THE MODE OF ACTION OF ω -AMINOACYLCHOLINES ON THE NEURO-MUSCULAR JUNCTION

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The theory of chemical transmission of acetylcholine is the fundamental thesis on which every pharmacologist constructs his thought of life science such as pharmacology. In the modern age when not only probable transmitters other than acetylcholine for the synaptic transmission in the central nervous system but also probable interaction between acetylcholine and catecholamine at the cholinergic nerve ending are presented, events of chemical transmission are not believed to be so simple as they were believed to be solved completely in the past.

There is no doubt that the neuromuscular junction is the best site for the study on the chemical transmission. Among very large amount of literatures Thesleff (1) broke the concept of the persistent depolarization of the end plate region of C_{10} and others in his enlightened work while he proved the lowered sensibility of end plate region against acetylcholine in the maximum effect of N-M blocking produced by so-called depolarizing agents. As far as the acetylcholine and its receptor are understood firmly as key and lock, the concept of the insensibility of membrane brings us in chaos in the fundamental understanding of events in the neuromuscular junction. Furthermore recent publications of Riker's group (2-4) shaken enough the concept of acetylcholine as transmitter in the neuromuscular junction hitherto in use for understanding of the competitive blocking by *d*-Tc.

In the progress of the study on the esters of γ -aminobutylic acid (8), ω -aminoacylcholine esters were studied in this laboratory. Authors described its pharmacological character in this paper, of which mode of action was between these of *d*-Tc and succinylcholine, even though authors have no confident thought for understanding the chemical events in the neuromuscular transmission.

METHODS

Twenty-one mongrel dogs, either sex, around 10 kg of body weight and 14 cats, either sex, around 2.5 kg of body weight were used. Dogs and few cats were anesthetized with sodium pentobarbital (30 mg/kg, intravenously). A majority of experiments in cats were done under α -chloralose anesthesia, 50 to 80 mg/kg, intravenously with the initial inhalation of ether.

Animals were placed on the animal board, not fixing tightly. The distal end of the femur was nailed and fixed tightly on the arm of the board. The tendon of the anterior tibial muscle was isolated and cut at the distal end. A cotton cord fastened at the cut end of the tendon was stretched to a steel pole for obtaining the isometric contraction. The tension developed was registered on the smoked drum by 10 times magnification. In a few experiments the tendon of the gastrocnemius-soleus muscle was simultaneously arranged in the same way for comparing the effect of drug between flexor and extensor muscles. The distal end of the cut tibial nerve placed on the stimulatory electrode was occasionally rinsed with Ringer's solution for keeping out of drying and the square wave current, 0.05 msec and 10 volts, was applied at every 10 seconds for the supramaximal stimulation and 10 to 30 cps in a period of 20 seconds for the tetanic stimulation. In the gastrocnemius-soleus muscle preparation, the sciatic nerve was stimulated.

The tracheal tube was cannulated and the artificial respiration was arranged in 15 cpm with a tidal volume of 10 ml per kg of body weight. The blood pressure was measured at the carotid artery. For observing the systemic action the drug solution dissolved in Ringer's solution was administered in a shot through a polyethylen tube inserted in the jugular vein followed by flushing with 0.5 ml of Ringer's solution. For observing the synergism and antagonism one another thin polyethylen catheter, 1 mm outer diameter was inserted into a small branch of the femoral artery, in which 0.1 ml of the drug solution was administered in one shot meanwhile the test drug was injected intravenously by use of the perfusing machine.

The compounds in question were chlorides of 6-aminocaproylcholine (II), analogues of one less methylen ring, 5-aminopentanoylcholine (I) and of one more methylen ring, 7-aminoheptanoylcholine (III) and 6-aminocaproyl- β -methylcholine. Authors called the

FIG. 1. The initial facilitation of twitch height by continuous infusion of compound (II).

former three compounds as *ω*-aminoacylcholines in this paper. *d*-Tubocurarine chloride (*d*-Tc) and succinylcholine chloride were used for comparison. Edrophonium chloride, tetramethyl-(TMA), tetraethyl-(TEA), tetrapropyl-(TPA), and tetrabutylammonium(TBA) chloride or bromide were tested for analysing the mode of action of these *ω*-aminoacylcholines. *ω*-Aminoacylcholines and 6-aminocaproyl- β -methylcholine chloride were synthesized in the Dai-ichi Seiyaku Laboratory.

RESULTS

The *ω*-aminoacylcholine showed two principal actions on the whole animal, the para-

FIG. 2. The dose-response relation of compounds (I), (II), (III) and succinylcholine on the dog's anterior tibial muscle preparation in reference to the effect on the systemic blood pressure by i.v. administration.

lysis of the skeletal muscle and elevation of blood pressure. The original causes of these effects were the block of neuromuscular transmission and the ganglion stimulation.

1) Doses and responses

The initial facilitation of the contraction of the tibial muscle was observed before induction of the neuromuscular block by use of these compounds. This phenomenon was easily obtained in the first or second injection, but it disappeared after repeated administration. When these compounds were infused continuously in a constant speed, there was a certain critical dose level to obtain this phenomenon over which dose level the facilitation was not observed (Fig. 1).

A little difference existed among three ω -aminoacylcholines in the effective dose levels to block the N-M transmission. One typical dose response relation obtained in

FIG. 3. The dose-response relation observed in the cat's anterior tibial (upper) and gastrocnemius-soleus muscle (lower).

the tibial muscle preparation of dog was shown in Fig. 2 in reference to the effect on the systemic blood pressure. Effects were compared to those of succinylcholine. The similar results were obtained in cats (Fig. 3). As a rule, compounds (I), (II) and (III) were less potent on the N-M junction and much potent on the ganglion stimulation compared with succinylcholine. Compounds (II) and (III) were almost equally effective on the N-M junction and on the blood pressure, while the potency of compound (I) was far weaker on blocking N-M junction but more potent on the ganglion stimulation than the former two compounds.

It must be worthy to note that the neuromuscular blocking action was induced more quickly by use of ω -aminoacylcholines and restored more completely and more rapidly to the preadministration level by discontinuing the infusion of drug than by use of succinylcholine, when the equivalent effect were compared. There was no difference on the mode of recovery even after three hours continuous infusion of compound (II) in a dose level which maintained the minimal contraction of the tibial muscle of cats and dogs.

β -Methyl analogue of compound (II) showed one-two hundredth potency of compound (II) on the neuromuscular block but the potency on the ganglion stimulation was almost same. It showed very definite but temporal stimulation of respiration (Fig. 4).

FIG. 4. The effects of β -methyl analogue of compound (II) on the respiration, blood pressure and N-M junction (the anterior tibial muscle) of dog.

2) Tetanic stimulation

The tetanic stimulation, 10 to 30 cps in a period of 20 seconds, was occasionally applied, when a sustained partial block of the N-M transmission was obtained by continuous infusion of ω -aminoacylcholines (Fig. 5).

FIG. 5. Changes of response pattern to the tetanic stimulation on the cat (30 cps). a) succinylcholine; b) compound (II); c) compound (III); and d) *d*-Tc.

In the nontreated preparation the tetanic contraction occurred at the impact of stimulation and showed a gradual increment of contraction height as long as the stimulation continued. In the continuous infusion of succinylcholine the tetanic contraction was well maintained similar to that in the nontreated preparation although a gradual decrement of the height of the tetanic contraction was observed (Fig. 5, a), while in cases of *d*-Tc the tetanic contraction could not be maintained its height and the muscle became flaccid soon after the maximal contraction occurred (Fig. 5, d). The posttetanic facilitation was observable in the partial block by use of *d*-Tc but not by use of succinylcholine.

When the tetanic stimulation was applied to the state of partial block by continuous infusion of ω -aminoacetylcholine, the pattern of the tetanic contraction and the occurrence

of the posttetanic facilitation were close to that observed by use of *d*-Tc in the majority of cases (Fig. 5, b). However in few cases the similar pattern to that of succinylcholine was obtained (Fig. 5, c).

The characteristic property of the quick restoration as described above was also displayed in the quick recovery of the pattern of the tetanic contraction to that in the preadministration period.

3) Antagonism and synergism

The antagonism between *d*-Tc and ω -aminoacylcholines was observed (Fig. 6). Edrophonium did not operate such a definite antagonism against the neuromuscular block

FIG. 6. Antagonisms between *d*-Tc and succinylcholine and between *d*-Tc and ω -aminoacylcholines on the dog's anterior tibial muscle.

FIG. 7. a) Effects of edrophonium on the N-M blocking induced by *d*-Tc, succinylcholine and compound (II); b) Effects of TEA on the same condition as above.

produced by infusion of compounds (I), (II) or (III) as that between edrophonium and *d*-Tc, rather edrophonium potentiated the effect of compounds (I), (II) and (III). Edrophonium potentiated the action of succinylcholine except in the late stage of administration when the dual block phenomenon had occurred (Fig. 7, a).

TEA was given in doses of 1–10 mg/kg in the partial block of the neuromuscular junction with continuous infusion of compound (II). The twitch height was apparently restored by TEA in proportion to the dose as shown in a typical result of Fig. 7, b. Such antagonism was observed also between TEA and compounds (I) or (III) and also between TEA and *d*-Tc. Meanwhile the antagonism between TEA and succinylcholine was not constant, rather weak and in a short period, even if there would have been.

TMA, TPA and TBA potentiated the neuromuscular blocking action of compounds (I), (II), (III) and succinylcholine, while *d*-Tc was antagonized by TMA (Fig. 8). It is

FIG. 8. Effects of TMA, TPA and TBA on the N-M blocking induced by *d*-Tc, compound (II) and succinylcholine (intraarterial administration).

FIG. 9. Effects of β -metnyl analogue on the N-M blocking induced by compound (II).

interesting that the definite antagonism was observed between compound (II) and its β -methyl analogue in some cases even though in the other cases both compounds showed synergism (Fig. 9).

4) Toxicity

The acute toxicity was determined on new hatched chickens and DD strain of mice. Drugs were given intramuscularly and LD_{50} 's were calculated by Wilcoxon-Litchfield

TABLE 1. Toxicity of ω -aminoacylcholines in mice and chickens in reference to succinylcholine and *d*-Tc.

	Mice	Chickens
I	3.4 mg/kg (3.3-3.9)	5.0 mg/kg (3.2-8.0)
II	10.7 mg/kg (8.6-13.3)	1.3 mg/kg (1.17-1.45)
III	48.5 mg/kg (43.0-54.7)	0.24 mg/kg (0.14-0.41)
SCC	2.0 mg/kg (1.02-3.8)	1.73 mg/kg (1.41-2.13)
<i>d</i> -Tc	0.35 mg/kg (0.25-0.49)	1.31 mg/kg (0.87-1.98)

method. Succinylcholine and *d*-Tc were used in comparison with these compounds. Chickens showed the spastic convulsion and the animals were killed by paralysis of the respiratory muscle. Animals recovered well showing few sign of acute poisoning when they were not killed directly. Results were summarized in Table 1.

It is worthy to note that the values of LD_{50} 's of compound (I), (II) and (III) were in the opposite relation between mice and chickens.

DISCUSSION

Since the classical concept of competitive blocking of *d*-tubocurarine has been challenged by Riker and his coworkers (2-4), it becomes not so easy to discuss on the pharmacological action of neuromuscular junction. That is because *d*-Tc is the standard compound for evaluating the effect of new compounds and also because the concept of the chemical transmission of acetylcholine was established on the observations by use of the curarized muscle. Accepted the chemical transmission of acetylcholine to be the actual event, the acetylcholine release from the motor nerve endings, the transfer to the receptor sites and the depolarization of the end plate regions are the basic thought. Standing on this concept, the event at the receptor site is a focus of the effect of *d*-Tc expressed as competitive blocking. On the other hand, the concept of the desensitization of the end plate regions was brought into the explanation of action of so-called depolarizing agents by Thesleff (1). Hutter (5) applied this concept for explanation of the action of *d*-Tc beforehand. It looks that both concepts

had different angles of vision, the one from the events of the transmission, the other from the character of the end plate regions.

The authors have gained an impression of dual mechanism of neuromuscular blocking when the close arterial injection of acetylcholine was tested in the partially curarized muscles. When acetylcholine was injected close-arterially, the responding twitch height actually diminished gradually as curarization went deeper until no response in the state of complete block. However, when acetylcholine was injected close-arterially to the partially curarized muscle during the tetanic stimulation, the twitch response was same to that to the pretetanic injection, indicating the absence of alteration in the sensibility of the end plate before and during the tetanic stimulation (The partially curarized muscle could not maintain contracture in response to the tetanic stimulation and the tension reduced to become even lesser than before curarization—Wedensky inhibition). At least in the tetanic stimulation the neuromuscular block by curare has its origin in the inhibition of the nerve endings to release acetylcholine. Identical investigation by Hutter (5) led him to the different conclusion that curare acted solely in desensitizing the muscle end plate, in the judgement that lesser amount of acetylcholine was released during the tetanic stimulation even in the non-curarized muscle, and that sensibility of end plate regions was sufficient enough to react to a lesser amount of acetylcholine in the non-curarized muscle, but not in the curarized muscle.

TEA which was established as the enlarger of acetylcholine release at the motor nerve endings [Stovner (6), Koketsu (7)] showed the antagonistic action not only in the twitching but also in the tetanic stimulation. This observation led the authors to support the thought of the active site of *d*-Tc in the motor nerve endings.

The desensitization of end plate region is a very terminological expression which is not so easy to understand in the scientific meaning. When not only depolarizing agents but also *d*-Tc would induce the phenomenon of desensitization, there may be some different character of desensitization between both types of N-M blocking because there is antagonism between them. The concept of dissociation coefficient at the receptor site may help the understanding of this antagonism, but how can the authors explain a dual block phenomenon of succinylcholine in a later stage of N-M blocking? TEA temporarily facilitated the twitch even in the partial block of succinylcholine, thus succinylcholine may act also on the motor nerve endings.

For the present, the authors are very dissatisfied with pharmacological explanation of neuromuscular blocking mechanism, but the assumption of dual sites of action of *d*-Tc in the depressor effect in the motor nerve endings and in the desensitizing the end plate regions looks for the authors the limit of satisfactory approach in this problem. The chemical transmission in the neuromuscular junction is a very hard subject to solve.

ω -Aminoacylcholines now under our consideration revealed themselves the intermediate character between curare and succinylcholine in the features of the tetanic response and drug antagonism. The principal action is, however, on the end plate region, affecting it in the mode of succinylcholine type. There is the initial facilitation of twitch response

and fibrillary twitches or fasciculation during the course of induction. Meanwhile there is the curare type action at the motor nerve ending. This type dual effect of *ω*-aminoacylcholines may correspond to their quick induction and quick recovery without so-called dual block phenomenon adopted in the neuromuscular blocking by use of succinylcholine.

SUMMARY

The effects of 5-aminopentanoyl-(I), 6-aminohexanoyl-(II) and 7-aminoheptanoyl-(III) cholines and 6-aminohexanoyl- β -methylcholine chlorides on the neuromuscular junction were studied in dogs' and cats' tibial and gastrocnemius muscles.

1. Compounds (II) and (III) have the same potency of neuromuscular blocking (one-tenth potency of succinylcholine), compound (I) and β -methyl analogue of (II) have one-fifth and one-two hundredth potency of compound (II).

2. The induction and the restoration were very rapid. No so-called dual block phenomenon was observed.

3. The initial facilitation of twitch, the fasciculation in the time of induction, the antagonism to *d*-Tc and the potentiation of N-M blocking by edrophonium, TMA, TPA and TBA were observed.

4. The pattern of tetanic contraction, and the antagonism between *ω*-aminoacylcholines and TEA were similar to those in the case of *d*-Tc.

5. β -Methyl analogue of compound (II) showed both antagonism and synergism to compound (II) in the different animals. The ratio of occurrence is fifty to fifty. This analogue showed also temporarily the stimulation of respiration and the increase of systemic blood pressure in the intravenous administration. *ω*-Aminoacylcholines have the same pressor effect in almost same potency.

6. The authors discussed the mechanism of these compounds in reference to the neuro-muscular blocking by *d*-Tc.

ADDENDUM

After the authors wrote the manuscript, two reports were found closely related to this manuscript; The reduction of acetylcholine release by *d*-Tc was proved when the tetanic stimulation was applied on the diaphragm treated with DFP [BEANI, L., BIANCHI, C. AND LEDDA, F.: *J. Physiol.* **174**, 172 (1964)] and the reduction of acetylcholine release by succinylcholine was also observed [EDWARDS, C. AND IKEDA, K.: *J. Pharmacol.* **138**, 322 (1962)].

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