Brit. J. Pharmacol. (1951), **6**, 540.

THE ACTION OF *d*-TUBOCURARINE ON THE MONOSYNAPTIC EXTENSOR REFLEX

BY

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(Received April 2, 1951)

It has been believed for nearly 150 years that curare has a stimulant action on the central nervous system in addition to its well-known peripheral effect on neuromuscular transmission. The evidence was conflicting until Tillie (1890) published the results of a systematic investigation of the effects of curare on the central nervous system of the frog. He showed that much of the previous confusion had been due to the unpredictable action of curare on the circulation. By means of intra-aortic injections and local application of curare to the spinal cord he was able to demonstrate a strong stimulant effect on the central nervous system. This effect often amounted to tetanus and was present in both parts of the spinal cord after subdivision. He further showed that the depression of reflex activity often seen in the intact frog after the subcutaneous injection of curare was due to an inhibitory influence by the higher centres. He also demonstrated that the " strychnine-like " action of curare was not due to contamination by strychnine.

McGuigan (1916) studied the effect of the local application of curare to the central nervous system in dogs. He confirmed the excitatory action of curare, but emphasized that the general thrashing movements observed were quite unlike the spasms produced by strychnine. This stimulating action was confirmed by numerous other workers, including Joseph and Meltzer (1911), Santesson (1920–1), Blume (1934), West (1937), and Euler and Wahlund (1941). The literature is fully discussed by McIntyre (1947).

Cohnberg (1946) studied the action of curare administered by subcutaneous, intravenous, intramuscular, and intraperitoneal injection into intact animals of various species. He observed reflex hyperexcitability and convulsions in all the species examined (rat, cat, and rabbit). He showed that asphyxia was not responsible, and concluded that curare produces convulsions in mammals by stimulation at a higher level of the C.N.S. than the spinal cord, in contrast to strychnine. Eccles (1946), however, applied curare to the isolated frog spinal cord and found that large doses set up sustained spontaneous convulsant activity which resembled the action of strychnine.

McCawley (1949) found that small doses of *d*-tubocurarine administered intravenously to cats produced muscular twitchings, convulsions, and changes in the electroencephalogram, and he showed that these effects could be prevented by the

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previous administration of pentobarbital, thiopentone, or dial-urethane. This author investigated the effects of *d*-tubocurarine on the monosynaptic reflexes in cats anaesthetized with pentobarbital and found only a depression of amplitude. Salama and Wright (1950) administered *d*-tubocurarine chloride by intraventricular, intracisternal, and intrathecal injection to chloralosed, decerebrate, and high spinal cats. They showed that small doses produce heightened reflex excitability of the spinal cord and later generalized convulsions. They concluded that "the effects on the spinal cord are due only to a minor extent to a direct action on the spinal centres: they are mainly the result of stimulation of the cells of origin of the facilitatory pathways in the brain."

Naess (1950), however, could not find any effect on the mono- and poly-synaptic reflexes in the cat after the administration of *d*-tubocurarine intravenously. In these experiments the cats were anaesthetized with dial.

Baisset, Laporte, and Grezes-Rueff (1949) report that *d*-tubocurarine, injected intravenously, gives a transient increase followed by a disappearance of the monosynaptic reflex in the decapitated unanaesthetized dog only when a rapid injection of curare is followed by a drop of the blood pressure from 40 to 25 mm. mercury.

In the course of other studies on the effect of *d*-tubocurarine on excitability changes in the spinal cord, we noted that the drug had a marked effect on the monosynaptic reflex response. This reflex appeared to be a sensitive indicator of part of the central effect of tubocurarine on the nervous system, and its action was studied in various types of preparation. The effect of *d*-tubocurarine on the polysynaptic reflex as well as a comparative study on the effect of strychnine is reported in another paper (Bernhard, Taverner, and Widén, 1951).

METHODS

Cats were used in all experiments. The preliminary decerebration or decapitation was performed under ether anaesthesia. The experimental procedures were begun more than two hours later so that the effect of the ether had disappeared. After the preliminary preparation lumbar laminectomy was performed and the appropriate roots were prepared intradurally. Usually the L_6, L_7, and S_1 ventral roots were cut distally and the L_7 or S_1 root was placed on the recording electrodes. The nerves to the two heads of the gastrocnemius muscle were dissected, cut distally, and one branch was placed on the stimulating electrodes. All exposed nervous structures were covered with paraffin oil. The electrical stimuli were applied through a transformer. Small supraliminal test responses were used throughout. The ventral root responses were amplified by a differential amplifier and recorded photographically from a cathode ray oscillograph. The curare preparation used was *d*-tubocurarine, 20 U (3 mg.)/c.c. (Abbott). All the injections were given slowly into a vein of the forelimb.

The procedure adopted was to set up the preparation and elicit a monosynaptic reflex response from one of the nerves to the gastrocnemius by electrical stimulation at intervals, usually of 2 seconds. When the response had settled to a fairly constant amplitude control records were taken for periods of 0.5–1 minute at a stimulus frequency of 30/min. The *d*-tubocurarine was then injected intravenously and sample records were taken for periods of approximately 30 seconds, at intervals of 1–2 minutes, for a total period of 10–20 minutes. The oscillograph screen was observed throughout, and any special events were recorded as required. The height of the monosynaptic reflex response was measured without magnification. In the decerebrate preparations artificial respiration was begun as soon as any evidence of respiratory failure appeared. All

other preparations received artificial respiration from an electrically driven pump throughout. In a series of experiments the blood pressure was recorded simultaneously from the carotid artery.

The effect of the experimental procedures upon the amplitude of the monosynaptic reflex response was expressed graphically in terms of the average pre-injection test response. Each point was obtained from the mean of the amplitude of five successive responses at 2-second intervals expressed as a percentage of the pre-injection response amplitude. In graphs which show the effect of repeated injections the stimulus strength remained constant throughout and the preparation was not disturbed. If necessary the amplifier gain was reduced, allowance being made for this in computing the results. The illustrations show frames, selected from the film records of the experiments, chosen to give a representative picture of the events under consideration.

RESULTS

Threshold dose of tubocurarine

Stimulation of the afferent fibres with lowest threshold in the gastrocnemius nerve is followed by a well-synchronized reflex potential in the ventral root. Fig. 1 A shows such a potential. The time marker represents intervals of 1 msec., and the interval between the shock artifact and the initiation of the reflex spike is 2.5 msec. It has been shown by Lloyd (1943) that in such a preparation afferent conduction to the spinal cord requires approximately 1.4 msec. and in the ventral root about 0.3 msec. By subtraction approximately 0.8 msec. is available for synaptic delay and central conduction. This short time interval suggests strongly that two-neurone arcs are involved (Lorente de Nó, 1935 ; Renshaw, 1940), i.e., monosynaptic reflex arcs. The same author has shown that this discharge (Group I reflex) reflects into the muscle, the large afferent fibres of which are stimulated (Lloyd, 1943), and therefore the reflex spike may be regarded as representing activity in the extensor motorneurones.

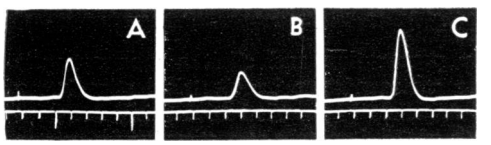

FIG. 1.—Reflex discharge (monosynaptic response) recorded in L_7 ventral root after stimulation of the ipsilateral gastrocnemius nerve (A) before, (B) 45 sec. and (C) 5 min. after, intravenous injection of 0.11 mg. tubocurarine per kg. Time in msec.

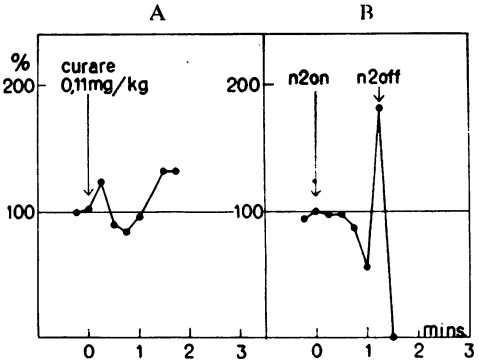

FIG. 2.—(A) Effect of 0.11 mg. tubocurarine per kg. on the monosynaptic response in a decerebrate cat. The amplitude of the monosynaptic spike in per cent of pre-injection test response; (B) Effect of asphyxia (N_2 on—N_2 off) on the amplitude of the monosynaptic reflex discharge.

In Fig. 1, B and C show the changes in amplitude of the monosynaptic response 45 seconds and 5 minutes respectively after the intravenous injection of 0.11 mg. tubocurarine per kg. body weight into a decerebrate cat. Fig. 2 A is a graph showing the changes in amplitude of the monosynaptic response plotted as a percentage of the pre-injection test response. There is a slight increase in amplitude during the first 15 seconds, followed by a slight depression which is maximal at 45 seconds. There is then a rise in amplitude to about 130 per cent of the average test response.

This pattern of response is similar to that obtained by asphyxia with nitrogen (Fig. 2 B). The possibility that the response in Fig. 2 A might be due to asphyxial effects resulting from interference with the respiratory excursions by tubocurarine was excluded in other experiments where the decerebrate preparation was given artificial respiration before the administration of tubocurarine. In such experiments similar effects were found.

Response in decerebrate preparations

If a larger initial dose of curare is given to a decerebrate cat the response is more striking. Fig. 3 shows the effect on the amplitude of the monosynaptic reflex response of the intravenous injection of 1.12 mg. *d*-tubocurarine per kg. body weight. The same initial changes occur, and there is a more marked and longer-lasting increase in amplitude to nearly 400 per cent of the test response.

A further effect of tubocurarine can be observed if a slower sweep is employed. Fig. 4 C shows an irregular repetitive discharge in the ventral roots, which may indicate increased background activity, occurring 1–3 minutes after the intravenous injection of 1.5 mg. *d*-tubocurarine per kg. into a decerebrate cat. The increase of the monosynaptic reflex response does not appear to be related to the appearance

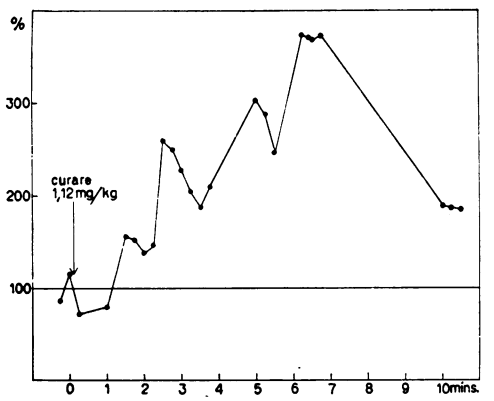

FIG. 3.—Effect of 1.12 mg. tubocurarine per kg. on the amplitude of the monosynaptic reflex response in a decerebrate cat. Ordinates: amplitude as percentage of pre-injection level. Abscissae: time in min.

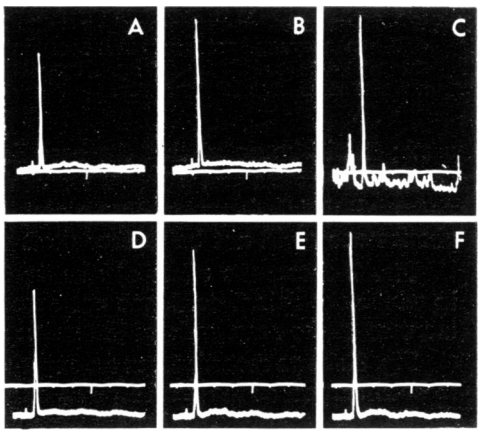

FIG. 4.—The monosynaptic reflex response in S_1 ventral root (A) before, (B) 30 sec. and (C) 1½ min. after, 1.5 mg. tubocurarine per kg. In series D–F, record D was obtained before, E 1 min. and F 2 min. after, a second dose of tubocurarine (1.5 mg. per kg.). Time in 10 msec.

of this activity. In Fig. 4, E and F show the effect of a further injection of the same dose of tubocurarine into the same preparation. There is a similar augmentation of the monosynaptic reflex response, but no sign of repetitive activity. The repetitive discharge in the ventral roots is only seen in decerebrate preparations, usually only after the first and occasionally after the second injection.

Response in spinal preparations

Fig. 5 shows the effect of tubocurarine injected intravenously into a decapitate preparation ; 1.57 mg. per kg. results in an increase in the amplitude of the monosynaptic reflex response to over 200 per cent. A second similar dose 15 minutes later gives a further slight increase in the amplitude. The initial depression observed in decerebrate preparations was seen more seldom in the spinal preparations. Fig. 6 shows a series of records from such an experiment, in which a D.C. amplifier was

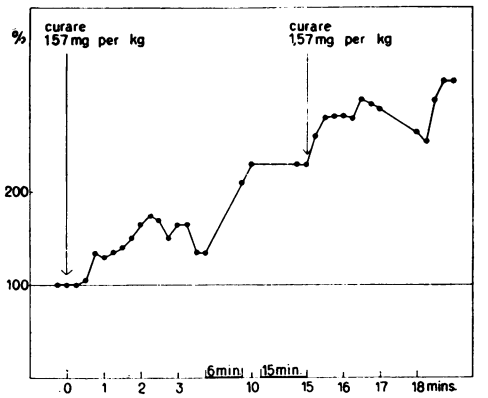

FIG. 5.—Effect of tubocurarine on the amplitude of the monosynaptic response in a decapitate cat in response to 1.57 mg. per kg. at zero time and the same dose 15 min. later.

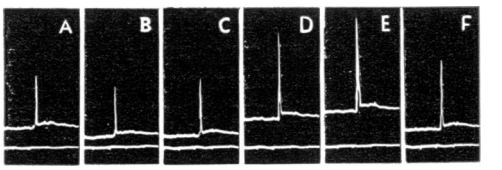

FIG. 6.—The monosynaptic response in L_7 ventral root (A) before, and (B) 25 sec., (C) 30 sec., (D) 1 min., (E) 2 min. and (F) 3 min. after, the injection of 1.7 mg. tubocurarine per kg. Decapitate preparation. Time in 10 msec.

used ; it will be noted that the base line moves up and down as the amplitude of the response rises and falls. This movement represents a change in the membrane potential, which decreases as the amplitude of the reflex response increases. This phenomenon will not be further dealt with in this article.

Fig. 7 shows the effect of smaller doses of curare on a decapitate preparation in which the spinal cord was cut across at the level $T_{11} - T_{12}$. There is again an increase in the amplitude of the reflex response but no early depression.

Fig. 8 shows records from an experiment in which a decapitate cat was subjected to cord section at the level $L_1 - L_2$ and the dorsal roots were all cut from $L_3 - S_3$; the L_6, L_7, and S_1 ventral roots were also cut on one side. The monosynaptic test response was elicited by weak stimulation of the S_1 dorsal root and recorded from the S_1 ventral root. Fig. 9 shows graphically the increase in the amplitude of the monosynaptic reflex response.

In none of the spinal preparations was any spontaneous repetitive activity observed in the ventral roots.

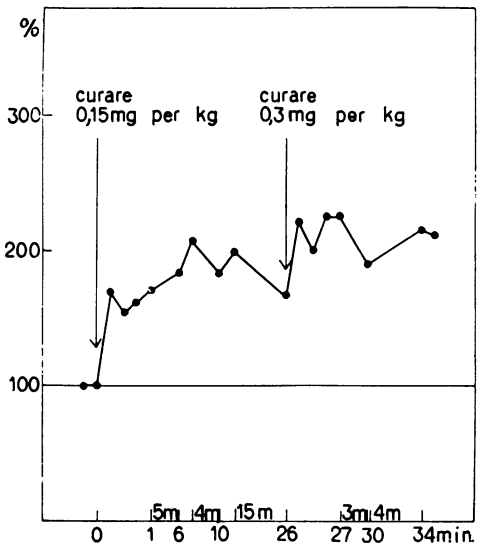

FIG. 7.—Effect of tubocurarine on the amplitude of monosynaptic response in a low spinal cat; 0.15 mg./kg. at zero time and 0.3 mg./kg. 26 min. later.

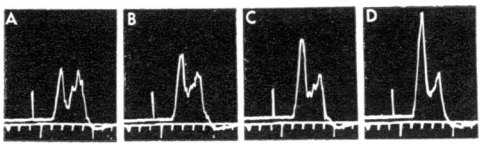

FIG. 8.—The reflex discharge in the S_1 ventral root after stimulation of the S_1 dorsal root (A) before, and (B) 7 min., (C) 11 min., and (D) 18 min. after, the injection of 1.04 mg. tubocurarine per kg. Low spinal preparation. Dorsal roots L_5–S_1 cut.

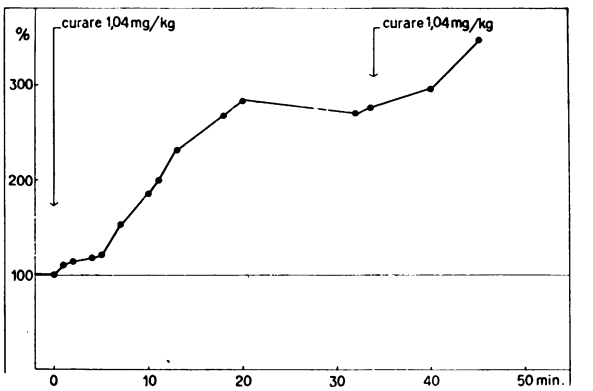

FIG. 9.—Effect of tubocurarine on the amplitude of the monosynaptic response in the L_7 ventral root after stimulation of the L_7 dorsal root in a deafferented low spinal preparation; 1.04 mg./kg. at zero time and the same dose 35 min. later.

Effect on threshold for reflex response

Fig. 10 shows three strength-amplitude curves for the monosynaptic reflex response obtained by stimulation of the nerve to one head of the gastrocnemius muscle in a decerebrate cat. The ordinates represent the amplitude of the reflex response in millivolts, and the abscissae are potentiometric units of stimulus strength. The right-hand curve shows the response to increasing strength of stimulus before the administration of tubocurarine. The middle curve was obtained after the intravenous injection of 0.75 mg. tubocurarine per kg., and the left-hand curve after a total of 3.7 mg. per kg. had been given. It will be observed that the threshold for reflex response remains unchanged, but the curves are increasingly steep, so that a smaller stimulus produces a larger reflex response, once threshold has been reached, after tubocurarine has been administered.

Fig. 11 is a similar curve obtained from a decerebrate cat by stimulating the nerve to one head of the gastrocnemius muscle and recording from the centrally cut S_1 dorsal root. The ordinates represent the amplitude of the earliest afferent volley in millivolts. The abscissae are potentiometric units of stimulus strength. This Figure shows that tubocurarine (0.6 and 1.2 mg./kg.) had no effect on the threshold amplitude of the afferent volleys.

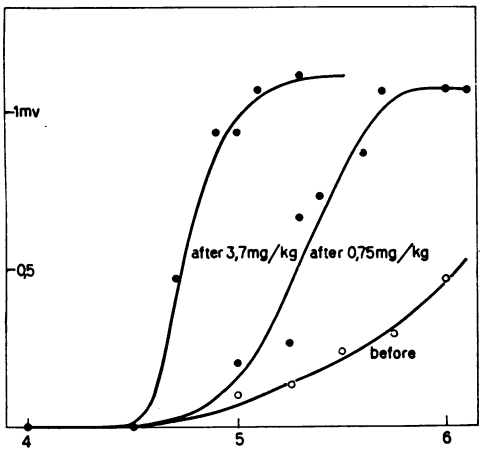

FIG. 10.—Amplitude of the monosynaptic response in mV. plotted against strength of stimulus (nerve to gastrocnemius) in potentiometric units before and after the injection of 0.75 mg. per kg. and 3.7 mg. per kg. of tubocurarine.

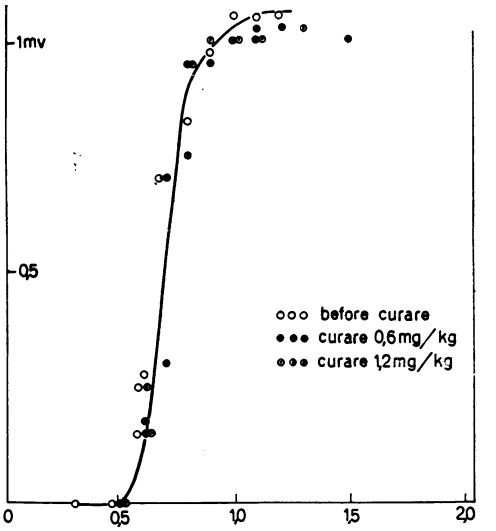

FIG. 11.—Amplitude of the S_1 dorsal root discharge in mV. plotted against strength of stimulus (nerve to gastrocnemius) before and after the injection of 0.6 mg. per kg. and 1.2 mg. per kg. of tubocurarine.

Effect of dial

If dial is injected intravenously 30 minutes before tubocurarine is given the increase in amplitude which is usually seen is either diminished or abolished entirely. Fig. 12 shows the result of such an experiment on a decapitate cat ; 35 mg. per kg. of dial were injected intravenously 30 minutes before the tubocurarine, which was also injected intravenously in a dose of 1.72 mg. per kg. A dose of this order given to a spinal preparation usually gives an increase in amplitude of the monosynaptic response amounting to 150–200 per cent of the average pre-injection test response within five minutes. In this experiment the large dose of tubocurarine given had no effect on the monosynaptic reflex after dial had been given. In other experiments a similar result was obtained, although on some occasions there was some increase in amplitude which, however, was usually short-lived.

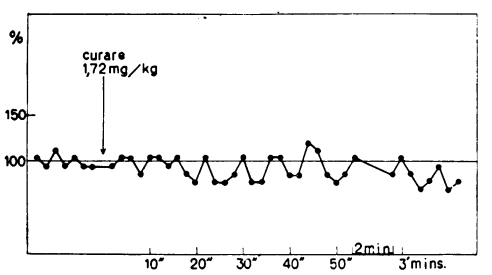

FIG. 12.—Effect of tubocurarine (1.72 mg./kg.) on the amplitude of the monosynaptic response in a high spinal preparation under the influence of dial (35 mg./kg.).

Simultaneous recording of reflex response and blood pressure

It is known that tubocurarine has an influence on the blood pressure, giving a rise after intraventricular or intracisternal injection (see, e.g., Salama and Wright, 1950) and a fall of pressure after intravenous application (see, e.g., Baisset *et al.*, 1949). Since aortic occlusion like asphyxia is followed by a temporary fall and a following increase of the monosynaptic reflexes (see Kirstein, 1951), it was of interest to see if the augmentation of the monosynaptic reflex after intravenous injection of tubocurarine is due to a change in blood pressure. Experiments were therefore performed in which the blood pressure was also recorded. The diagram in Fig. 13

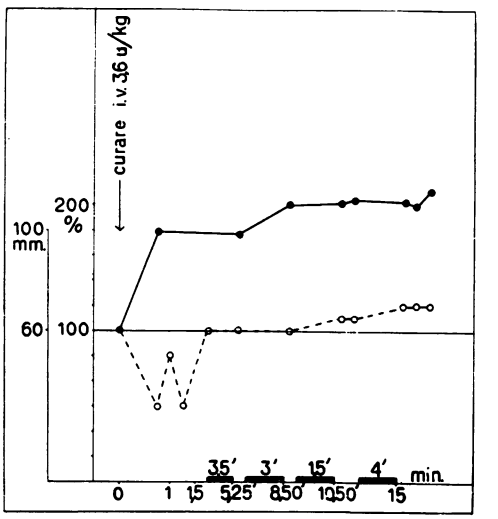

FIG. 13.—Effect of tubocurarine (0.54 mg./kg.) on the amplitude of the monosynaptic reflex response (unbroken line) and the blood pressure (broken line) in a decapitate cat.

shows the results from such an experiment on a decerebrate preparation, the arterial blood pressure being recorded from the common carotid artery. The injection of 0.54 mg. per kg. was followed by a transient fall of the blood pressure during the first two minutes from 60 to about 40, followed by a slow rise to about 70 mm. Hg. The monosynaptic reflex increased to about 200 per cent, and obviously this augmentation developed quite independently of the variations in the blood pressure. When the blood pressure reached its lowest value, 0.5 minute after the injection, the reflex was increased, but the same increase was found also when the pressure had reached the pre-injection value after five minutes.

During the next 10 minutes the blood pressure remained at the pre-injection value and even rose slowly to about 70 mm. Hg. In spite of that the reflex was still augmented to about 200 per cent 15 minutes after the injection. Thus the reflex changes are not related in time to the changes in blood pressure. If, however, in bad preparations the blood pressure was low (about 40 mm. or less) the reflex response became weaker or disappeared. In such preparations the injection of tubocurarine may give a serious drop of the blood pressure followed by a disappearance of the monosynaptic reflex. Such experiments were discarded.

Discussion

It is known that intravenously injected *d*-tubocurarine causes a fall in blood pressure which is partly related to the rate of injection. Baisset, Laporte, and Grezes-Rueff (1949) showed that, when the injection of tubocurarine evokes a sudden and deep fall of the blood pressure, the monosynaptic reflex in the ventral root occurring after dorsal root stimulation may show a transient rise followed by a fall and disappearance of the reflex. This effect, which can be compared with the effect of asphyxia and aortic occlusion (see Kirstein, 1951), was also found in some of our experiments, especially in preparations in which a low blood pressure was recorded before the injections. We found, however, that there is a rise in the amplitude of the monosynaptic extensor reflex which is independent of the transient fall of the blood pressure. Further, the increase of the monosynaptic extensor reflex was shown to persist for a long period after the injection. Our observations are in agreement with the findings of Salama and Wright (1950), who employed the classical technique of reflex study and found that *locally* applied tubocurarine is followed by an increase in the amplitude of the knee jerk. In the decerebrate preparation larger doses of tubocurarine produce spontanous repetitive activity in the ventral roots which appears to be unrelated to the degree of enhancement of the reflex response. It is possible that this effect is related to an influence of tubocurarine on the supraspinal centres as suggested by Salama and Wright (1950).

The experiments on preparations with spinal section at various levels, and on deafferented preparations, indicate that tubocurarine exerts its influence on all levels of the central nervous system and that the augmentation of the reflex response is not attributable only to stimulation of special " facilitatory centres " in the brain.

The fact that Baisset *et al.* did not find any change in the monosynaptic reflex after the injection of tubocurarine which was not correlated to the change of blood pressure may be due to the fact that the blood pressure was about 40 mm. Hg before the injection was given. In the cat at this blood pressure level the drug also has little effect on the reflex response. The negative results of Naess (1950) are apparently due to the use of dial preparations, because we found that dial itself abolishes the response to tubocurarine injections.

The action of tubocurarine on ganglionic and neuromuscular transmission is always depressant, never excitatory. It would be satisfactory to attempt to explain the central effect by a similar blocking action. Alternatively tubocurarine may have central effects totally different from its peripheral action. The phenomenon observed would then be due to a stimulatory effect of the drug on central nervous structures at all levels. This view is difficult to reconcile with the strength-amplitude curve in Fig. 11. This shows that the threshold is unaffected by tubocurarine, and suggests that the increased response is due to an increase in excitability of cells in the subliminal fringe and the recruitment of cells which were previously inexcitable. This might well indicate the removal of inhibitory influences, but is hardly compatible with the idea of a direct excitatory effect of tubocurarine on central structures. In such a case the threshold for reflex response would be expected to fall by a detectable amount.

If the effect on the spinal cord is attributed to a blocking or paralysing action then tubocurarine must have a selective effect on certain structures which normally have a continuous inhibitory action on the monosynaptic extensor reflex. This

concept receives some support from the action of dial, which diminishes or abolishes the central effect of tubocurarine. It has been suggested by Renshaw (1940) that dial depresses interneurone activity, and presumably such depression would make the " inhibitory structures " insusceptible to tubocurarine.

Finally, since both the fall in blood pressure and a liberation of histamine will evoke a secretion of adrenaline it may be argued that the augmentation of the monosynaptic extensor reflex is due to liberated adrenaline. It has earlier been shown that adrenaline influences spinal reflexes (see, e.g., Bülbring and Burn, 1941 ; Bernhard, Skoglund, and Therman, 1947 ; Bülbring, Burn, and Skoglund, 1948). In this connexion it must be emphasized that tubocurarine facilitates the monosynaptic extensor reflex but that we did not find any effect of tubocurarine on the multisynaptic flexor reflex (Bernhard, Taverner, and Widén, 1951) nor on the monosynaptic flexor reflex (unpublished observations). Such an explanation of the action of tubocurarine would imply that adrenaline has a selective effect on the extensor reflex (cf. Bernhard and Therman, 1947 ; Bernhard, Skoglund, and Therman, 1947). The observations that the augmentation of the monosynaptic extensor reflex lasts for a long period (in Fig. 9 more than half an hour) and that the effect is abolished in narcotized preparations (Fig. 13) make it difficult to attribute the effect described merely to the action of liberated adrenaline.

Further experimental results with special reference to the comparison between the central effects of strychnine and curare will be published later.

SUMMARY

1. The results are described of an investigation into the effects of intravenously injected *d*-tubocurarine on the monosynaptic extensor reflex response in the cat in unanaesthetized, decerebrate, decapitate, low spinal, and deafferented preparations and in spinal preparations under the influence of dial. The blood pressure changes were observed and their relationship to the changes in the reflex established.

2. In all the unanaesthetized preparations intravenously injected *d*-tubocurarine was found to increase the amplitude of the monosynaptic extensor reflex. This effect, which was most marked in decerebrate and high spinal and least in deafferented low spinal preparations, was shown not to be correlated with the changes in blood pressure.

3. Spontaneous repetitive activity was observed in the ventral roots of unanaesthetized decerebrate preparations only.

4. Dial injected intravenously before the *d*-tubocurarine was shown to diminish or abolish the effects described.

5. Possible mechanisms of the central action of *d*-tubocurarine are discussed.

This work has been supported by grants from the Rockefeller Foundation and the Swedish Medical Research Council.

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