THE EFFECT OF DEFICIENCY AND SMALL EXCESS OF THIAMINE ON THE RAT PHRENIC NERVE DIAPHRAGM PREPARATION

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Preparations made from thiamine deficient rats showed subnormal twitch tensions in response to nerve stimulation but gave normal twitch tensions when directly stimulated. Tensions developed during tetanus were subnormal whether elicited directly or indirectly. Sensitivity to an excess of potassium ion was reduced but that to tubocurarine remained unchanged. Excess of thiamine, in near physiological concentrations, was almost devoid of curare-like action except in the presence of an excess of thyroid hormone.

IT has been suggested (von Muralt, 1947) that thiamine may play an important part in the transmission of excitation from the motor nerve terminals to voluntary muscle, and may also influence the contractile process in muscle fibres, because Minz (1938) found that cholinergic fibres liberate thiamine as well as acetylcholine when excited electrically. Our purpose has therefore been to make investigation of the possible functions of thiamine at the neuromuscular junction and in the contraction of muscle fibres using the rat phrenic nerve diaphragm preparation.

EXPERIMENTAL

Methods

Female rats of a single Wistar strain, weighing 200–250 g., were used. Those adrenalectomised drank 0·4 per cent NaCl in tap water, were fed diet 41 b of Stein, and were used for experiments on the fourth to sixth postoperative day. All other rats were fed a basic diet of: corn starch, 60; casein, 18; corn oil, 6; dried whole liver powder, 5; dried yeast, 7 and U.S.P. XII salt mixture No. 2, 4 per cent. This diet was prepared in bulk and was stored at room temperature in sealed containers: the thiamine content, estimated by the thiochrome method, was 137 μg./100 g. at the time of use. Thiamine deficient diet was prepared by mixing sodium metabisulphite into the standard diet to 0·6 per cent w/w, at room temperature; this was used in the interval of two to eight weeks after its preparation. The treatment with metabisulphite reduced the thiamine content to less than 1 μg./100 g. in 2 weeks. Weighed amounts of basic and sulphite-treated diets were mixed to stiff pastes, daily, with water and were supplied in narrow troughs fixed to the sides of the cages. Animals fed on these diets each received 0·5 ml. cod liver oil orally, weekly, by pipette.

Rats fed on the sulphited-treated diet ceased to gain weight in the third or fourth week, then lost weight and developed severe polyneuritis and bradycardia in the fifth or sixth week. They were used for experiments only when signs of advanced thiamine deficiency had become evident.

Adrenalectomy. Bilateral adrenalectomy was performed as described by Venning, Kazmin and Bell (1946) under pentobarbitone anaesthesia.

Phrenic nerve diaphragm peparations were made as described by Bülbring (1946) and were suspended in a bath containing a measured volume (80–90 ml.) of aerated Tyrode's fluid at 27 ± 1° which contained twice the glucose stated in the original formula. Contractions of the diaphragm were induced either by stimulation of the phrenic nerve or, in curarised preparations, directly. Rectangular pulses of 100 μ-sec. duration were delivered at intervals of 10 sec. (twitches) or at a frequency of 100/sec. (tetanus) from a voltage source 1·5 times that required to induce maximum tension. This source did not exceed 10 volts during nerve, or 100 volts during direct stimulation.

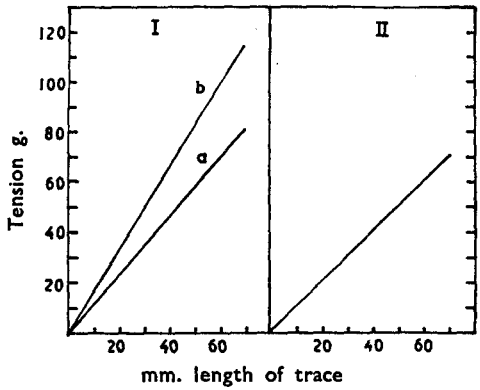

FIG. 1. The graphs show regression lines which are calibration curves relating length of trace in mm. to g. hung from each of two isometric levers (I and II) at a distance of 1 cm. from the fulcrum. a and b refer to two different magnifications used with lever I.

The design of experiments. A. Calibration curves for two Palmer isometric levers used are shown in Fig. 1. Preparations were suspended from these levers from a point 1 cm. from the fulcrum and were subjected to an initial tension of 10 g. In each experiment maximum tensions developed in response to single shocks and tetanus delivered through the phrenic nerve were measured, as was the effect of 5 min. of exposure to an additional 0·3 ml. 15 per cent KCl per 80 ml. bath fluid on twitch tension. These measurements were then repeated after full curarisation during direct stimulation (Figs. 2, 3). *B.* Preparations were suspended from heart levers and maximum twitch tension was elicited every 10 sec. in response to nerve stimulation. A fixed dose of tubocurarine, sub-maximal in effect, was added to the bath fluid every 40 min. for a contact period of 10 min. When a constant response to the fixed dose of tubocurarine became evident another drug was added to the bath fluid 5 min. before the standard dose of tubocurarine was due, and was washed out together with the tubocurarine 15 min. later. Return was then made to tubocurarine alone. Change in twitch height after 10 min. exposure to

tubocurarine has been expressed as a per cent of initial twitch height. Modification of response to tubocurarine by a second drug has been referred to the mean of flanking responses to tubocurarine alone.

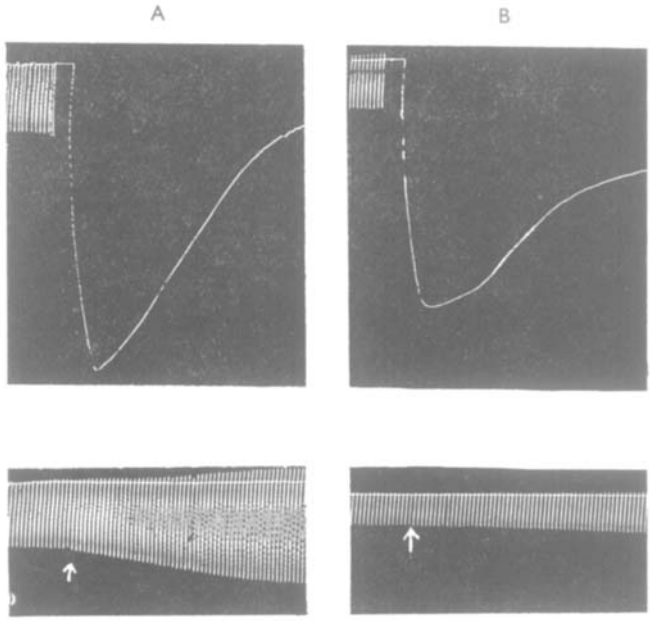

FIG. 2. The tracings were made by the contractions of a nerve diaphragm preparation from a normal rat. The diaphragm was attached 1 cm. from the fulcrum of an isometric lever and was subjected to an initial tension of 10 g. Above, maximum twitch and tetanus, below, the effect of added KCl (at arrow), 0·3 ml. 15 per cent, to 80 ml. bath fluid. A, stimulation through the nerve; B, direct stimulation, fully curarised.

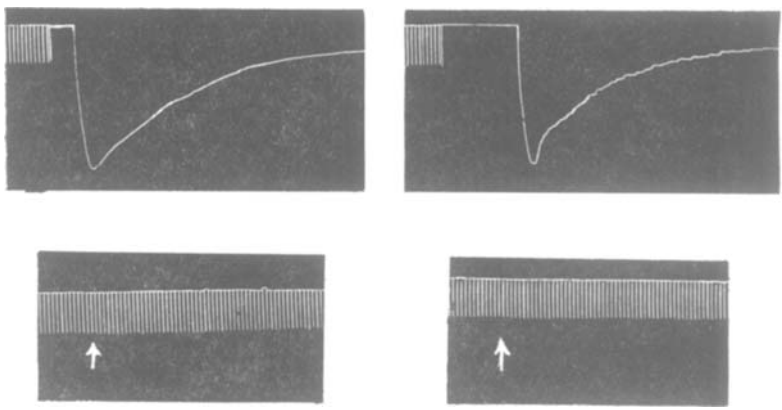

FIG. 3. As Fig. 2, except that the preparation used was taken from a rat in advanced deficiency of thiamine.

RESULTS

The Effect of Deficiency of Thiamine on Tensions Developed During Twitch and Tetanus by Rat Diaphragms in Reponse Both to Direct and Indirect Stimulation

Single preparations taken from each of six normal rats, and six made from four rats in advanced thiamine insufficiency, were used in experiments which yielded the data summarised in Tables I and II. The maximum

TABLE I

A COMPARISON OF TENSIONS DEVELOPED DURING MAXIMUM TWITCH AND TETANUS, AND OF SENSITIVITY TO K^+ EXCESS, IN CURARISED DIAPHRAGM PREPARATIONS MADE FROM NORMAL AND THIAMINE DEFICIENT RATS AND STIMULATED DIRECTLY

	g. tension developed in response to direct stimulation			
			Maximum twitches	
Wt. of rat g.	Maximum twitch	Maximum tetanus	Normal	Added K^+
Thiamine deficient—				
150	13·5	15·0	13·5	13·5
160	20·0	21·0	18·5	18·5
165	13·0	16·5	13·0	13·0
165	14·0	30·5	12·5	12·0
165	16·0	35·0	16·0	16·0
155	17·0	36·5	17·0	17·0
Normal—				
170	18·0	72·0	17·5	19·0
180	26·0	111·0	25·0	29·0
165	15·0	48·0	14·0	22·0
170	17·0	70·0	17·0	23·0
150	13·0	57·5	13·0	15·5
175	16·0	50·5	14·0	15·5
Thiamine deficient—				
159 ± 2·1	15·6 ± 1·2	25·9 ± 3·8	15·1 ± 1·0	15·0 ± 1·0
Normal—				
168 ± 4·3	17·5 ± 1·9	68·2 ± 9·4	16·8 ± 1·8	22·3 ± 2·1
t calc. (n = 10)—	1·27	9·95	1·26	4·09

twitch tensions given by thiamine deficient preparations equalled the normal only when curarised muscles were stimulated directly (Table I). Twitch tension was found subnormal in the deficient preparations when these were excited through the nerve (Table II). The maximum tensions developed during tetanus, and the ratio of maximum tetanus/twitch tensions were subnormal in thiamine deficient preparations whether excited by direct stimulation or through the nerve (Tables I and II). Whereas normal preparations responded to the excess concentration of potassium ions used by increase in twitch tension, whether stimulated directly or through the nerve, the thiamine deficient preparations did not (Tables I and II). The diaphragms taken from the rats deficient in thiamine looked thinner, and had less adherent fatty tissue than did those from normal rats.

The Effect of Deficiency and Excess of Thiamine on the Response of the Rat Diaphragm to Nerve Stimulation, and on Sensitivity to Tubocurarine

Four nerve diaphragm preparations made from normal rats, weighing 185 ± 7 g., developed maximum twitch tensions in response to single

shocks to the phrenic nerve which were recorded as contractions measuring 36·0 ± 3·8 (4) mm. on a smoked drum by semi-isometric levers. Four similar preparations made from animals of the same age group which were in an advanced stage of thiamine deficiency (body weight 110 ± 5 g.) gave maximum twitch heights of 29·0 ± 4·8 (4) mm. on the same lever systems; in addition they developed less tension (t calc. = 2·4, P = <0·05) during a maximal twitch than did normal diaphragms.

TABLE II

A COMPARISON OF TENSIONS DEVELOPED DURING MAXIMUM TWITCH AND TETANUS, AND OF SENSITIVITY TO K^+ EXCESS, IN PHRENIC NERVE DIAPHRAGM PREPARATIONS MADE FROM NORMAL AND THIAMINE DEFICIENT RATS AND STIMULATED THROUGH THE PHRENIC NERVES

Wt. of rat g.	g. tensions developed in response to nerve stimulation			
	Maximum twitch	Maximum tetanus	Maximum twitches	
			Normal	Added K^+
Thiamine deficient—				
150	13·5	24·0	13·5	13·5
160	22·5	42·0	20·5	20·5
165	14·0	29·0	15·5	15·5
160	14·0	29·5	14·0	14·0
165	18·0	39·0	18·0	18·0
155	17·0	37·5	17·0	18·0
Normal—				
170	27·0	87·0	26·0	35·0
180	34·0	122·0	31·0	35·5
165	21·0	70·0	22·0	31·0
170	18·5	54·0	18·0	22·5
150	23·0	82·0	19·5	30·0
175	23·0	91·5	23·0	28·0
Thiamine deficient—				
159 ± 2·1	16·5 ± 1·4	33·5 ± 1·2	16·6 ± 0·8	16·7 ± 0·9
Normal—				
168 ± 4·3	24·4 ± 2·2	84·4 ± 3·0	23·3 ± 1·9	30·3 ± 2·0
t *calc.* (n = 10)—	2·98	15·9	3·14	4·85

The neuromuscular blocking action of tubocurarine did not differ in these two groups of nerve diaphragm preparations. Tubocurarine, 0·85 ± 0·08 μg./ml. reduced the maximum twitch tension by 63·6 ± 3·9 per cent of the control values in a 10 min. period of contact with the normal diaphragms. A concentration of 0·87 ± 0·02 μg. tubocurarine/ml. reduced the maximum twitch height by 65·5 ± 3·9 per cent of control values, when preparations from thiamine deficient animals were similarly examined.

Thiamine hydrochloride, 5 to 20 μg., added to bath volumes of fluid ranging from 60 to 100 ml. was without effect on twitch tensions whether the nerve diaphragm preparations had been taken from normal rats or from those deficient in thiamine. These concentrations of thiamine did in some cases cause a slight increase in the blocking action of tubocurarine in preparations made from both normal and from thiamine deficient animals (Table III); this, since it occurred in seven out of eight preparations, was significant by t test. The calculated values of t are shown at the foot of Table III.

TABLE III

THE EFFECT OF THIAMINE HYDROCHLORIDE IN THE BATH FLUID ON THE SENSITIVITY OF PHRENIC NERVE DIAPHRAGM FROM NORMAL RATS AND FROM RATS DEFICIENT IN THIAMINE ON THE BLOCK PRODUCED BY A FIXED DOSE OF TUBOCURARINE

μg. tubocurarine used	Per cent inhibition of twitch tension resulting from 10 min. exposure to a fixed concentration of tubocurarine			
	In the absence of thiamine			In the presence of thiamine 5–10 μg./100 ml.
	Control 1	Control 2	Mean control value	
Normal rats—				
I 75	60·0	57·5	58·8	54·5
II 75	61·5	60·0	60·8	65·5
III 75	56·8	62·2	59·5	66·6
IV 75	60·5	57·1	58·8	67·5
Rats deficient in thiamine—				
I 75	62·0	71·0	66·5	75·0
II 60	64·0	68·0	66·0	69·0
III 100	69·0	78·0	73·5	77·0
IV 60	50·0	61·0	55·5	59·0

Significance of differences have been examined by *t* tests in which each preparation has served as its own control.
Values of *t* calc. for effect of thiamine:—
Preparations from normal rats, $t = 2{\cdot}00$, n = 3, P = <0·1.
Preparations from deficient rats, $t = 3{\cdot}74$, n = 3, P = <0·05.
Together, $t = 2{\cdot}54$, n = 6, P = <0·05.

The Effect of an Excess of Triiodothyronine on the Sensitivity of the Phrenic Nerve Diaphragm Preparation to the Blocking Action of Tubocurarine

Pickens and Lockett (1961) have shown a reduction in the quantity of acetylcholine liberated per nerve impulse from the phrenic nerve diaphragm preparation when this preparation is bathed in a fluid containing 0·05 μg. triiodothyronine per 100 ml. It was therefore of interest to discover whether the small reduction of acetylcholine output occasioned by this excess of triiodothyronine was detectable as an increased sensitivity to tubocurarine, and, if so, whether the effects of triiodothyronine and thiamine excess could summate.

TABLE IV

THE EFFECT OF L-TRIIODOTHYRONINE, 0·05 μg./100 ml. AND TRIIODOTHYRONINE WITH THIAMINE HYDROCHLORIDE, 5–10 μg./90 ml., ON THE SENSITIVITY OF PHRENIC NERVE DIAPHRAGM PREPARATIONS FROM NORMAL AND ADRENALECTOMISED RATS TO TUBOCURARINE

Drugs		Effect of tubocurarine alone (decrease in twitch tension as per cent initial twitch tension)	Change in per cent response to curare induced by other drugs	*t* calc.
Tubocurarine μg./ml.	Other drugs			
Normal rats—				
0·75 ± 0·08 (6)	triiodothyronine (T_3)	59·2 ± 6·3	+6·2 ± 3·8	2·8
Adrenalectomised rats—				
0·73 ± 0·09 (7)	thiamine (2)			
	cocarboxylase (5)	55·3 ± 5·4	+1·9 ± 1·18	0·86
	thiamine or cocarb. + T_3	63·7 ± 4·3	−0·3 ± 2·39	0·33
Thiamine deficient rats—				
0·86 ± 0·05 (3)	thiamine	74·1 ± 10·9	+3·1 ± 0·07	3·12
	thiamine + T_3	70·2 ± 16·8	+6·5 ± 1·95	3·33

t calculated as in Table I.

Comparison has therefore been made of the intensity of the neuromuscular block caused by a fixed dose of tubocurarine in the absence of other drug and in the presence of triiodothyronine (0·05 μg./100 ml.) alone and with thiamine hydrochloride (5 to 10 μg./100 ml.). The results of these experiments are seen in Table IV. Triiodothyronine caused just significant potentiation of the blocking action of tubocurarine in preparations from normal rats and appeared to sum in effect with thiamine in this respect in preparations made from thiamine deficient animals. It was not found possible to demonstrate potentiation of the action of tubocurarine by thiamine and triiodothyronine in those preparations made from adrenalectomised salt maintained animals.

Discussion

The results summarised in Table I show that curarised voluntary muscle from thiamine deficient animals is capable of producing a short lived twitch of normal tension but cannot develop or sustain normal tensions when in tetanus. This reduction in power is most probably to be attributed to reduction in muscle adenosine triphosphate by reason of a depression of activity in the Krebs cycle for lack of co-carboxylase. The fact that thiamine deficient muscle was abnormally insensitive to an excess of extracellular potassium ion may indicate a greater than normal stability of membrane polarisation. The results shown in Table II contribute one additional fact. Twitch tension in response to nerve stimulation is reduced during thiamine insufficiency, but that in response to direct stimulation is not. This observation may possibly be explained by the facts that the quantities of acetylcholine and of acetylcoenzyme A (Bhagat and Lockett, 1962) found in nerve tissue are reduced in late thiamine deficiency. It therefore follows that there may be a reduction in the amount of acetylcholine liberated per nerve impulse from the motor nerves to voluntary muscle during thiamine deficiency. Any such reduction cannot, however, have been great, since it was insufficient to increase sensitivity to tubocurarine (Table III).

It was possible to demonstrate a slight curare-like action of quantities of excess thiamine which could be considered to approach physiological, augmented by the presence of an excess of triiodothyronine. The curare-like actions of thiamine are well known, but have been elicited in cats, for instance, only by the intravenous injection of huge quantities of the compound 20 mg./kg. (Ngai, Ginsburg and Katz, 1961). A number of other studies have been made of the pharmacological effects of large amounts of thiamine at the myoneural junction of voluntary muscle, for both acetylcholine and thiamine are quaternary compounds which have a free hydroxyl group. Torda and Wolff (1944) found that very large concentrations of thiamine (5×10^{-3} M, upward) induced contracture in frog skeletal muscle, and Cheymol, Bourillet, Levassort and Kerp (1957) showed that exceedingly high concentrations of thiamine (5×10^{-2}) could anul the effects of stimulation of the phrenic nerve on isolated rat diaphragms.

Our experiments indicate that loss of muscular power during deficiency in thiamine is very largely attributable to lack of muscle co-carboxylase, and that possible physiological excesses of thiamine would be likely to affect neuromuscular transmission only in the presence of hyperthyroid state.

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References

Bhagat, B. and Lockett, M. F. (1962). *J. Pharm. Pharmacol.*, **14**, 37–40.
Bülbring, E. (1946). *Brit. J. Pharmacol.*, **1**, 38–61.
Cheymol, J., Bourillet, T., Levassort, C. and Kerp, L. (1957). *Arch. int. Pharmacodyn.*, **111**, 36–59.
Minz, B. (1938). *C.R. soc. Biol. Paris*, **127**, 1251–1253.
Muralt, A. von (1947). *Vit. Hormones*, **5**, 93–118.
Ngai, S. H., Ginsburg, S. and Katz, R. L. (1961). *Biochem. Pharmacol.*, **7**, 256–264.
Pickens, J. and Lockett, M. F. (1961). *J. Endocrinol.*, **23**, 231–236.
Torda, C. and Wolf, H. G. (1944). *Proc. Soc. exp. Biol. N.Y.*, **56**, 88–89.
Venning, E. H., Kazmin, V. E. and Bell, J. C. (1946). *Endocrinol.*, **38**, 79–89.