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# ACTION OF THIAMINE AND ITS ANALOG ON NEUROMUSCULAR TRANSMISSION IN THE CAT*

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**Abstract**—The action of thiamine and a number of its analogs on tibialis twitch response to peroneal nerve stimulation, respiration, and arterial pressure, as well as their effect on the neuromuscular block produced by *d*-tubocurarine and decamethonium, were studied in anesthetized cats.

It was found that thiamine, pyrithiamine, and pyrithiamine analogs in which there is a hydroxy group on the pyridinium ring caused a decrease in the twitch without initial potentiation, depressed respiration, and lowered arterial pressure. These compounds also antagonized both *d*-tubocurarine and decamethonium.

On the other hand, methyl thiazolium iodide, methyl pyridinium iodide, and the analogs of thiamine and pyrithiamine in which there is no hydroxy group on the ring bearing the quarternary nitrogen, produced an increase in the twitch with muscular fasciculation, and with larger doses, a decrease in the twitch. Changes in respiratory movements generally paralleled those of the twitch. Arterial pressure was usually elevated. These compounds antagonized *d*-tubocurarine and potentiated decamethonium.

The thiazolium fragment, at the dose level studied, increased the twitch only slightly. It also antagonized *d*-tubocurarine and potentiated decamethonium. The pyrimidine fragment seemed to have no effect on the twitch.

It appears that the activity of thiamine and pyrithiamine on the neuromuscular junction is related to the quarternary nitrogen, the "pyrimidyl" coupling, and the presence of a hydroxy group on the onium-bearing ring. The antagonistic action of these compounds against *d*-tubocurarine and decamethonium also depends on the same structural components.

THE action of thiamine in blocking neuromuscular transmission has been reported by several groups of investigators.[1–4] Cheymol *et al.*[5] and di Palma and Hitchcock[6] have extended study of this action of thiamine to include some of its derivatives.

In the cat, thiamine antagonized decamethonium, succinylcholine and *d*-tubocurarine, but, with successive doses, finally potentiated *d*-tubocurarine.[5] On the other hand, in the rat diaphragm preparation *in vitro*, thiamine consistently potentiated *d*-tubocurarine, decamethonium and succinylcholine.[4]

The study here reported undertook to re-examine the action of thiamine on the neuromuscular transmission and its influence on the block produced by *d*-tubocurarine

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and decamethonium, using the cat peroneal nerve–tibialis preparation. In addition, pyrithiamine, oxythiamine, and a number of their analogs were similarly tested. This study further attempted to correlate the structures of these compounds with their pharmacologic activities.

## METHODS

Eighty-nine cats, each weighing between 2 and 4 kg, were used. The animals were anesthetized with 36 mg/kg of pentobarbital sodium administered intraperitoneally. The peroneal nerve was separated from the sciatic trunk, ligated at the mid-thigh level, and a shielded Palmer bipolar electrode applied to its peripheral end. At 10-sec intervals, electric stimuli consisting of rectangular pulses of 0·1-msec duration and 3–5-V intensity were delivered to the nerve by a Grass stimulator (model S4C) via a stimulus isolation unit. The resulting twitch responses of the tibialis muscle were measured with a Grass force displacement transducer (model FT-03, loaded with 300-g springs). Arterial pressure was measured with a Statham transducer (P23A) from the femoral artery on the other side. An accordion type pneumograph placed around the lower thorax registered respiratory movements. All recordings were made on a Grass model 5 polygraph. When necessary, artificial ventilation was provided with a Palmer ideal pump.

All drugs were injected intravenously through an in-dwelling catheter in a femoral vein. When the action of thiamine or its analogs was investigated in the presence of *d*-tubocurarine or decamethonium, one of the latter two was injected first to produce a partial or a complete paralysis of short duration, and one of the former was then injected during the recovery phase. When several compounds were tested in a single animal, an interval of at least 1 hr was provided between the paired injections. The sequence of testing these compounds was varied from animal to animal.

Table 1 depicts the structures of compounds tested in the study. Compounds other than thiamine, pyrithiamine, and oxythiamine were synthesized in the laboratory of the Department of Neurology by one of the authors (S.G.).*

**Methods of synthesis*

*Method* 1. Methyl pyridinium iodide was prepared in the usual manner by reacting pyridine with methyl iodide under cooling.

Methyl thiazolium iodide and 3:4-dimethyl-5-β-hydroxyethyl thiazolium iodide were prepared by refluxing for 30 min a solution of the corresponding base in dimethylformamide with an excess methyl iodide. Precipitation of the quaternary salt was completed through addition of absolute ether. For preparation of 4-methyl-5-β-hydroxyethyl thiazole from commercial thiamine see Williams *et al.*[7]

*Method* 2. 4-Amino-2-methyl-5-bromomethyl pyridine hydrobromide, which was used for the preparation of the quaternary compounds, was kindly supplied by Dr. Karl Pfister of Merck & Co., Inc. Five millimoles of the "pyrimidine" were dissolved in 12 ml of dimethylformamide and the corresponding base was added using a 40–50 per cent excess. After a short while, precipitation of the quaternary salt started; the mixture was left at room temperature for 1 day, the solids were filtered off and dissolved in boiling methanol. When the solution had cooled a small amount of absolute ether was added to promote precipitation of the pure salt. Yields ranged from 40 to 60 per cent of the theoretical.

For the preparation of 1-(4-amino-2-methyl pyrimidyl-5-methyl)-3-hydroxy pyridinium bromide hydrobromide, 3-acetoxy pyridine (prepared by acetylation of 3-pyridol with acetic anhydride) was used for quaternization. The crude compound was subsequently de-acetylated by refluxing for 1 hr with an aqueous-methanolic solution of HBr (about 1·5 M), evaporated to dryness, and finally recrystallized from methanol–ether. Melting of all compounds except the thiazole derivative were too high to be determined.

*Analysis.* Halogen content was determined by the Volhard method and neutral equivalents were measured by potentiometric titration.

TABLE 1.

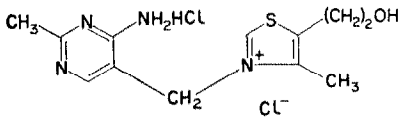
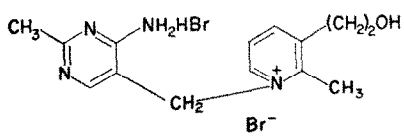
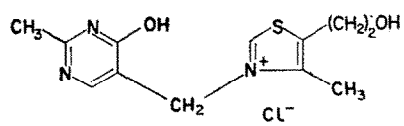
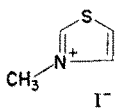
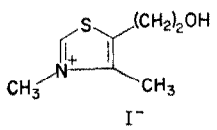
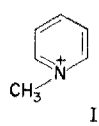
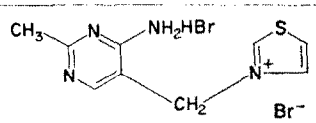
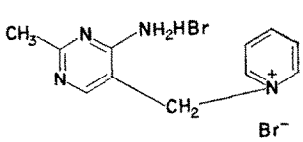
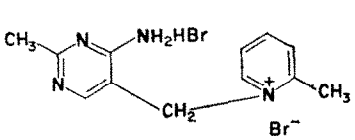
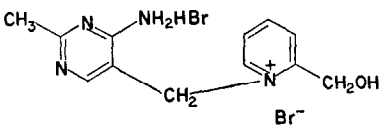
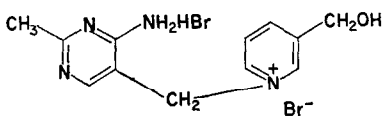
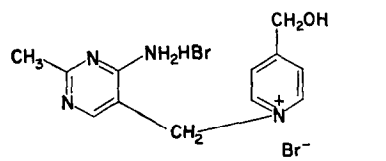
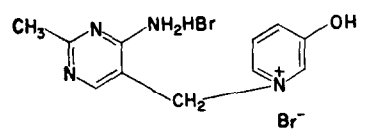
| Structural formulae | Names | M.P. (°C) | Method of synthesis* |
|---|---|---|---|
|  | Thiamine HCl, 3-(4-amino-2-methyl pyrimidyl-5-methyl)-4-methyl-5-β-hydroxyethyl thiazolium chloride hydrochloride (M.W. = 337) | 248 (decomp) | commercial |
|  | Pyrithiamine HBr, 1-(4-amino-2-methyl pyrimidyl-5-methyl)-2-methyl-3-β-hydroxyethyl pyridinium bromide hydrobromide (M.W. = 420) | 218–220 | commercial |
|  | Oxythiamine Cl, 3-(4-hydroxy-2-methyl pyrimidyl-5-methyl)-4-methyl-5-β-hydroxyethyl thiazolium chloride (M.W. = 302) | 195 (decomp.) | commercial |
|  | Methyl thiazolium iodide (M.W. = 227) | 151–152 | 1 |
|  | 3:4-Dimethyl-5-β-hydroxyethyl thiazolium iodide (M.W. = 285) | — | 1 |
|  | Methyl pyridinium iodide (M.W. = 221) | 116 | 1 |
|  | 3-(4-Amino-2-methyl pyrimidyl-5-methyl)-thiazolium bromide hydrobromide (M.W. = 368) | 247 | 2 |
|  | 1-(4-Amino-2-methyl pyrimidyl-5-methyl)-pyridinium bromide hydrobromide (M.W. = 362) | — | 2 |
|  | 1-(4-Amino-2-methyl pyrimidyl-5-methyl)-2-methyl pyridinium bromide hydrobromide (M.W. = 376) | — | 2 |

TABLE 1.—*continued.*

| Structural formulae | Names | M.P. (°C) | Method of synthesis* |
|---|---|---|---|
|  | 1-(4-Amino-2-methyl pyrimidyl-5-methyl)-2-hydroxymethyl pyridinium bromide hydrobromide (M.W. = 392) | — | 2 |
|  | 1-(4-Amino-2-methyl pyrimidyl-5-methyl)-3-hydroxymethyl pyridinium bromide hydrobromide (M.W. = 392) | — | 2 |
|  | 1-(4-Amino-2-methyl pyrimidyl-5-methyl)-4-hydroxymethyl pyridinium bromide hydrobromide (M.W. = 392) | — | 2 |
|  | 1-(4-Amino-2-methyl pyrimidyl-5-methyl)-3-hydroxy pyridinium bromide hydrobromide (M.W. = 378) | — | 2 |

* See footnote under Methods.

## RESULTS

To facilitate the presention of experimental findings and discussion, the compounds are divided into four categories:

(A) Thiamine, pyrithiamine and oxythiamine.
(B) Fragments of thiamine and pyrithiamine.
(C) Thiamine and pyrithiamine analogs in which there is no hydroxy group on the ring bearing the quarternary nitrogen.
(D) Pyrithiamine analogs in which there is a hydroxy group on the pyridinium ring.

### A. *Thiamine, pyrithiamine and oxythiamine*

1. *Effect on tibialis twitch response, respiration and arterial pressure.* Thiamine hydrochloride in doses up to 10 mg/kg had no effect on the tibialis twitch response to stimulation of the peroneal nerve. In two of four instances, 20 mg of thiamine per kg caused a transient, minimal decrease in the twitch. Administration of 40 mg or more of thimaine per kg resulted in complete or nearly complete paraylisis and apnea, both effects lasting for 15–20 min. The decrease in the twitch was never preceded by an increase, nor was muscular fasciculation ever observed (Fig. 1). In two preparations, 0·02 mg of neostigmine methylsulfate per kg partially antagonized the action of thiamine.

The arterial pressure fell by 40–50 mm Hg with 5 mg of thiamine per kg. With larger doses, hypotension was more pronounced. Recovery was gradual, requiring 10–15 min to reach the pre-injection level or a steady level lower than that of the control.

Four milligrams of pyrithiamine hydrobromide per kg resulted in a slight and transient decrease in the twitch response; 40 mg/kg reduced the twitch to approximately 50 per cent of control. The respiratory movements were depressed, requiring the institution of artificial respiration. With the dose levels studied, there was a moderate drop of arterial pressure.

Oxythiamine hydrochloride, in doses up to 50 mg/kg, had no effect on twitch response and respiration. However, it produced a fall in arterial pressure comparable to that caused by thiamine.

2. *Effect on neuromuscular block produced by* d-*tubocurarine and decamethonium.* In ten instances, thiamine was administered during the recovery phase of neuromuscular block produced by *d*-tubocurarine. In seven of these a dose of thiamine of from 2 to 10 mg/kg resulted in a definite increase in the twitch response (Fig. 2, top) and in the remaining three, 10 mg of thiamine per kg potentiated the action of *d*-tubocurarine.

Pyrithiamine and oxythiamine, in doses of 5–15 mg/kg, also antagonized *d*-tubocurarine but were less potent than thiamine.

Thiamine, pyrithiamine, and oxythiamine, in doses of 5–15 mg/kg, regularly antagonized the action of decamethonium (Fig. 2, bottom). In no instance was potentiation observed even after repeated administration of these compounds.

B. *Fragments of thiamine and pyrithiamine.*

Methyl thiazolium iodide, 3:4-dimethyl-5-$\beta$-hydroxyethyl thiazolium iodide, methyl pyridinium iodide, and 4-amino-2-methyl-5-bromomethyl pyrimidine hydrobromide.

1. *Effect on tibialis twitch response, respiration, and arterial pressure.* Both methyl thiazolium iodide and methyl pyridinium iodide increased the twitch response at dose levels of 1–10 mg/kg and 4–20 mg/kg, respectively. With larger doses (20 mg/kg and 40 mg/kg, respectively) an initial increase in the twitch and muscular fasciculation was followed by partial paralysis. Usually both the rate and amplitude of respiration increased, except that after 40 mg of methyl pyridinium iodide per kg, the respiration became depressed after initial stimulation. The arterial pressure was markedly elevated (Fig. 3).

3:4-Dimethyl-5-$\beta$-hydroxyethyl thiazolium iodide in doses of 10–50 mg/kg increased the twitch response slightly. The repiration was not affected significantly. The arterial pressure showed a fall of from 20 to 50 mm Hg.

4-Amino-2-methyl-5-bromomethyl pyrimidine hydrobromide in doses up to 50 mg/kg had no apparent effect on twitch response and respiration. There was a slight fall in arterial pressure.

2. *Effect on neuromuscular block produced by* d-*tubocurarine and decamethonium.* Methyl thiazolium iodide, methyl pyridinium iodide and 3:4-dimethyl-5-$\beta$-hydroxyethyl thiazolium iodide, in decreasing order of potency, antagonized *d*-tubocurarine and potentiated decamethonium (Fig. 4).

4-Amino-2-methyl-5-bromomethyl pyrimidine hydrobromide had no influence on the block produced by either *d*-tubocurarine or decamethonium.

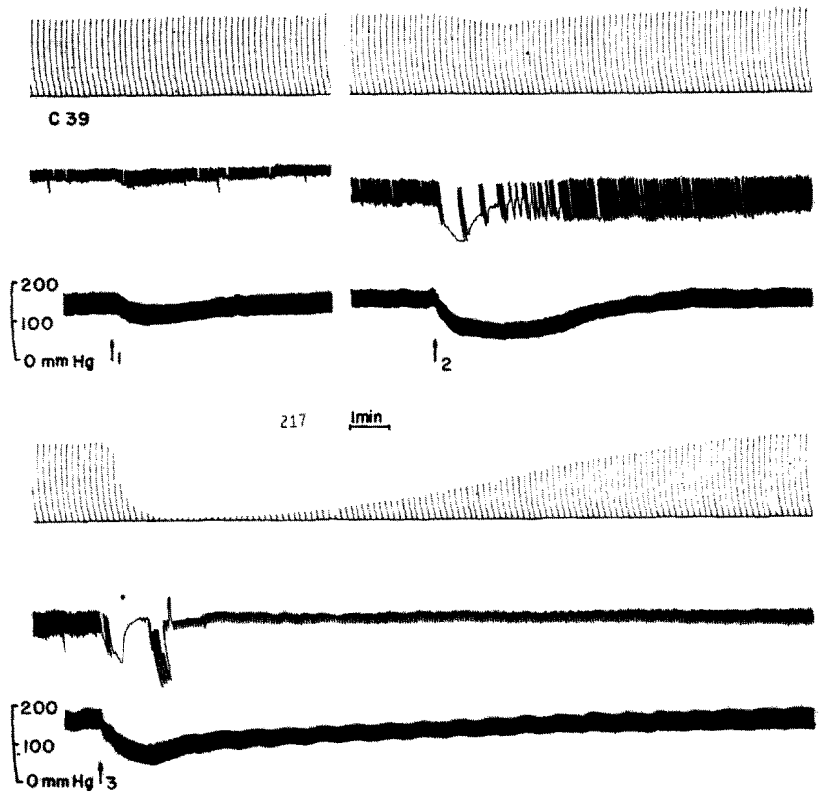



FIG. 1. Cat 39, 3 kg Pentobarbital anesthesia, 36 mg/kg, given intraperitoneally. *Top trace*, tibialis muscle twitch response to peroneal nerve stimulation; *middle trace*, respiratory movements; *bottom trace*, arterial pressure. Same anesthesia and parameters for all subsequent figures except those of the middle and bottom traces, which may be in reverse order. At arrow 1, 10 mg/kg; at arrow 2, 20 mg/kg and at arrow 3, 60 mg/kg of thiamine, intravenously. Respiration failed within 1 min after the last injection. Artificial respiration continued to the end of tracings.

*facing page 260*

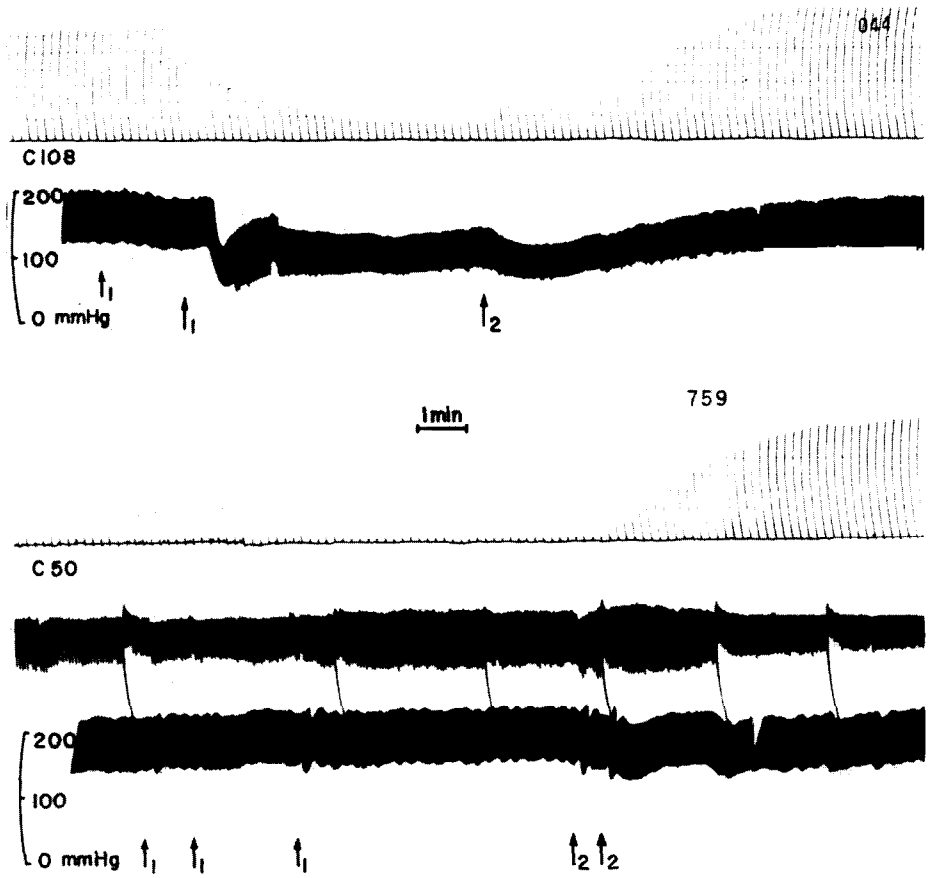



FIG. 2. *Top panel.* Cat 108, 3 kg. At each of arrows 1, 100 μg of *d*-tubocurarine per kg, intravenously. At arrow 2, thiamine, 10 mg/kg, intravenously. *Bottom panel.* Cat 50, 3·8 kg. At each of arrows 1, 5 μg of decamethonium per kg, intravenously. At each of arrows 2, 5 mg of thiamine per kg, intravenously.

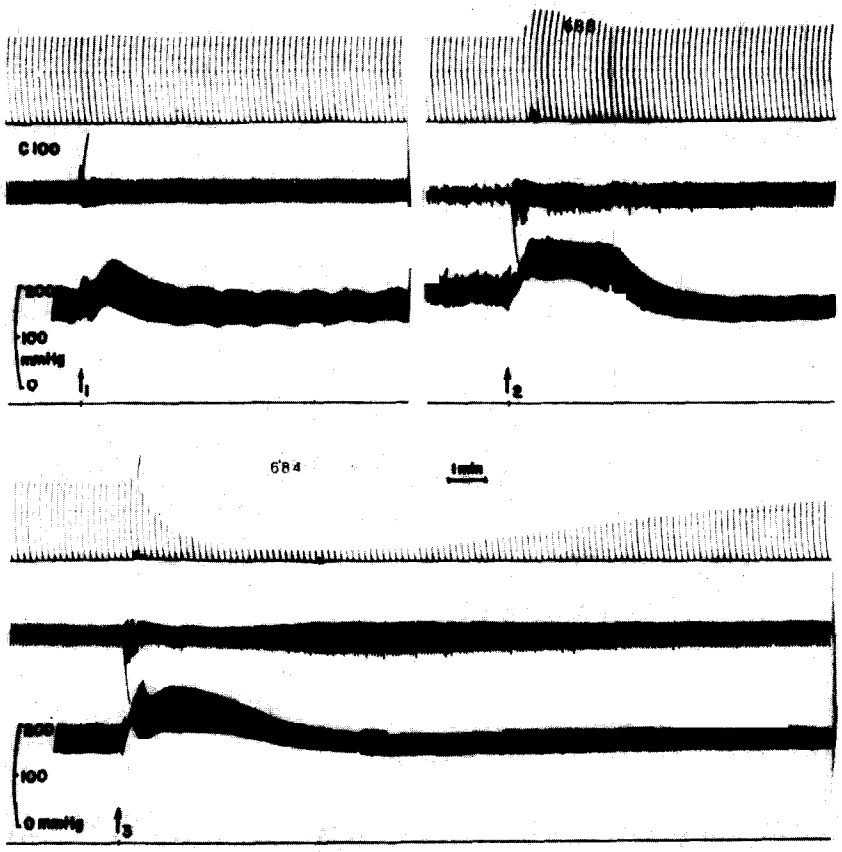



FIG. 3. Cat 100, 2·9 kg. At arrow 1, methyl thiazolium iodide, 2 mg/kg, intravenously; at arrow 2, 10 mg/kg. Note the increase in twitch response, respiratory rate and amplitude and arterial pressure. After 20 mg of the same compound per kg, injected at arrow 3, the brief initial increase in twitch was followed by partial paralysis.

the antagonistic effect was comparable to that of thiamine. With *d*-tubocurarine, these compounds are less potent than thiamine. As shown in the bottom panel of Fig. 8, 1-(4-amino-2-methyl pyrimidyl-5-methyl)-3-hydroxymethyl pyridinium bromide, 5 mg/kg, increased the twitch response only slightly. The twitch remained at this new level with no further progressive recovery, and a second dose of the same compound had no effect.

1-(4-Amino-2-methyl pyrimidyl-5-methyl)-2-hydroxymethyl pyridinium bromide antagonized *d*-tubocurarine. When tested against decamethonium, the results were inconsistent. In four of six instances there was no apparent effect; transient potentiation was observed in the fifth; antagonism in the sixth.

## DISCUSSION

The neuromuscular blocking action of thiamine as observed in this study is similar to that observed by Smith *et al.*,[3] Gjone,[4] Cheymol *et al.*[5] and di Palma and Hitchock.[6] The nature of the blockade appears to resemble that produced by *d*-tubocurarine. Nastuk and Kahn[8] found that in frog sciatic nerve–sartorius preparations, thiamine reduced the twitch response without initially potentiating it. The threshold concentration of acetylcholine required to activate the muscle was elevated (see also Torda and Wolff[1]). Membrane potential studies revealed that thiamine did not significantly alter the resting membrane potential but that a 2–3 millimolar solution of this compound reduced the amplitude, and decreased the rate of decay, of the end-plate potential; at the 4-millimolar level, thiamine blocked axonal conduction. Thus, it seems that thiamine possesses multiple actions, one of which is neuromuscular blockade through competitive inhibition.

It has been suggested that the neuromuscular blocking activity of thiamine resides in the thiazolium portion, which contains the quarternary nitrogen.[6] This seems indeed to be the case, as the pyrimidine fraction apparently has no effect on the tibialis twitch response. However, the action of methyl thiazolium iodide is distinctly different from that of thiamine. The results indicate that methyl thiazolium iodide, being an onium derivative, activates the neuromuscular junction and, in larger doses, produces neuromuscular blockage through depolarization. The addition of $\beta$-hydroxyethyl and methyl groups to thiazolium (3:4-dimethyl-5-$\beta$-hydroxyethyl thiazolium iodide) significantly reduces its potency (see above). This finding is in agreement with the thesis of Riker[9] that the addition of a hydroxy group to onium ions reduces their excitatory action at the neuromuscular junction.

The coupling of thiazolium with pyrimidine produces a compound which differs structurally from thiamine only by the absence of the $\beta$-hydroxethyl and methyl groups in the thiazolium ring. This coupling with pyrimidine increases the potency of thiazolium but does not qualitatively alter the nature of its activity at the neuromuscular junction, namely, depolarization.

Parallel results were obtained with methyl pyridinium iodide, 1-(4-amino-2-methyl pyrimidyl-5-methyl)-pyridinium bromide and pyrithiamine hydrobromide in respect to the nature of the neuromuscular blockade produced and their relative potency.

It appears, therefore, that the action of thiamine and pyrithiamine depends upon several components of their complex molecules. The quarternary nitrogen in the thiazolium or the pyridinium ring is the component which imparts the neuromuscular

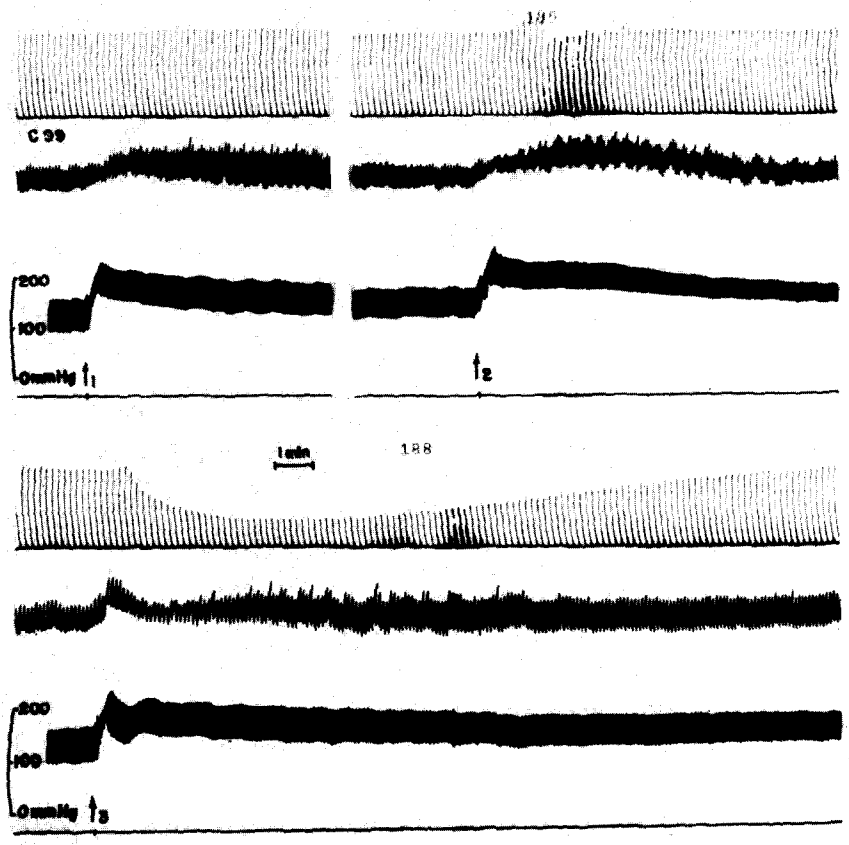



FIG. 5. Cat 99, 2·5 kg. Intravenous injections of 3-(4-amino-2-methyl pyrimidyl-5-methyl)-thiazolium bromide; at arrow 1, 0·6 mg/kg; at arrow 2, 1 mg/kg; and at arrow 3, 2 mg/kg. The effects on parameters measured were similar to those of methyl thiazolium iodide, as shown in Fig. 3.

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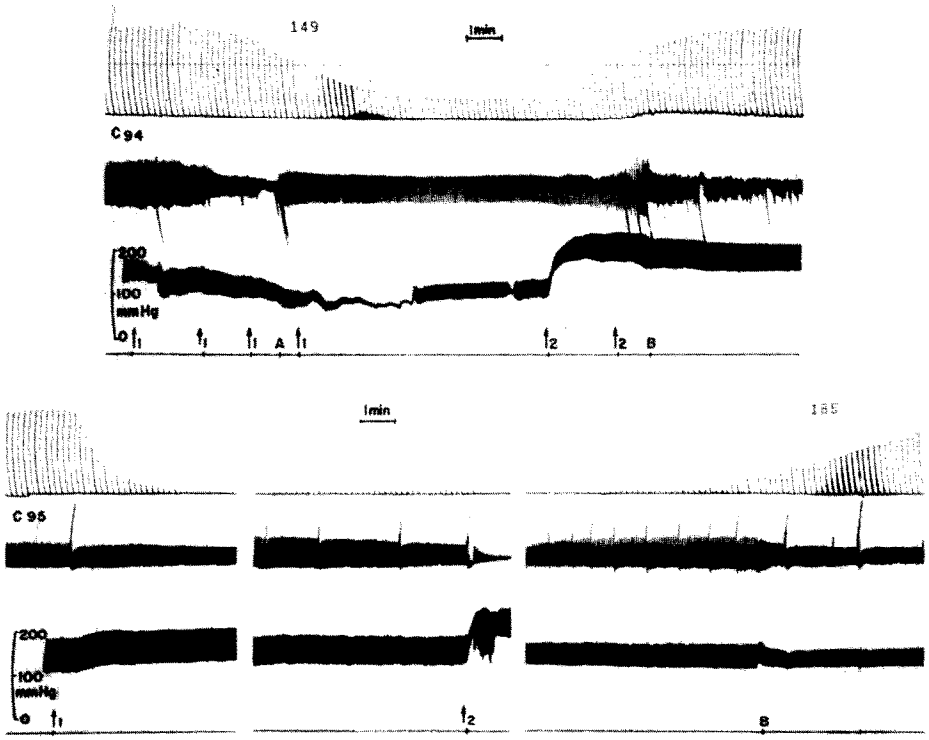


FIG. 6. *Top panel.* Cat 94, 2 kg. At each of arrows 1, 100 $\mu$g of *d*-tubocurarine per kg, intravenously. At each arrows 2, 2 mg of 3-(4-amino-2-methyl pyrimidyl-5-methyl)-thiazolium bromide per kg, intravenously. Between points *A* and *B*, artificial respiration. *Bottom panels.* Cat 95, 2·5 kg. At arrow 1, 20 $\mu$g of decamethonium per kg, intravenously. At arrow 2, 2 mg of 3-(4-amino-2-methyl pyrimidyl-5-methyl)-thiazolium bromide per kg, intravenously. Thirteen-minute interval between left and center panels; 17-min interval between center and right panels. Artificial respiration was started right after the center panel; stopped at point *B*.

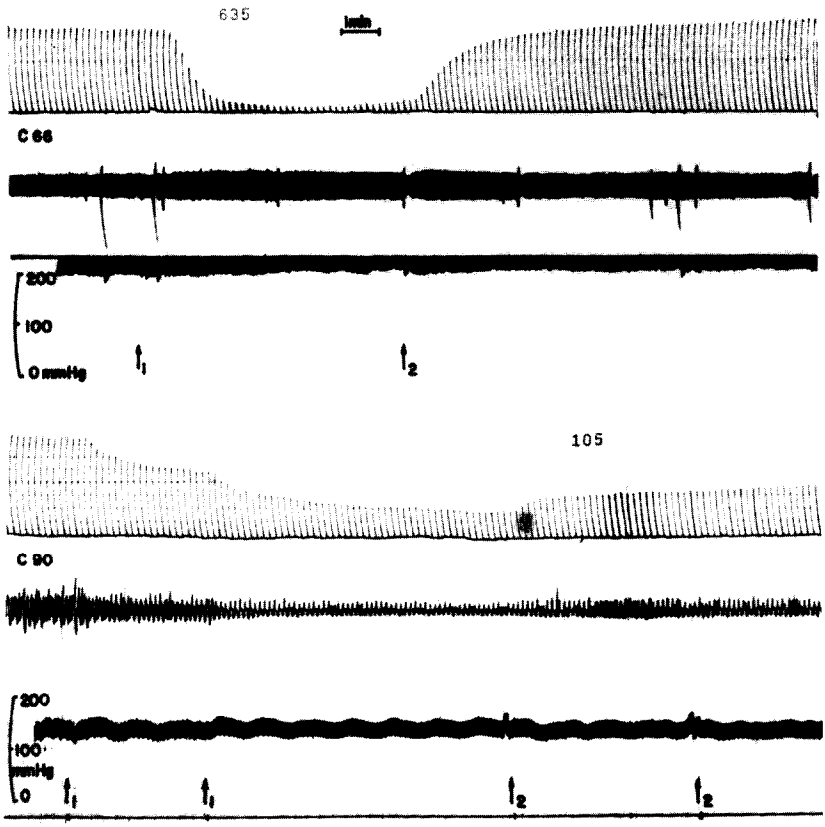


FIG. 7. *Top panel.* Cat 66, 2·5 kg. At arrow 1, 20 μg of decamethonium per kg, intravenously. At arrow 2, 5 mg of 1-(4-amino-2-methyl pyrimidyl-5-methyl)-3-hydroxymethyl pyridinium bromide per kg, intravenously. Twitch recovered gradually. No significant change in respiration and arterial pressure. *Bottom panel.* Cat 90, 2·5 kg. At each of arrows 1, 100 μg of *d*-tubocurarine per kg, intravenously. At each of arrows 2, 5 mg of 1-(4-amino-2-methyl pyrimidyl-5-methyl)-3-hydroxymethyl pyridinium bromide per kg, intravenously. Note the slight increase in twitch and an increase in respiratory amplitude after the first dose. Twitch stayed at the new level without further progressive recovery. The second dose was without effect.

junctional activity. Coupling of thiazolium or pyridinium with pyrimidine increases its potency. Further addition of a hydroxy group to the quarternary nitrogen-bearing ring radically modifies the nature of the junctional activity to resemble that of *d*-tubocurarine.

It is interesting that thiamine, pyrithiamine and pyrithiamine analogs *with* a hydroxy group on the pyridinium ring usually caused a fall in arterial pressure. This fall is probably due to their ganglionic blocking activity, as demonstrated by di Palma and Hitchcock[6] (thiamine and pyrithiamine). On the other hand, thiazolium, pyridinium and the "pyrimidyl" analog of thiazolium *without* the hydroxy group on the quarternary nitrogen-bearing ring always produced a marked elevation of arterial pressure. Although the mechanism of this action was not investigated in the present study, it is most likely that these compounds have a ganglionic stimulating action in addition to their neuromuscular junctional activity.

The influence of thiamine, pyrithiamine and their analogs on the neuromuscular block produced by *d*-tubocurarine and decamethonium is definitely related to their molecular structures. Thus, thiazolium, pyridinium and their "pyrimidyl" analogs *without* the hydroxy group on the quarternary nitrogen-bearing ring, always antagonized *d*-tubocurarine and potentiated decamethonium. Such effects of these compounds are not surprising, in view of the fact that they appear to have an excitatory action at the neuromuscular junction. Thiamine, pyrithiamine, oxythiamine and pyrithiamine analogs *with* a hydroxy group on the pyridinium ring, with one exception (see results), usually antagonized the action of both *d*-tubocurarine and decamethonium.

The antagonistic effect of this group of compounds on both types of neuromuscular block is interesting in that their own action resembles that of *d*-tubocurarine. Results from the present study provide no clue as to the mechanisms of this action. However, it would appear difficult to explain these findings within the context of the present concept of neuromuscular transmission and of the mode of action of *d*-tubocurarine and decamethonium. It should be pointed out that the antagonistic action of thiamine is evident only in *in vivo*-experiments. Thiamine potentiates the action of *d*-tubocurarine, decamethonium, and succinylcholine in rat diaphragm preparation *in vitro*.[4] Also, the antagonistic action of thiamine against *d*-tubocurarine is reversed with larger doses (10–15 mg/kg) or by its successive administration (see also Cheymol *et al.*[5]).

A few other compounds have been reported capable of antagonizing both *d*-tubocurarine and decamethonium. These are 1:5-bis-(allyldimethyl ammonium phenyl) penta-3-one dibromide (B.W. drug no. 53–67 or drug no. 284-C-51, an anti-acetylcholinesterase),[10, 11] N:N′-bis-(2-diethylaminoethyl)oxamide bis-2-methoxybenzyl chloride) WIN 8078,[12, 13] and tetraethylammonium chloride.[14–16] The molecular structures of these compounds are different from one another and distinct from that of thiamine and its analogs. Their mode of action at the neuromuscular junction and the mechanisms for their antagonistic action against the relaxants are probably also different. These problems remain to be investigated.

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