

EFFECTS ON THE SPINAL CORD OF THE CAT BY DRUGS KNOWN TO INFLUENCE NEUROMUSCULAR TRANSMISSION

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In 1960, Feldberg & Fleischhauer, while studying the uptake of bromophenol blue by the brain of the cat after intraventricular administration, noted that when the dye came in contact with the upper cervical spinal cord movements of the hind-legs resembling those of a scratch reflex were elicited. In a subsequent series of experiments in which the bromophenol blue was applied topically to the cord at C1, scratching movements were likewise produced. Sections of the spinal cord at this level showed intense staining of the zona gelatinosa Rolandi. They also found that tubocurarine applied at this level elicited the same response. It was concluded that these two substances selectively activate long, descending, propriospinal neurones in the spinal cord. These same neurones are activated on stimulation of the skin and mediate the scratching movements of the hind-legs which were described by Sherrington & Laslett (1903).

Domer & Feldberg (1960) enlarged upon the observations made with tubocurarine. The principal findings were: (1) when applied at C2, concentrations higher than those effective at C1 were necessary to elicit the movements; (2) the pattern of movements produced in decerebrate preparations was different from that in cats anaesthetized with pentobarbitone sodium; (3) systemic administration of pentobarbitone sodium to anaesthetized or decerebrate preparations greatly diminished the strength of the movements, and chloralose completely abolished them; (4) transection of the medulla just below the obex greatly diminished the strength of the movements, but did not abolish them.

Tubocurarine is known to block neuromuscular transmission by competing with acetylcholine and the present experiments were done to ascertain the activity on the spinal cord of other drugs which are known to affect cholinergic function at peripheral sites.

METHODS

The experiments were performed on fifty cats of either sex weighing 2.0 to 4.4 kg. They were either anaesthetized with pentobarbitone sodium (35 mg/kg, intraperitoneally) or decerebrated during preliminary ethyl chloride-ether anaesthesia by removing the brain above the mid-collicular level.

The head was immobilized and the body supported in a standing position. The hind-legs were immobilized with drill bits inserted into the lateral protuberances at each end of the fibulae. A string-lever system was attached to the sectioned tendon of each tibialis anterior muscle and movements of the muscles were recorded on smoked paper. A 100-g weight was suspended from each lever to maintain a constant tension upon the muscles. The recorded excursions, therefore, represented changes in muscle length and tension and were not a linearly related measure of the force developed.

The drugs were dissolved in an artificial cerebrospinal fluid of the composition given by Merlis (1940). Small pledgets of cotton wool were soaked in the solution containing the drug and these were placed directly on the exposed dorsal surface of the cervical spinal cord at the desired level. If no response were obtained in 30 to 40 min with any given concentration of drug, the cord was then washed for 30 min with pledgets of cotton wool soaked in artificial cerebrospinal fluid solution before the next drug concentration was applied.

A response was considered to be positive if tension was developed in one, or both, of the tibialis anterior muscles. This was usually rhythmic in character and in one muscle at a time rather than simultaneously. These movements which occurred during and after drug application without any other stimulus will hereafter be referred to as "spontaneous." Movements during drug application could also be evoked by rubbing the pinna of the ear or the skin surrounding it. These will be referred to as the "scratch reflex" and its associated "scratching movements."

The compounds tested were: hexafluorenum bromide, edrophonium chloride, neostigmine methylsulphate, gallamine triethiodide, decamethonium bromide and suxamethonium chloride. Concentrations (w/v) refer to the salts.

RESULTS

Before, or in the absence of, drug application, the tibialis anterior muscles either remained quiescent or, in the anaesthetized animals, occasionally developed a tremor upon which any later drug-induced movements were superimposed. Before drug application, rubbing the pinna of the ear did not evoke movements of the tibialis anterior muscle.

Effects of hexafluorenum and edrophonium

Pentobarbitone sodium anaesthesia. A concentration of 1% hexafluorenum or edrophonium applied at C1 for 30 min did not result in any spontaneous movements of the tibialis anterior muscle. However, 15 min after washing of the cord had begun, following 1 hr application of hexafluorenum, numerous small bursts of spontaneous activity occurred and persisted for 45 min. Although the muscles on both sides exhibited these bursts of activity, they always alternated, that is one side was quiescent during movement of the contralateral side.

With hexafluorenum, a concentration of 1% was the lowest with which the scratch reflex could be elicited by rubbing the pinna of the ear or the surrounding skin. The scratching movements could be evoked 10 to 40 min after the first application of hexafluorenum and persisted for as long as 50 min after the start of washing with artificial cerebrospinal fluid.

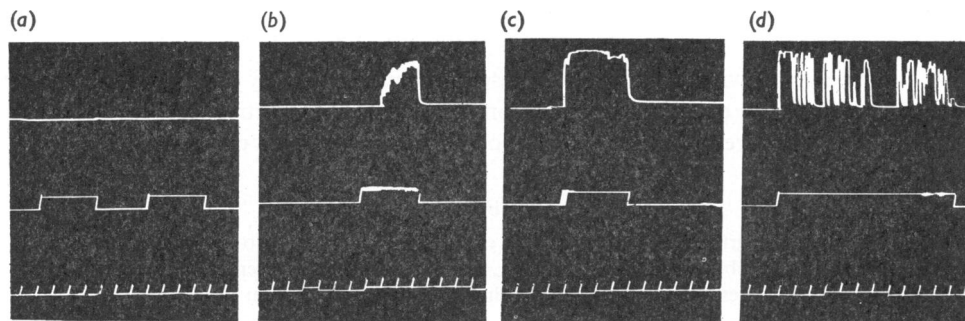


Fig. 1. Records of the left anterior tibialis muscle of a cat anaesthetized with pentobarbitone sodium (top trace). There was no response during rubbing of the pinna of the left ear (a) before application of hexafluorenum bromide. Various patterns (b, c and d) of evoked responses were obtained 40, 65 and 85 min after application of 0.1% at C1. Stimulus marker (middle trace). Time marker, 10 sec (bottom trace).

The evoked movements began either immediately or within 10 sec after the skin stimulation was started and ceased abruptly when the stimulation was stopped. The pattern of these evoked movements varied greatly, as can be seen in Fig. 1. As mentioned before, rubbing the pinna of the ear before drug application caused no movements of the tibialis anterior (*a*), whereas during and following the drug application a similar stimulus resulted in various patterns (*b*, *c* and *d*). Thus it was not possible to characterize a typical pattern of evoked movements.

No movements were evoked during application of edrophonium to the cervical spinal cord. Neither spontaneous nor evoked movements of the tibialis anterior muscle were obtained when either hexafluorenum or edrophonium were applied at the level of C2 in concentrations as great as 1%.

Decerebration. A concentration of 0.02% hexafluorenum applied at C1 itself elicited spontaneous movements in one of two decerebrate cats. These movements occurred 15 to 30 min after the application and continued for 12 min after the removal of the drug. In two of three cats to which 0.1% hexafluorenum was applied at C1, spontaneous movements occurred after 17 and 31 min, and persisted for 13 and 50 min respectively after the drug application was stopped and the washing begun.

The character of the spontaneous movements of decerebrate cats differed in two respects from that in animals anaesthetized with pentobarbitone sodium. In the decerebrate cats there was a greater amount of tension developed and sustained for a longer period of time, and movements occurred simultaneously in both legs rather than in each leg alternately, as found in the anaesthetized animals. With evoked responses these same two differences were observed, and, in addition, a series of rapid oscillations was often superimposed on the sustained contraction (Fig. 2).

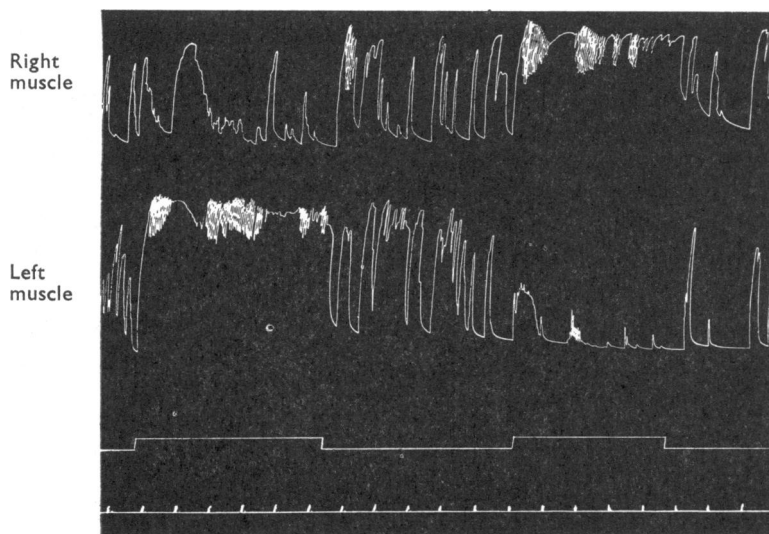


Fig. 2. Records of right (uppermost trace) and left (second trace) anterior tibialis muscles of a decerebrate preparation 30 min after 1% hexafluorenum bromide had been applied to C1. The third trace indicates the period of rubbing the pinna of the left ear, a pause and then a period of rubbing the pinna of the right ear. Time marker, 5 sec (bottom line).

In one experiment in which hexafluorenum was applied at the level of C2, a concentration of 1% was necessary before the scratching movements could be elicited by rubbing the ears. No movements occurred in the absence of ear stimulation.

When 1% hexafluorenum was applied on one side of the cervical spinal cord, spontaneous scratching movements occurred on the ipsilateral side only.

Transection of the medulla just below the obex altered the character of both spontaneous and evoked movements. There was a decreased tension developed and an increase in the frequency of contractions (Fig. 3).

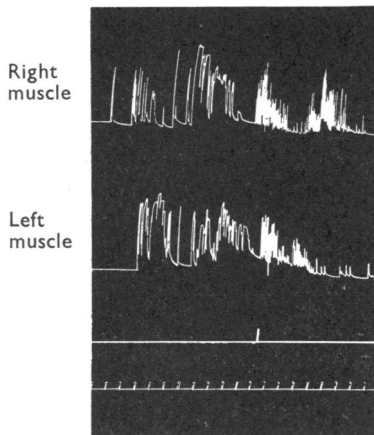


Fig. 3. Records of right (top trace) and left (second trace) anterior tibialis muscles of a decerebrate cat showing the effect on spontaneous movements of transection of the medulla just below the obex. Hexafluorenum bromide, 1%, had been applied at C1 for a period of 25 min before the transection (indicated by the marker on the third trace). Time marker, 10 sec (bottom line).

A concentration of 0.2% edrophonium chloride placed at C1 for 30 to 35 min failed to produce movements or to activate the reflex response. However, movements occurred 9 to 18 min after applying a concentration of 1%. The movements ceased within 5 min of removal of the drug, but could be evoked for a further 30 to 45 min by rubbing the ears. The tension and duration of the spontaneous movements produced by edrophonium were similar to those produced by hexafluorenum, but edrophonium caused more pronounced high-frequency movements superimposed on the slow contraction.

In one of two animals to which a concentration of 2% was applied at the level of C2, spontaneous movements began after 35 min. In both of these animals, rubbing the pinna of the ear elicited scratching movements after 30 min of drug application. The spontaneous activity ceased within 5 min of removal of the drug, but the reflex response could be evoked for 45 min. The pattern of movements was the same as that produced when the drug was applied at C1.

When 2% edrophonium was applied to one side of the cervical spinal cord, spontaneous movements occurred on the ipsilateral side only (Fig. 4,a). If the edrophonium were then removed from the side of initial application and placed on the other side, spontaneous activity occurred on the side to which the drug was currently being applied while it ceased on the contralateral side (Fig. 4,b).

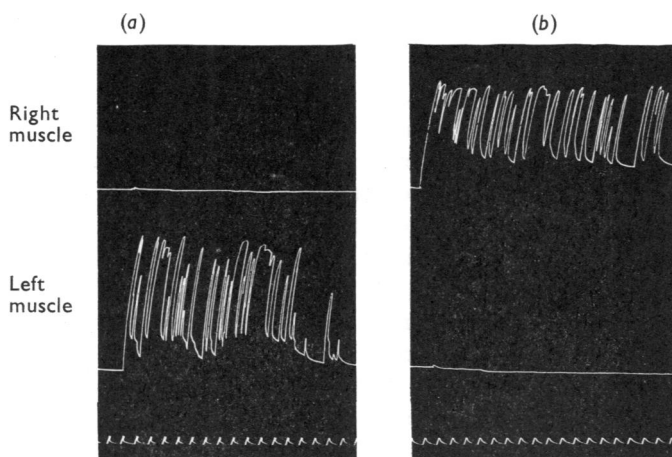


Fig. 4. Records of right (top trace) and left (middle trace) anterior tibialis muscles of a decerebrate cat. (a) Spontaneous movements occurring on the left side a few minutes after 2% edrophonium chloride had been applied to the left side of the spinal cord at C1. (b) Spontaneous movements on the right side while the left was quiescent after the changing of the edrophonium chloride application from the left to the right side. Time marker, 10 sec.

Transection of the medulla just below the obex did not alter the tension developed during contraction, although the number of contractions per unit time increased.

Effects of other drugs

The four other compounds investigated in this study—suxamethonium chloride, decamethonium bromide, gallamine triethiodide and neostigmine methylsulphate—were each applied to the cervical spinal cord in a concentration of 1% for a period of 30 min in cats anaesthetized with pentobarbitone sodium and in decerebrate cats. In no instance was there spontaneous movement of the hind-legs, nor could the reflex response be elicited by rubbing the pinna of the ear. Numerous signs of systemic effects were noted with all the drugs but suxamethonium. After application of neostigmine the animal salivated, lacrimated, urinated, defaecated and had generalized muscular fasciculations. During application of decamethonium muscular fasciculations similar to those seen with neostigmine were noted. With gallamine triethiodide, fasciculations of the facial musculature occurred which resulted in movements of the ears.

DISCUSSION

There are a number of similarities between the action of tubocurarine as found in previous experiments (Domer & Feldberg, 1960) and in the actions of hexafluorenum bromide and edrophonium chloride in the present experiments. In each case the pentobarbitone sodium anaesthesia caused a decrease in the force and duration of contractions of the anterior tibialis muscle when compared to the movements elicited by drug application to the spinal cord of decerebrate cats. The minimal concentration of drug necessary to elicit the spontaneous response in the anaesthetized animals was greater than in decerebrate animals. Both of these results suggest that midbrain activity plays a role in the character of the scratch reflex. In both the previous experiments (Domer & Feldberg, 1960) and the present series, rubbing the pinna of the ear or the skin around it could initiate the scratch

reflex during application of tubocurarine. This is the area which Sherrington (1910) found to be most sensitive in initiating the scratch reflex. The threshold concentration necessary to elicit the reflex was higher when the drug was applied at the level of C2 than that required at the level of C1. This may reflect a real difference in threshold, or may simply be due to the fact that the neurones or synapses which are being activated are nearer the surface at C1. As there is no ventral root at C1 in the cat, the applied drugs may be acting on the sensory rootlets entering from the C1 dorsal root ganglion or possibly on the zona gelatinosa Rolandi, as suggested by Feldberg & Fleischhauer (1960). In both the previous (Domer & Feldberg, 1960) and present experiments it was found that drug application to one side of the spinal cord resulted in scratching movements on the ipsilateral side only. Also, the length of time during which the reflex was active after removal of the drug from the spinal cord and washing with artificial cerebrospinal fluid tended to vary directly with the length of time that the drug had been applied.

There were two differences between the results found with tubocurarine (Domer & Feldberg, 1960) and the present experiments. First, in the previous experiments the scratching movements occurred only on one side at a time. In the present experiments in which hexafluorenum and edrophonium elicited the same type of scratching movements in decerebrate animals, simultaneous movements of both hind-legs occurred frequently. Second, transection of the medulla just below the obex decreased the strength of contraction during application of tubocurarine in the previous series and with hexafluorenum in the present series of experiments. During application of edrophonium the amount of tension developed was unaffected after the transection. The lack of effect on the tension developed during application of edrophonium indicates that it may not act at all of the sites common to tubocurarine and hexafluorenum.

Thus, although three drugs were capable of eliciting the scratching movements when they were applied to the cervical spinal cord of the cat, individual differences did exist. In addition, other agents which possess neuromuscular activity—suxamethonium chloride, decamethonium bromide, neostigmine methylsulphate and gallamine triethiodide—were inactive in eliciting the scratching movements. It might be argued that the relative strength of these organic cations and the lipoid solubility of the rest of the molecule might be sufficiently different to explain this variation in activity. In this way a barrier might prevent, or hinder, the penetration of one compound more than another. This may well be the case, but it will be necessary to do further experiments to clarify this point.

It is of interest that edrophonium, a compound which is employed therapeutically as an antagonist of tubocurarine, produced the same effect as tubocurarine when it was applied to the spinal cord and it did not block the action of subsequently applied tubocurarine.

In the previous report (Domer & Feldberg, 1960) it was suggested that the ear bars by which the animal's head was immobilized might have lowered the threshold to ear stimulation. This was not a factor in the present experiments in which the headholder touched only the mouth and jaw.

Just what do the scratching movements represent? Koenigstein (1948) studied a number of substances which he injected into the cisterna magna of cats and rabbits. He found that scratching occurred at a lower dose than did convulsive seizures. In other words, the scratching movements were essentially a pre-seizure syndrome or might be considered to be a very circumscribed area of seizure activity which spread as the dose was increased.

MacLean, Dua & Denniston (1963) found a similar stimulus strength/response relationship when they electrically stimulated various points along the spinothalamic tract of monkeys. A weak stimulus caused scratching of various body parts whereas increasing the intensity of the stimulus caused struggling and vocalization. Thus, in the present experiments, the degree of central stimulation which these substances with neuromuscular activity are known to possess (Feldberg & Sherwood, 1954; Sakuma, 1959) may have manifested itself first as a scratching movement and then would develop, with increasing concentrations of the substances, into frank seizures.

SUMMARY

1. Application of hexafluorenum bromide or edrophonium chloride to the dorsal surface of the cervical spinal cord of the cat produced spontaneous scratching movements of the hind legs and facilitated the scratch reflex elicited by rubbing the pinna of the ear or the surrounding skin. This activity was produced when the drugs were applied at the level of C1 or C2. The lower level required a higher concentration in order to elicit the same effect.

2. Neostigmine methylsulphate, suxamethonium chloride, decamethonium bromide and gallamine triethiodide produced no scratching movements when applied to the cervical spinal cord.

3. The responses elicited during hexafluorenum bromide application in animals anaesthetized with pentobarbitone sodium were decreased in strength and shorter in duration than those obtained in decerebrate animals. No movements were elicited during edrophonium chloride application in cats anaesthetized with pentobarbitone sodium.

4. Transection of the medulla just below the obex diminished the strength and increased the frequency of contractions during hexafluorenum bromide application. Transection during edrophonium chloride application increased the frequency but had no effect upon the strength of the subsequent contractions.

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