine. (B) Disappearance of spontaneous and evoked responses 5 min after application of 5% GABA. (C) An evoked response of the left tibialis anterior muscle appeared after 20 min of application of 0.1% tubocurarine following 5% GABA for 30 min. (D) Absence of evoked responses of both muscles during application of 0.1% tubocurarine for 30 min following the application of 10% GABA for 30 min. Signals indicate periods of rubbing the pinna of the right (R) and left (L) ears. Time marker, 5 sec.

of both evoked and spontaneous contractions (Fig. 3). In other experiments the effects of a mixture of tubocurarine 0.1% and GABA in concentrations ranging from 1% to 10% were observed. It was found that the higher the concentration of GABA the more delayed was the onset of spontaneous or evoked movements. With a concentration of 10% GABA there was always a complete blockade of the effect of tubocurarine.

Topical application of 0.1 to 10% GABA at C-1 to decerebrate cats had no effect in producing evoked or spontaneous contractions of the tibialis anterior muscles. In a concentration of 1% GABA blocked the effect of simultaneously applied tubocurarine (0.02%) (Fig. 4).

In some experiments GABA in concentrations of 5 to 10% applied during the height of spontaneous contractions of the tibialis anterior muscles, occurring during tubocurarine application at C-1, caused complete cessation of activity within 3 min. The tubocurarine-

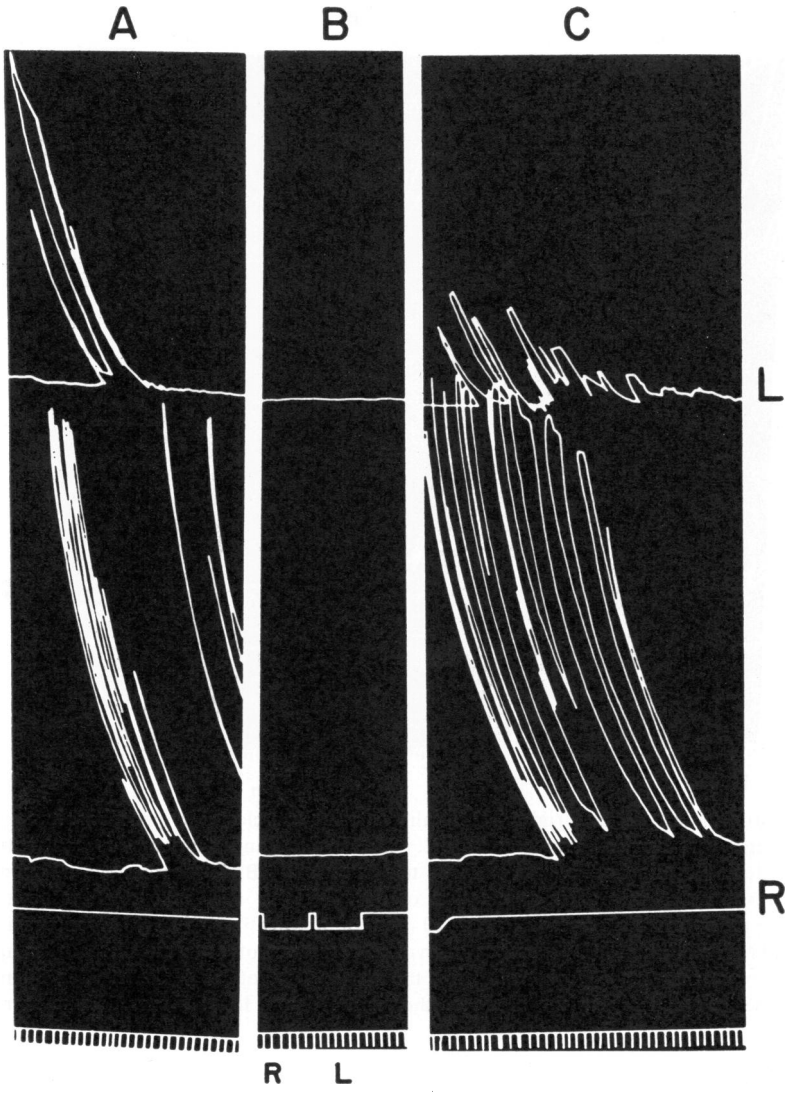

Fig. 4. Records of left (L) and right (R) tibialis anterior muscles of a decerebrated cat. Drugs were applied topically at C-1. (A) Spontaneous contractions of both muscles during application of 0.1% tubocurarine for 20 min. (B) Absence of spontaneous or evoked responses during application of a solution of 0.02% tubocurarine+1% GABA, for 30 min. (C) Recovery of spontaneous activity in both muscles 4 min after washing the cord at C-1. Signals indicate periods of rubbing the pinna of the right (R) and left (L) ears. Time marker, 5 sec.

induced movements reappeared when GABA was washed off the cord. It might be noted that 5% sodium chloride added to the artificial c.s.f. did not result in muscular movements when applied to the cervical spinal cord.

GABA in doses up to 50 mg/kg administered intravenously had no effect on the response to tubocurarine applied topically to the spinal cord.

DISCUSSION

Our knowledge of chemical transmission in the central nervous system is still a matter of speculation and controversy except for the Renshaw cell in the ventral root of the spinal cord which has been shown to be cholinergically innervated (Eccles, 1957). The possibility of the presence of cells of the Renshaw type at the C-1 level used in the present experiments is unlikely due to the absence of ventral roots at that level in cats.

The probability that there might be other cholinergic synapses in the spinal cord is compatible with the work of others. Feldberg, Gray & Perry (1953), using close arterial injection of acetylcholine into the cervical spinal cord, suggested that acetylcholine acts predominantly at interneurones. Fernandez de Molina, Gray & Palmer (1958), with close arterial injection of acetylcholine into the lumbosacral spinal cord, demonstrated both its excitatory and inhibitory action. From this they inferred that acetylcholine must have an action in the spinal cord in addition to that on the Renshaw cell.

Since Curtis & Ryall (1964) have shown that the Renshaw cell responds pharmacologically as though it were a ganglionic cell rather than a cell at the neuromuscular junction, it was presumed that other cholinergic synapses in the spinal cord might behave in a similar manner. It is, however, surprising that hexamethonium did not cause spontaneous movements of the tibialis anterior muscle or facilitate the scratch reflex. This could mean that hexamethonium belongs to the group of inactive compounds mentioned before, e.g. suxamethonium or decamethonium (Domer & Dyas, 1965). However, topical application of hexamethonium in high concentrations subsequent to tubocurarine application did produce spontaneous contractions of the tibialis anterior muscles and did facilitate the movements evoked by rubbing the ear. This action was seen in decerebrate cats and not in pentobarbitone-anaesthetized cats and is probably due to the fact that the former are more sensitive and respond to the small quantities of tubocurarine present. Two possible explanations for this may be as follows. The hexamethonium may act at the receptors only to a subthreshold extent. Coupled with the active residium of previously applied tubocurarine this is sufficient to cause the response. The second possibility may be that tubocurarine occupies two receptor sites at the spinal synapse. One of these is the active site for producing the observed movements. During the washing the artificial c.s.f. following tubocurarine application the tubocurarine is dislodged from the active site while it lingers at the inactive sites. The subsequently applied hexamethonium competes successfully with the tubocurarine at the inactive sites and dislodges it from the receptors. The tubocurarine thus released may then go to the active receptor site and produce its action.

Hexamethonium (2%) applied in decerebrate cats was frequently lethal. This was presumably due to respiratory failure following systemic absorption. Prior application of tubocurarine seemed to protect animals given hexamethonium. The absence of such a toxic effect of hexamethonium in the anaesthetized cats may reflect a relatively greater stability of the respiratory centre as compared to the decerebrate cats.

Intracerebral injection of histamine is known to cause clonic seizures in mice (Haley, 1957) and the sedative action of antihistaminics has been attributed to their antagonism of histamine in the c.n.s. (Rosenberg & Savarie, 1964). Hence, production of powerful spontaneous contractions of the tibialis anterior muscles during application of histamine

to the spinal cord in decerebrate preparations would be compatible with this c.n.s. stimulant activity. However, the effects of histamine differed in two important respects from those of tubocurarine. Histamine did not facilitate the evoked reflex and a second application of histamine did not induce spontaneous contractions. To the contrary, in the majority of cases following the initial stimulation, the spinal cord became insensitive to any stimuli employed. Premedication with the anti-histaminic drug, diphenhydramine, blocked the effect of topically applied histamine but not that of tubocurarine. Hence liberation of histamine by tubocurarine from the dorsal roots of the spinal cord may be one of the components of its action, but it does not seem to contribute a great deal to the overall activity. This is further borne out by the fact that other compounds that are known to liberate histamine, e.g. decamethonium or suxamethonium (Paton, 1957), are ineffective in eliciting scratching movements when applied to the spinal cord (Domer & Dyas, 1965).

There was no effect of topical application to the spinal cord of histamine in anaesthetized cats. This difference in response to histamine in the two preparations may be due to the greater sensitivity of the decerebrate preparations and to the depressant effect of pentobarbitone on the spinal cord (Domer & Feldberg, 1960).

The inhibitory effect of GABA in the c.n.s. has been convincingly demonstrated by different groups of workers in recent years (Purpura, Girado & Grundfest, 1957 ; Eidelberg *et al.*, 1960 ; Hayashi, 1960 ; Jasper, 1960) although its role as possible inhibitory transmitter is still controversial (Curtis, 1963).

While McLennan (1957) and Honour and McLennan (1960) did not find any effect on the monosynaptic knee jerk reflex with topical application of GABA to the spinal cord of cats, Bhargava and Shrivastava (1964) reported inhibition of both mono- and polysynaptic reflexes with intrathecal injection of GABA. In the present studies GABA blocked the effect of tubocurarine on the evoked scratch reflex as well as the spontaneous contractions of the tibialis anterior muscles when they were topically applied to the spinal cord at C-1. Thus GABA does have an inhibitory effect on this polysynaptic reflex. Further, since there was a degree of dose-response interaction between GABA and tubocurarine, the possibility exists that there may be competition between the two drugs for the same receptor sites. This was evident in that GABA caused an inhibition when applied prior to, in combination with, or at the height of tubocurarine activity. On intravenous administration GABA did not block the tubocurarine effect. This may reflect its poor penetration through the blood-brain barrier in cats which has been reported by other workers (Purpura, Girado, Smith & Gomez, 1958 ; Gelder & Elliott, 1958 ; Stanton, 1963).

The effect of GABA was reversible in that, even after prolonged blockade of the spontaneous and evoked activity during topical applications of GABA plus tubocurarine, washing the spinal cord with an artificial c.s.f. resulted in the reappearance of the tubocurarine effect within 5 min. However, if no fresh drug were applied and the previously applied drugs were not washed off, the inhibitory effect of GABA persisted much longer. This delay in the reappearance of the tubocurarine effect may be attributed to the metabolism of GABA at the sites of action or to its slow diffusion from these sites. The mode of action of GABA may be due to a competition with some excitatory transmitter released by tubocurarine or by competing with tubocurarine itself for the receptor. It

may also be that the action of GABA is due to a nonspecific action on the spinal neurones as inferred by Curtis and Watkins (1959). Conversely, it may be that tubocurarine is acting by inhibiting naturally occurring inhibitory substances such as GABA or closely related compounds. Studies to clarify these interactions are presently in progress.

SUMMARY

1. The spontaneous and evoked muscular movement caused by application of tubocurarine to the cervical spinal cord of cats anaesthetized with pentobarbitone or decerebrated has proved to be a useful tool with which to study drug interactions in the spinal cord.

2. Although topical application of hexamethonium itself did not have demonstrable effects, following the topical application of tubocurarine it was possible to obtain both spontaneous and evoked muscular movements. This is further evidence for a possible action of tubocurarine at a cholinergic synapse.

3. Histamine application resulted in spontaneous movements only in decerebrate preparations. This activity was blocked by an antihistamine. Histamine liberation is not an important component of tubocurarine's activity as it was unaffected by the antihistamine.

4. Simultaneous or prior application of GABA blocked the muscular movements normally seen during tubocurarine application. The interaction seemed to be competitive in that larger concentrations of GABA were required to block the larger doses of tubocurarine. This would suggest that tubocurarine may be exerting its effect by inhibiting some inhibitory substance.

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REFERENCES

ALAM, M., ANREP, G. V., BARSOUM, G. S., TALAT, M. & WEININGER, E. (1939). Liberation of histamine from the skeletal muscle by curare. *J. Physiol. (Lond.)*, **95**, 148–158.

BHARGAVA, K. P. & SHRIVASTAVA, R. K. (1964). Nonspecific depressant action of γ-aminobutyric acid on somatic reflexes. *Br. J. Pharmac. Chemother.*, **23**, 391–398.

CURTIS, D. R. (1963). The pharmacology of central and peripheral inhibition. *Pharmac. Rev.*, **15**, 333–364.

CURTIS, D. R. & RYALL, R. W. (1964). As quoted by Eccles, J. C. in *The Physiology of Synapses*, p. **67**. New York: Academic Press.

CURTIS, D. R. & WATKINS, J. C. (1959). The depression of spinal neurones by γ-amino-n-butyric acid and B-alanine. *J. Physiol. (Lond.)*, **146**, 185–203.

DOMER, F. R. & DYAS, E. C. (1965). Effects on the spinal cord of the cat by drugs known to influence neuromuscular transmission. *Br. J. Pharmac. Chemother.*, **25**, 411–417.

DOMER, F. R. & FELDBERG, W. (1960). Scratching movements and facilitation of the scratch reflex produced by tubocurarine in cats. *J. Physiol. (Lond.)*, **153**, 35–51.

ECCLES, J. C. (1957). *The Physiology of Nerve Cells*, p. 163. Baltimore: The Johns Hopkins Press.

EIDELBERG, E., BAXTER, C. S., RABELS, E. & SALDIAS, C. A. (1960). *Inhibition in the Nervous System and Gamma Aminobutyric Acid*, p. 365. New York: Pergamon Press.

FELDBERG, W. & FLEISCHHAUER, K. (1960). Scratching movements evoked by drugs applied to the upper cervical cord. *J. Physiol. (Lond.)*, **151**, 502–517.

FELDBERG, W., GRAY, J. A. B. & PERRY, W. L. M. (1953). Effects of close arterial injections of acetylcholine on the activity of the cervical spinal cord of the cat. *J. Physiol. (Lond.)*, **119**, 428–438.

FERNANDEZ DE MOLINA, A., GRAY, J. A. B. & PALMER, J. F. (1958). Effects of acetylcholine on the activity of the lumbosacral cord of the cat. *J. Physiol. (Lond.)*, **141**, 169–176.

GELDER, N. M. VAN & ELLIOTT, K. A. C. (1958). Disposition of γ-aminobutyric acid administered to mammals. *J. Neurochem.*, **3**, 139–143.

HALEY, T. J. (1957). Pharmacological effects produced by intracerebral administration of drugs of unrelated structure to conscious mice. *Arch. Int. Pharmacodyn. Ther.*, **110**, 239–244.

HAYASHI, T. (1960). *Inhibition in the Nervous System and Gamma Aminobutyric Acid*, p, 515. New York: Pergamon Press.

HONOUR, A. J. & MCLENNAN, H. (1960). The effects of γ-aminobutyric acid and other compounds on structures of the mammalian nervous system which are inhibited by factor I. *J. Physiol. (Lond).*, **150**, 306–318.

JASPER, H. H. P. (1960). *Inhibition in the Nervous System and Gamma Aminobutyric Acid*, p. 544. New York: Pergamon Press.

MCLENNAN, H. (1957). A comparison of some physiological properties of an inhibitory factor from brain (Factor I) and of γ-aminobutyric acid and related compounds. *J. Physiol. (Lond.)*, **139**, 79–86.

MERLIS, J. K. (1940). The effect of changes in the calcium content of the cerebrospinal fluid on spinal reflex activity in the dog. *Am. J. Physiol.*, **131**, 67–72.

MONGAR, J. L. (1956). *Histamine—Ciba Foundation Symposium*, p. 74. Boston: Little, Brown & Company.

PATON, W. D. M. (1957). Histamine release by compounds of simple chemical structure. *Pharmac. Rev.*, **9** 269–328.

PURPURA, D. P., GIRADO, M. & GRUNDFEST, H. (1957). Selective blockade of excitatory synapses in the cat brain by γ-aminobutyric acid. *Science, N.Y.*, **125**, 1200–1201.

PURPURA, D. P., GIRADO, M., SMITH, T. G. & GOMEZ, J. A. (1958). Synaptic effects of systemic γ-aminobutyric acid in cortical regions of increased vascular permeability. *Proc. Soc. exp. Biol. Med.*, **97**, 348–353.

ROSENBERG, F. J. & SAVARIE, P. J. (1964). Histamine and the reversal of chlorpromazine-induced depression. *J. Pharmac. exp. Ther.*, **146**, 180–185.

STANTON, H. C. (1963). Mode of action of gamma aminobutyric acid on the cardiovascular system. *Arch., Int. Pharmacodyn. Ther.*, **143**, 195–204.