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THE ACTION OF HYPNOTIC DRUGS ON FROG SKELETAL MUSCLE

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Barbiturate drugs with a short duration of action are used extensively both clinically and in animal experiments, and it is of importance to know how they modify the responses of the skeletal neuromuscular junction. Preliminary experiments showed that barbiturates could augment the "twitch" of isolated frog muscles excited by their motor nerves, so it was decided to study their action upon frog muscle in greater detail.

The ilio-fibularis muscle was used, since Kuffler and his associates (see Kuffler, 1946, 1953) had studied in detail its dual innervation by both large and small diameter motor nerve fibres. The isolated muscle responds with a twitch to electrical stimulation of its motor nerve, and with a contracture to acetylcholine (ACh) added to the fluid bathing it. An appraisal of the pharmacological reactions of this preparation was therefore of considerable interest.

Evidence is presented that the sodium salts of thiopentone, pentobarbitone and hexobarbitone augment the twitch of the ilio-fibularis muscle in response to either indirect or direct stimulation and at the same time decrease or abolish the ACh contracture. These effects are compared with the actions of the barbiturates on the nerve-sartorius and rectus abdominis preparations of the frog, and on the phrenic nerve-diaphragm preparation of the rat. The action of some non-barbiturate hypnotics on muscle is also reported. Some of the results were communicated to the British Pharmacological Society in January, 1952, and January, 1953.

METHODS

The ilio-fibularis muscle was exposed in the dorsum of the frog's thigh by retraction of the vastus externus and the semi-membranosus muscles. Its fascial sheath was carefully stripped, avoiding injury to its motor nerve—a fine branch of the sciatic entering the muscle in its proximal one-third on its antero-medial aspect. The muscle, with its nerve and parent sciatic trunk, was isolated and transferred to a 10 ml. bath of oxygenated frog Ringer solution at room temperature. Condenser discharges, through platinum electrodes, provided maximal stimuli at 15 sec. intervals to the sciatic nerve, or at 30 sec. intervals to the extremities of the muscle directly (M). The contractions to electrical stimuli, and the ACh contractures, were recorded isotonically by a weighted gimbal-lever. The muscle was loaded at 2 g., the amplification of the lever system being tenfold. ACh, acting for 60 sec., produced a contracture (Fig. 1). Responses to electrical stimulation, and sensitivity to ACh, were well maintained for many hours.

With the non-barbiturate hypnotics, single maximal stimuli were applied every 15 sec., alternately to the nerve and to the muscle, and the twitches, initially of equal height from both forms of stimulation, were recorded isotonically. A similar method was used for the nerve-sartorius preparation.

The technique used for the isolated frog rectus abdominis muscle was described by Quilliam and Strong (1949) and that for the isolated rat phrenic nerve-diaphragm preparation was a modification of Bülbring's (1946) method but with Krebs solution aerated with 5% CO_2 and 95% O_2.

RESULTS

The Isolated Frog Sciatic Nerve Ilio-fibularis Muscle Preparation.

In the normal muscle the twitches in response to both maximal indirect and direct stimulation were equal in height; 50 μg. of ACh produced a contracture.

Thiopentone Sodium.—Soon after adding the drug, in a concentration of 5×10^{-5}, there was a slow progressive increase in the contractions elicited by indirect stimulation, reaching a maximum in about 45 min. At this time the response to 50 μg. ACh was markedly reduced, though the addition of 1 mg. ACh produced a large contracture.

When the barbiturate concentration was increased to 1×10^{-4}, a further augmentation of the responses to nerve and to direct stimulation occurred; after 25 min. the addition of 50 μg. ACh produced a transient increase in the twitches but no contracture. The contracture caused by 1 mg. ACh was reduced

to that seen with the normal muscle after only 50 μg. ACh—a twentyfold decrease in ACh sensitivity. When the thiopentone concentration was raised to 2×10^{-4}, a further increase of the twitch occurred, but 1 mg. ACh now failed to produce contracture and temporarily reduced the muscle twitches. The contracture, originally evoked in the normal muscle by 10 mg. potassium chloride (KCl), was also abolished. Further increases of thiopentone concentration (to 3×10^{-4}, 5×10^{-4} and to 1×10^{-3}) produced little change in the height of the muscle twitches; 1 mg. ACh no longer caused contracture, but temporarily decreased the twitch responses. At a final concentration of 2×10^{-3}, there was a sharp decline in the responses to nerve stimulation, terminating in complete neuromuscular block. At this stage the direct muscle response was reduced to about one-half of the maximal twitch height seen. Repeated washing now partially restored the muscle twitch.

Pentobarbitone Sodium.—The augmentation of the muscle twitch with pentobarbitone sodium (Fig. 1) was considerably less than with thiopentone. At a concentration of 6×10^{-4}, neuromuscular block appeared—becoming complete at a concentration of 1.4×10^{-3}. With pentobarbitone, partial neuromuscular block occurred at a lower concentration (6×10^{-4}) than with thiopentone. No reduction was seen in the direct muscle response until the pentobarbitone concentration reached 1.4×10^{-3}. At this stage repeated washing restored the muscle responses to direct and indirect stimulation. The contracture from 20 μg. ACh was decreased in stages as the concentration of pentobarbitone was increased, and was abolished only at 6×10^{-4}. At the highest concentration attained (1.4×10^{-3}), 1 mg. ACh still evoked a contracture, but this was considerably smaller than that with 10 μg. ACh in the fresh muscle. With this barbiturate concentration, no contracture occurred with 5 mg. of KCl.

Hexobarbitone Sodium.—With this drug, the augmentation of the twitch response to indirect and direct stimulation followed the pattern seen with pentobarbitone sodium except that the neuromuscular block, which appeared at 1×10^{-3}, was never complete—even at the highest concentration used (1.5×10^{-3}), when the indirect response was reduced to about 60% of the direct response. The contracture elicited by 25 μg. ACh was less readily suppressed than with pentobarbitone, and only at the highest hexobarbitone concentration was it abolished; at this stage 1 mg. ACh produced a contracture much smaller than did a test dose of 25 μg. in the fresh muscle.

Comparison of Effects of Barbiturates on the Muscle Twitch and on the ACh Contracture.—In Fig. 2 the increase in the muscle twitch in response to indirect stimulation is plotted against the con-

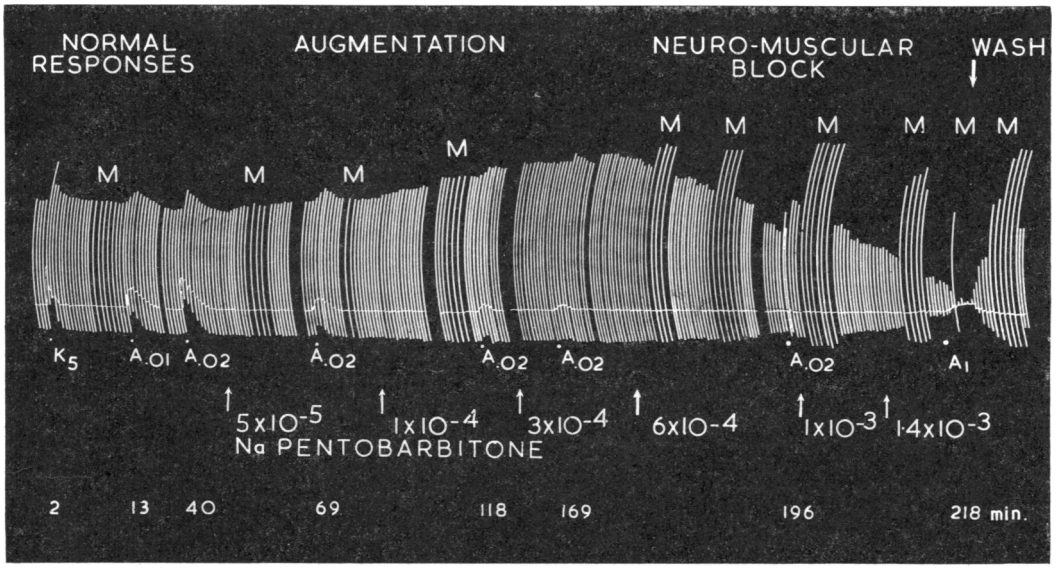

FIG. 1.—Isolated frog sciatic nerve ilio-fibularis muscle preparation. Record of isotonic twitches evoked by indirect or direct (M) single maximal stimuli. The contracture response to 0.01 mg. (A 0.01), 0.02 mg. (A 0.02), and 1 mg. (A1) ACh and to 5 mg. (K5) KCl are recorded at intervals. The record illustrates the action of pentobarbitone sodium which first augments the muscle twitch; it then produces neuromuscular block and decreases the contracture response to added ACh.

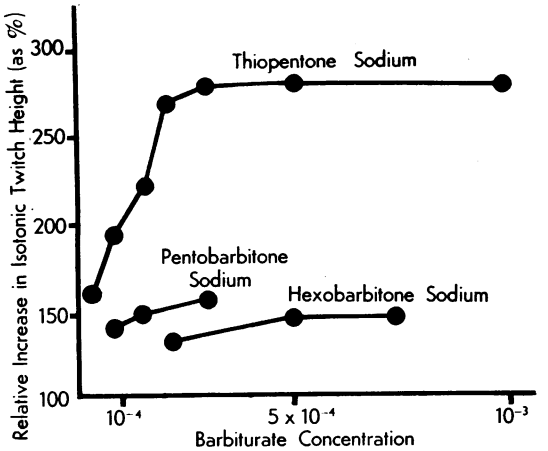

FIG. 2.—Isotonic twitches were recorded from the isolated frog ilio-fibularis muscle stimulated indirectly. The graph shows the relation between concentration of barbiturate and percentage increase in twitch height.

centration of barbiturate. The graphs were constructed from measurements obtained from Fig. 1 and from typical experiments with thiopentone and hexobarbitone.

The twitch heights progressively increased as the drug concentrations rose. With each barbiturate, a concentration was reached above which no further augmentation occurred. Thiopentone sodium differed from the other two compounds in giving nearly twice as much augmentation.

The contracture elicited by a small dose of ACh (usually 20 to 50 μg./10 ml.) was decreased by low and abolished by high concentrations of the three barbiturates. The effects of 1 mg. ACh were decreased by high concentrations of all three barbiturates, but were abolished only by thiopentone sodium. Thiopentone was thus the most active agent in reducing the ACh contracture, pentobarbitone was intermediate, and hexobarbitone was the least active.

Other Isolated Muscle Preparations

Experiments with the frog sartorius and rectus and the rat diaphragm muscles were conducted along the same general lines as those used with the ilio-fibularis muscle. In most instances, 4 to 7 experiments were made to provide each average figure shown in Table I.

The order of activity of the barbiturates in producing firstly an initial and secondly a maximum increase in twitch height in all muscles examined was thiopentone, then pentobarbitone and lastly hexobarbitone. In the production of neuromuscular block, the most active drug was, with the ilio-fibularis and the diaphragm, pentobarbitone and, with the sartorius, thiopentone. No neuromuscular block could be obtained with thiopentone in the rat diaphragm, since the responses to both direct and indirect stimulation were decreased equally as the concentration rose, the strength chosen for direct muscle stimulation being sufficient to excite a fully curarized muscle.

Other Barbiturates

Other barbiturates were less active than the three just described in producing the characteristic augmentation of the muscle twitch response and a reduction of the acetylcholine contracture in frog muscle (Table II). Even with the rectus, which was the most sensitive of the three test tissues, the concentrations of barbiturates required were too high to permit detailed quantitative study. Large doses of the barbiturates listed in Table II produced an augmentation of the twitch response in the rat diaphragm stimulated via its nerve.

Control Experiments with the Isolated Frog Sartorius and the Ilio-fibularis Muscle Preparations

As dilute solutions of the sodium salts of barbiturates are alkaline, measurements were made of the effect of sodium and hydroxyl ions on the

TABLE I

EFFECTS OF BARBITURATES ON MUSCLE TWITCH RESPONSES

Average Dose Required to Cause:	Thiopentone Sodium			Pentobarbitone Sodium			Hexobarbitone Sodium		
	Initial Increase in Twitch Height (μg./ml.)	Maximum Increase in Twitch Height (μg./ml.)	Neuro-muscular Block (μg./ml.)	Initial Increase in Twitch Height (μg./ml.)	Maximum Increase in Twitch Height (μg./ml.)	Neuro-muscular Block (μg./ml.)	Initial Increase in Twitch Height (μg./ml.)	Maximum Increase in Twitch Height (μg./ml.)	Neuro-muscular Block (μg./ml.)
Frog muscle:									
Ilio-fibularis ..	50	150 (250)	2,000	50	300 (150)	570	300	1,000 (140)	680
Rectus abdominis ..	25	310 (330)		100	1,500 (230)		200	2,500 (200)	
Sartorius	25	50 (160)	100	100	500 (150)	500	100	1,000 (150)	1,000
Rat muscle:									
Diaphragm ..	12·5	85 (150)		17·5	172 (120)	220	98	440 (140)	570

The figures in parentheses indicate the maximum % increase in twitch height.

TABLE II

AVERAGE THRESHOLD CONCENTRATIONS REQUIRED TO PRODUCE AUGMENTATION OF THE MUSCLE TWITCH

	Frog Muscle		Rat Muscle
	Ilio-fibularis (mg./ml.)	Rectus (mg./ml.)	Diaphragm (mg./ml.)
Butobarbitone sodium ..	20*	2*	0·16
Allobarbitone „ ..	30*	4*	0·16
Barbitone „ ..	50*	10	0·60
Phenobarbitone „ ..	50	10	0·30

* Indicates that ACh-contracture was decreased at this concentration.

muscle twitch. When the sodium chloride concentration was raised by 1 mg./ml. in the frog Ringer solution bathing a fresh muscle, there was little change in the response to either direct or indirect stimulation. When the sodium bicarbonate content of the Ringer solution was raised by 100 μg./ml. no alteration of the responses of the muscle occurred, although larger amounts (1 mg./ml.) decreased the twitch elicited by nerve or muscle stimulation, an effect which was completely reversed by washing. If the *p*H of the Ringer solution was raised by adding 100 μg./ml. sodium hydroxide, there was an abrupt augmentation of the responses to both types of stimulation. When the *p*H was returned to 7.2 by bubbling a mixture of 5% CO_2 and 95% O_2 through the bath liquid, the height of the muscle twitches returned to the original level. However, larger quantities of sodium hydroxide (200 μg./ml.) decreased both types of muscle response, but adjustment of the *p*H to 7.2 restored the original amplitude of the twitches.

If pentobarbitone sodium (final concentration 100 μg./ml.) was added to the Ringer bathing a fresh muscle, the *p*H of the fluid rose above 8.8 and the muscle twitches in response to nerve and muscle stimulation were augmented equally. When this augmentation had reached its maximum, adjustment of the *p*H to 7.2 with CO_2 produced a further augmentation of the responses to both types of stimulation. With higher concentrations of pentobarbitone, it was not possible to return the bath fluid to *p*H 7.2 with CO_2. Similar effects were observed with thiopentone and hexobarbitone.

Non-Barbiturate Hypnotic Drugs

Paraldehyde, added to the fluid bathing a fresh ilio-fibularis muscle, to give a final concentration of 0.5 mg./ml., caused a slight decrease in the height of the muscle twitch elicited by a single maximal stimulus applied to the motor nerve. As the paraldehyde concentration was raised, neuromuscular block increased and became complete at 1.0 to 1.5 mg./ml.; the response to direct muscle stimulation remained unchanged. When the amplitude of the indirect response had fallen to a constant value, the addition of 50 μg. of ACh, or of 50 μg. of decamethonium iodide, caused a transient increase in twitch height. The block from paraldehyde was not relieved by eserine, but washing restored the normal response of the preparation.

Methylpentynol exerted an action upon the ilio-fibularis muscle similar to that seen with paraldehyde. The addition of 0.5 mg./ml. caused a small degree of neuromuscular block (Fig. 3). When the concentration was increased to 1.0 mg./ml., a more rapidly developing neuromuscular block set in which could be partly relieved, but only for the short period of 5 min., by adding either 50 μg. decamethonium or 50 μg. ACh to the 10 ml. bath. As with paraldehyde, prolonged exposure to decamethonium always produced an increase of the neuromuscular block. After washing in frog Ringer solution, the amplitude of the muscle twitch in response to nerve stimulation was restored to that seen in the fresh muscle.

Chlorbutol was similar in action to paraldehyde except that it produced a complete neuromuscular block in a concentration of only 0.5 mg./ml.

Urethane produced neuromuscular block at 5 mg./ml., and was thus similar to, but less potent than, paraldehyde.

Chloralose in concentrations of 1.5 to 2.5 mg./ml. caused a progressive augmentation of the muscle twitch in response to both direct and indirect

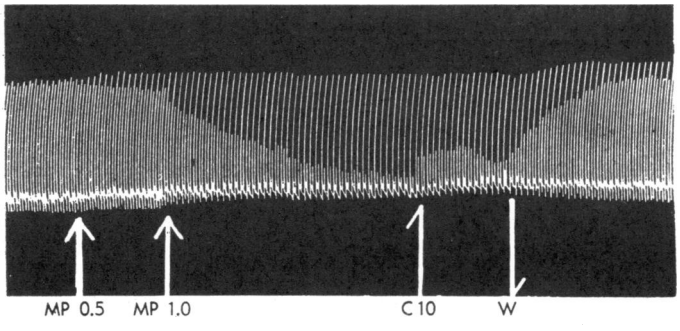

FIG. 3.—The isolated frog sciatic nerve ilio-fibularis muscle preparation. A single maximal stimulus was applied every 15 sec. alternately to the nerve or to the muscle directly. Methylpentynol 0.5 mg./ml. (MP 0.5) and 1 mg./ml. (MP 1.0) caused neuromuscular block which was partly relieved by 50 μg. of decamethonium (C10). Washing (W) restored the responses. Bath volume—10 ml.

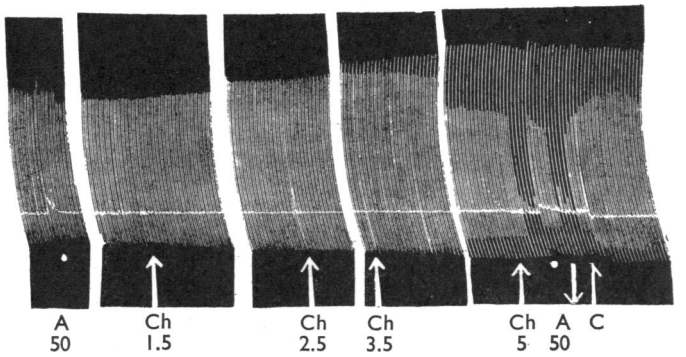

FIG. 4.—Record as in Fig. 3. Chloralose 1.5 mg./ml. (Ch 1.5) and 2.5 mg./ml. (Ch 2.5) caused an augmentation of the muscle twitch in response to indirect or direct stimulation. With 3.5 mg./ml. (Ch 3.5) and 5.0 mg./ml. (Ch 5), a neuromuscular block developed which was partly but transiently relieved by 50 μg. ACh (A50) or by 25 μg. decamethonium (C). At downward pointing arrow, the muscle was washed with frog Ringer solution containing 5 mg./ml. of chloralose. The contracture following ACh (A50) was only slightly reduced by chloralose.

stimulation, like the barbiturates. With higher concentrations there was a further augmentation of the response to direct stimulation but a decline of that to nerve stimulation. At a concentration of 5 mg./ml. complete neuromuscular block developed rapidly; it was reversed immediately but temporarily by 50 μg. ACh or by 25 μg. decamethonium. Fig. 4 illustrates these effects. Washing removed the effects of chloralose.

The effects of *carbromal*, in concentrations of 0.06 mg./ml. and 0.12 mg./ml., are shown in Fig. 5. The responses to both forms of stimulation were at first augmented; neuromuscular block then appeared and soon became complete. The responses to direct stimulation continued to be augmented, reaching nearly twice their initial value, an effect that was particularly marked with carbromal. At this stage, neither 50 μg. ACh, 50

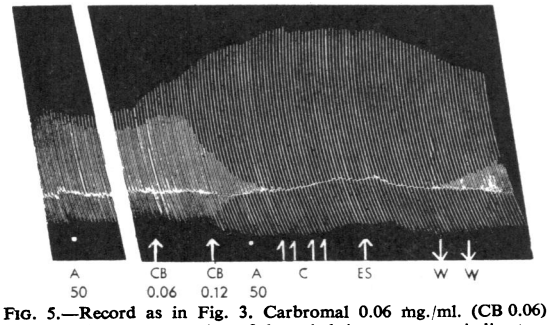

FIG. 5.—Record as in Fig. 3. Carbromal 0.06 mg./ml. (CB 0.06) caused an augmentation of the twitch in response to indirect or direct stimulation. Carbromal 0.12 mg./ml. (CB 0.12) produced a neuromuscular block associated with a marked augmentation of the direct muscle response. Neither 50 μg. ACh (A50), four doses of 50, 50, 100, and 100 μg. decamethonium (C) nor 100 μg. eserine (ES) relieved the block. ACh and decamethonium were followed by a contracture. Washing (W) restored the responses.

μg. decamethonium, or 100 μg. eserine, relieved the block though they did so at lower concentrations of the hypnotic. Washing usually restored the responses of the preparation to their original size.

Chloral hydrate did not cause neuromuscular block but, in doses of 0.2 to 2.5 mg./ml., decreased equally the twitches evoked by direct and by indirect stimulation.

Non-barbiturate drugs, then, in concentrations which produced a marked degree of neuromuscular block, caused little change in the ACh contracture of the ilio-fibularis muscle.

In the rat diaphragm, paraldehyde, methylpentynol, chloralose and carbromal produced neuromuscular block in concentrations comparable with those required to block the amphibian neuromuscular junction.

DISCUSSION

One action of barbiturates upon isolated frog skeletal muscles was to augment the twitch arising from nerve stimulation. A similar effect was observed with the rat diaphragm. Other workers have also seen this response in mammalian muscle (Huston, Martin, and Dille, 1947; Riedel and Huston, 1949; Secher, 1951; Kraatz, Gluckman, and Shields, 1953). Thus Secher (1951) reported this action with the rat diaphragm and Huston, Martin, and Dille (1947) observed an increase in the contractions of the gastrocnemius muscle elicited by indirect stimulation when pentobarbitone was added to the defibrinated blood perfusing a dog's isolated hind limb. In the rat, intraperitoneal administration of amylobarbitone sodium was followed by an increased twitch from the sciatic nerve-gastrocnemius muscle preparation (Riedel and Huston, 1949) and Kraatz, Gluckman, and Shields (1953) saw, with thiopentone and pentobarbitone, a similar augmentation in tension in the indirectly stimulated anterior tibial muscles of cats and dogs.

The depression of the ACh contracture in frog muscle with barbiturates has received no attention except for the report of Torda and Wolff (1947), who demonstrated a reduction in the ACh contracture of the rectus abdominis muscle with pentobarbitone and amylobarbitone.

The control experiments showed that neither sodium nor hydroxyl ions contributed to the aug-

mentation by barbiturates of the twitch heights of isolated frog muscles, but that these ions would be expected to oppose the observed muscle responses. The simple gimbal lever, lightly weighted, was used to record the isotonic twitches in isolated frog muscles because concurrent records of the ACh contractures were required. The mechanical limitations of such a lever system must not be overlooked even though they were reduced by the strict application of a comparative technique.

TABLE III

A CLASSIFICATION OF HYPNOTIC DRUGS BASED UPON THEIR ACTION UPON THE MUSCLE TWITCH RESPONSE OF THE ISOLATED SCIATIC NERVE ILIO-FIBULARIS MUSCLE PREPARATION

Group	Hypnotic	Threshold Concentration (mg./ml.)
(*a*) Augmentation of muscle twitches evoked by indirect and direct stimulation	Thiopentone sodium Pentobarbitone ,, Hexobarbitone ,,	0·05 0·05 0·30
(*b*) Neuromuscular block with response of muscle to direct stimulation unaltered	Chlorbutol Paraldehyde Methylpentynol Urethane	0·1 0·5 0·5 5·0
(*c*) Neuromuscular block with an augmented response from direct muscle stimulation	Carbromal Chloralose	0·12 3·50
(*d*) Depression of twitches in response to indirect and direct stimulation	Chloral hydrate	0·20

Whereas the first action of barbiturates upon amphibian and mammalian muscle was to produce an augmentation of the muscle twitch, that of non-barbiturate hypnotics was to impair neuromuscular transmission. On the basis of these peripheral actions, it was possible to divide hypnotics into four main classes (Table III). Both urethane in group (*b*) and chloralose in group (*c*) are distinguished by their low neuromuscular blocking activity.

The actions of hypnotics at the skeletal neuromuscular junction may be regarded as side effects. It was of some interest to consider what relation the concentrations used in these experiments bore to the mean human pharmacopoeial dose, expressed either as mg./kg. in a man weighing 70 kg. or as a concentration in mg./ml., assuming that it might be theoretically possible to distribute the dose equally throughout a blood volume of 5 l. In Table IV an estimate of these concentrations is made. The ratio of the human therapeutic dose (mg./kg.) to the threshold dose of the hypnotic (mg./ml.) required to produce an effect in the isolated frog ilio-fibularis muscle ranges, with four exceptions, between 77.5 and 143: with hexobarbitone it is 29, with methylpentynol 14.3, with urethane 4.3, and with chloralose 1.6. There is a positive correlation between the theoretical blood concentration and the *in vitro* dose required, except with the four drugs just mentioned. The discrepancy with methylpentynol might arise from too low an estimate of the human therapeutic dose: further experience with this hypnotic in man might decide this point. The larger discrepancies with urethane and chloralose are specially interesting, for these two compounds are used to produce long-lasting general anaesthesia in animals. Exley (1954) found that chloralose was almost devoid of ganglion-blocking activity. Thus chloralose commends itself as a general anaesthetic in laboratory experiments in which neuromuscular or ganglionic transmission is to be studied, the drug being virtually without action on both structures.

Mechanism of Action of Hypnotics on Muscle

The augmentation by barbiturates of the muscle twitch elicited by indirect stimulation might be

TABLE IV

RELATION OF HUMAN THERAPEUTIC DOSE TO THRESHOLD CONCENTRATION REQUIRED FOR AN ACTION ON THE ISOLATED FROG ILIO-FIBULARIS MUSCLE PREPARATION

The mean of the human therapeutic dose is expressed both as mg./kg. in a man weighing 70 kg. and as the concentration of the hypnotic which might be attained if it were theoretically possible to distribute it equally throughout a blood volume of 5 l.

Group and Compound	Mean of Human Therapeutic Dose		Theoretical Concentration in Blood mg./ml.	Threshold Concentration for Action on Ilio-fibularis mg./ml.	Ratio Human Therapeutic Dose (mg./kg.)/ Threshold Dose for Ilio-fibularis
	g. or ml.	mg./kg.			
(*a*) Thiopentone sodium	0·3	4·3	0·06	0·05	86
Pentobarbitone ,,	0·3	4·3	0·06	0·05	86
Hexobarbitone ,,	0·6	8·6	0·12	0·3	29
(*b*) Chlorbutol	0·75	10·7	0·15	0·1	107
Paraldehyde	5·0	71·4	1·0	0·5	143
Methylpentynol	0·5	7·14	0·1	0·5	14·3
Urethane	1·5	21·4	0·3	5·0	4·3
(*c*) Carbromal	0·65	9·3	0·13	0·12	77·5
Chloralose	0·4	5·7	0·08	3·50	1·6
(*d*) Chloral hydrate	1·6	23·1	0·32	0·20	115

expected to arise from either an anticholinesterase activity or an altered response of the muscle cell. The observed depression by barbiturates of the ACh contracture affords evidence that an anticholinesterase action is not implicated. As the effect of thiopentone is consistent with a decrease in the rate of propagation of the muscle action potential in the rat gracilis muscle (Quilliam, 1955), the mode of action of barbiturates on the " twitch muscle system " appears to reside in altered response of the muscle fibre.

The neuromuscular block caused by non-barbiturate hypnotics could arise from a decreased production of the local hormone at the nerve endings, an increased cholinesterase activity, a competitive blocking action or a long-lasting depolarization of the muscle fibre in the region of the neuromuscular junction. The present experiments throw no light upon the ACh release at the nerve endings nor upon the depolarizing activity of the drugs ; but the absence of a material change in the amplitude of the ACh contracture appears to eliminate increased cholinesterase activity as a major cause. The relief of the block by added ACh or decamethonium lends support to the view that the block is competitive in nature, but the absence of an effect with eserine in this connexion is puzzling. The mechanism of the augmentation of the response to direct stimulation probably resides in an altered response on the part of the muscle fibre itself because of the neuromuscular blocking action of the non-barbiturate hypnotics.

Before a final view of the mode of action of these groups of hypnotics upon the skeletal neuromuscular junction can be taken, a further study of the electrical changes associated with the action of these drugs upon the muscle cell must be undertaken.

The present work shows that the isolated frog sciatic nerve ilio-fibularis muscle preparation can be used to provide concurrent information on the contracture response evoked from frog muscle by ACh added to the bath fluid and on the twitch elicited by maximal stimulation of the motor nerve or of the muscle directly. Although it is outside the province of this paper to discuss in detail the relationship between these responses and those of the dual skeletal nerve-muscle systems in the frog (Kuffler, 1953), it is sufficient here to draw an analogy between the known effect of stimulation of the " slow skeletal muscle system " and the addition of ACh on the one hand, and between the effect of stimulation of the " twitch system " and the response of the ilio-fibularis muscle to single stimuli applied to its motor nerve on the other hand, and to point out the action of drugs on these effects.

Summary

1. The actions of the sodium salts of thiopentone, pentobarbitone, and hexobarbitone, upon the contractures elicited by ACh, and upon twitches evoked by electrical stimulation in the isolated frog sciatic nerve ilio-fibularis muscle, were compared with those in the isolated frog motor-nerve sartorius and the rectus abdominis muscle preparations.

2. In the frog ilio-fibularis, the contractures elicited by small doses of ACh were reduced by low and abolished by high concentrations of the three barbiturates. The contracture from a large dose of ACh was decreased by high concentrations of the three barbiturates but was abolished only by thiopentone. In these actions thiopentone was the most active agent ; pentobarbitone was next, and hexobarbitone was least.

3. The three barbiturates augmented the height of the muscle twitch elicited by indirect electrical stimulation of isolated frog muscles and of the isolated rat diaphragm. In this direction also, thiopentone was the most active, hexobarbitone the least active, with pentobarbitone occupying an intermediate position. High concentrations of the three barbiturates caused a block in neuromuscular transmission in all the muscles except the rat diaphragm, in which thiopentone reduced the responses to indirect and direct stimulation equally.

4. Large doses of the sodium salts of butobarbitone, allobarbitone, barbitone and phenobarbitone reduced the ACh contracture in frog muscle and augmented the twitch in response to indirect and direct electrical stimulation in frog and rat muscles.

5. Non-barbiturate hypnotics, such as paraldehyde, methylpentynol, chlorbutol and urethane, produced a neuromuscular block in concentrations which cause little, if any, reduction of the ACh contracture in the frog ilio-fibularis muscle. With chloralose and carbromal, the neuromuscular block is associated with an augmentation of the muscle twitch following direct electrical stimulation.

6. A classification of hypnotics is proposed, based upon their ability to augment or to reduce the height of the muscle twitch in response to nerve stimulation. The significance of these effects and the possible modes of action of these drugs are discussed.

7. The high concentrations of chloralose and of urethane required to reduce the response to nerve stimulation commend them as general anaesthetics for laboratory animals when studies of neuromuscular transmission are being made.

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