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SHORT COMMUNICATION

Modification of Acetylcholine Receptor-Mediated Ion Permeability by Thiamine

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SUMMARY

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Thiamine has been found to be active in an acetylcholine receptor-rich postsynaptic membrane preparation from *Torpedo californica* electroplax by inhibiting the rate of carbamylcholine-induced acetylcholine receptor-mediated ^{22}Na efflux. Thiamine reduces the maximal response to carbamylcholine without changing the affinity of the receptor for carbamylcholine.

INTRODUCTION

We have found thiamine to be active in a cholinergic postsynaptic membrane fraction from *Torpedo californica* electroplax. Thiamine is shown to inhibit the rate of carbamylcholine stimulated ^{22}Na efflux in these AChR[1]-rich resealed membrane vesicles. Thiamine decreases the maximal rate of ^{22}Na efflux without a significant effect on the apparent dissociation constant for the binding of carbamylcholine to the receptor. This is an indication at the biochemical level that thiamine can affect the ion pulses associated with nerve function.

Thiamine and its phosphate esters have been implicated in axonal and synaptic neural events (1-4). However, no clearcut analysis of the locale and function has been made. Thiamine was recently reported to alter synaptic transmission in *Torpedo* electroplax possibly by affecting ACh[1] release (3). The results reported here indicate that thiamine is also active at the postsynaptic membrane.

An AChR-rich postsynaptic membrane preparation (5, 6) from *Torpedo* electroplax was utilized in this study. These membranes bind cholinergic agonists and antagonists and they respond to cholinergic agonists with dose-dependent increases in permeability to Na^+, K^+, and Ca^{2+} ions. The permeability increases are blocked by specific cholinergic antagonists such as the α neurotoxin from *Naja naja siamensis*. These membranes also display the property of pharmacologial desensitization in response to cholinergic agonists (7, 8). Kinetic analysis of the rates of efflux of permeant ions from the membranes (9, 10) yield kinetic parameters which correlate well with values determined for intact cells (11). In this report we will use a kinetic method to investigate the mode of action of thiamine.

Figure 1 shows ^{22}Na efflux curves in the absence and presence of 10^{-4} M carbamylcholine. In the control experiment, the efflux curve is characterized by an initial fast phase followed by a slower phase of ^{22}Na loss. In the presence of carbamylcholine, there is an initial very rapid loss of ^{22}Na

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[1] The abbreviations used are: AChR, acetylcholine receptor; ACh, acetylcholine; TDP, thiamine diphosphate; TTP, thiamine triphosphate.

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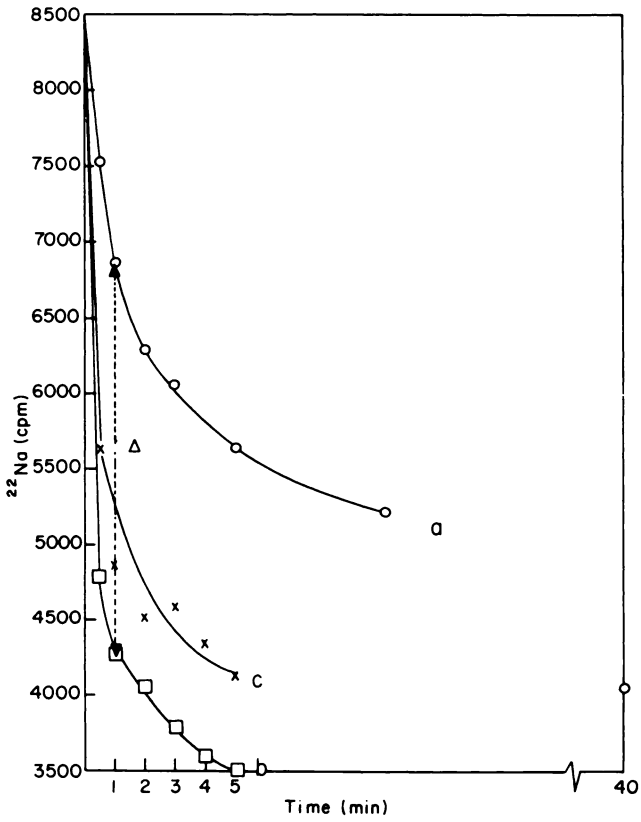

FIG. 1. *Sodium-22 efflux curves in the absence (a) and presence (b) of 10^{-4} M carbamylcholine (a synthetic cholinergic agonist not degraded by AChE) in the dilution medium*

C shows the efflux curve in the presence of 10^{-4} M carbamylcholine and 10^{-4} M thiamine in the dilution medium. A suspension of 0.1 ml of postsynaptic membranes preequilibrated in ^{22}NaCl was diluted 1:100 at zero time into a medium of 255 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$, 1.5 mM phosphate buffer (pH 7.0). At the indicated times 1.0 ml samples were rapidly filtered through HAWP 02400 Millipore filters, washed 3 times with 2.5 ml of the dilution medium, dried and the radioactivity remaining on the filter determined by liquid scintillation counting in a toluene based fluor. All curves were normalized to correct for small errors in dilution. The membranes were prepared essentially as described by Hazelbauer and Changeaux using freshly excised tissue from *Torpedo californica* or tissue that had been quick frozen in liquid nitrogen (6). It was determined that freezing in liquid nitrogen did not affect the activity of the membranes. The difference (Δ) between ^{22}Na retained in the control experiment at 1 min and ^{22}Na retained in the presence of carbamylcholine at 1 min was taken as a measure of the initial rate of ^{22}Na efflux. Preparation 39: toxin binding sites = 0.7 nmoles/mg protein, protein concentration = 15.0 mg/ml.

after which the curve becomes very similar in appearance to the control efflux. This initial loss of ^{22}Na provides a sensitive empirical measure of agonist induced AChR-mediated ^{22}Na efflux (5, 9)[2] and permits evaluation of initial efflux rates prior to the onset of desensitization. Figure 1 also shows the inhibitory effect of thiamine.

[2] Andreasen, T. J., D. R. Doerge and M. G. McNamee, unpublished observations.

Table 1 shows the dose dependence of thiamine's inhibitory effect in the concentration range of 10^{-4} M to 10^{-6} M. It was determined that 10^{-4} M thiamine was without effect on the control efflux rate. The thiamine content in *Torpedo* tissue has been determined and is on the order of 10^{-5}–10^{-6} M (3).

Table 2 shows the results of a kinetic analysis of thiamine's inhibitory effect. Although the response is not a direct measure

of the binding process, analyses of carbamylcholine responses fit typical Michaelis-Menton schemes (9, 10).[2] The noncompetitive nature of inhibition is similar to the effect of local anesthetics on ^{22}Na efflux from *Torpedo* membrane vesicles (5).

The effects of the polyphosphate esters of thiamine have been briefly investigated. TDP[1] is inhibitory in the same concentration range as thiamine and TTP[1] is noninhibitory. It is known that thiamine is released from nerve tissue upon excitation (1, 2). This presumably results from a dephosphorylation of the TDP and TTP by specific phosphatase action. The release of thiamine into the synaptic cleft and subsequent action on the postsynaptic membrane would alter the effects of ACh in a manner not yet fully understood. We reported previously that thiamine acts as an inhibitor of eel acetylcholinesterase with a K_I of 4×10^{-4} M (12). Thiamine also inhibits the *Torpedo* esterase with a similar inhibition constant. Further investigation on the mechanism of thiamine's postsynaptic effect is in progress.

TABLE 1

Dose dependence of thiamine's inhibition of the carbamylcholine-induced ^{22}Na efflux rate

The ⟨Δ⟩ values were determined by difference between means of 4 trials (standard deviation ≤5% for all sets). A suspension of 0.015 ml of membranes preequilibrated in $^{22}NaCl$ was diluted 1:100 into the dilution buffer containing the indicated thiamine concentration and 10^{-4} M carbamylcholine. At 1 min after dilution, a 1.0 ml aliquot was filtered, washed, dried and the radioactivity on the filter determined as before. Preparation 44: toxin binding sites = 0.8 nmoles/mg protein, protein concentration = 12.6 mg/ml.

[Thiamine]	⟨Δ⟩
(M)	*(cpm/min)*
0	8016
10^{-7}	7846
10^{-6}	7452
10^{-5}	7164
10^{-4}	6891

TABLE 2

Kinetic analysis of thiamine's inhibition of the carbamylcholine induced ^{22}Na efflux rate

These data obtained as in Table 1 were plotted in the linear form of the equation

$$\Delta = \Delta_{max} \frac{[carb]}{[carb] + K_D(App)}$$

Where Δ_{max} is the maximum rate of ^{22}Na efflux (extrapolated to infinite carbamylcholine concentration) (10). From the double reciprocal plot, $1/\Delta$ vs $1/[carb]$, the slopes and intercepts are obtained by a linear least squares analysis of the data points. From these numbers, the apparent dissociation constant $K_D(App)$, for the carbamylcholine-receptor complex can be determined. Within our error analysis, thiamine has no effect on the apparent K_D for carbamylcholine. The effect is seen on the Δ_{max} indicating a noncompetitive inhibition of the carbamylcholine-induced AChR-mediated ^{22}Na efflux rate by thiamine. Preparation 45: toxin binding sites = 0.8 nmoles/mg protein, protein concentration = 11.5 mg/ml.

[Thiamine]	Δ_{max}	K_D ($\times 10^5$)
(M)	*(cpm/min)*	
0	6999	1.32
10^{-6}	6779	1.20
10^{-5}	6138	2.53
10^{-4}	5305	1.32

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