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Tetrahydroaminoacridine and physostigmine have opposing effects on probability of transmitter release at the frog neuromuscular junction

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The effect of 1,2,3,4-tetrahydro-9-aminoacridine (THA) on quantal transmitter release was examined at the frog neuromuscular junction. THA (3 μM) caused an increase in m (no. of quanta released) as measured by K^+-evoked miniature endplate potential (MEPP) frequency. This was due to an increase in p (probability of release), as n (no. of functional release sites) was unchanged. The increase in p was dose-dependent over a range of 0.3–10 μM. By contrast, physostigmine (3 μM) caused a decrease in p, and neostigmine, which does not cross the nerve membrane, had no consistent effect on p. At the postsynaptic site, neostigmine produced the largest increase in MEPP size (79.2%), and THA produced the smallest (17.5%). The divergent effects of THA and physostigmine on p indicate a fundamental difference in their actions at the nerve terminal.

One of the features of Alzheimer's disease is a pronounced loss of cholinergic neurons in the nucleus basalis of Meynert [8, 11]. Attempts to enhance synaptic transmission at remaining neurons in this pathway have included the use of centrally acting anti-cholinesterases (anti-ChEs) such as physostigmine and 1,2,3,4-tetrahydro-9-aminoacridine (THA). Administration of physostigmine has produced some improvement in cognitive function [5, 19, 22], but the effect has been brief, presumably due to the short half-life of the drug. Other studies employing the longer-acting THA have met with mixed success [4, 6, 20], with positive results appearing more often in high-dose, longer-term trials [10].

The strategy of using physostigmine or THA to enhance cholinergic transmission is based on the ability of anti-ChEs to prolong the postsynaptic action of acetylcholine (ACh). This approach, however, is complicated by the fact that physostigmine causes a decrease in transmitter output [1, 2] which may offset the enhancement produced at the postsynaptic site. Because of this possibility and the interest in THA as a 'long-acting physostigmine', it was important to determine whether THA also inhibits transmitter release. We have found that THA has an effect opposite to that of physostigmine and that the actions of both agents are due to alterations in p.

Standard microelectrode techniques were used to record miniature endplate potentials (MEPPs) from frog (*Rana pipiens*) cutaneous pectoris neuromuscular junctions, as previously described [15]. Muscles were mounted in a 1 ml Plexiglas chamber, continuously perfused (1.5 ml/min) with Ringer solution. The normal Ringer contained (mM): NaCl 110, KCl 2.5, $CaCl_2$ 1.8, Tris 2.0 (to pH 7.2), and glucose 5.6. To increase basal MEPP frequency, $[K^+]$ was elevated to 10 mM by equimolar substitution of KCl for NaCl, and preparations equilibrated for at least 30 min before use. All results were recorded on FM tape for subsequent playback and analysis. The oscilloscope was set for 50 ms sweeps which were photographed across the width of moving film, and the number of MEPPs/sweep used in place of evoked quantal content (m). A total of 500 sequential sweeps were recorded for each sample, and data analyzed in subgroups of 100 [14]. Results which were nonstationary or not binomial were rejected. The program for unbiased quantal release parameters [13] was written in BASIC and processed on a Zenith 248 computer with 1 Megabyte of RAM. MEPP amplitudes were measured with the aid of a photographic enlarger, and 100 samples used for each estimate. Values were corrected for shifts in membrane potential [7], after recalculation of the reversal potential for ion changes in the Ringer [21]. Adjustment of mean MEPP amplitudes for samples lost

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in baseline noise was not necessary because of the large signal-to-noise ratio.

The effect of THA (3 μM) on transmitter release was first examined as a function of time. Results were obtained from continuous single cell recordings in 10 mM K^+ Ringer, in which data were collected every 5 min, following a 10 min stabilization period. The outcome from six such trials is shown in Fig. 1. For comparative purposes, identical experiments were performed using equimolar concentrations (3 μM) of physostigmine and neostigmine (6 trials each). Over the 20 min period examined, THA and neostigmine produced monotonic increases in m (23.1 and 29%, respectively), whereas physostigmine produced a monotonic decrease in m (18.2%) (Fig. 1A). The increase in m produced by THA was due to an increase in p (Fig. 1B), as n was essentially unchanged (Fig. 1C). Similarly, the decrease in m with physostigmine was due to a decrease in p (Fig. 1B), as n was either unchanged or increased (Fig. 1C). The results with neostigmine were more complex, as there was an oscillation in p, which seemed to mimic the effect on p of physostigmine (Fig. 1B). Nonetheless, the increase in m appeared to be associated primarily with n, which was increased or unchanged but never decreased (Fig. 1C). Finally, there was no systematic effect by any agent on spatial variance in p (var_s p) (Fig. 1D). The consistently negative values for var_s p were presumably an artifact of the method due to a small amount of temporal variance occurring during the 30 s of recording [14].

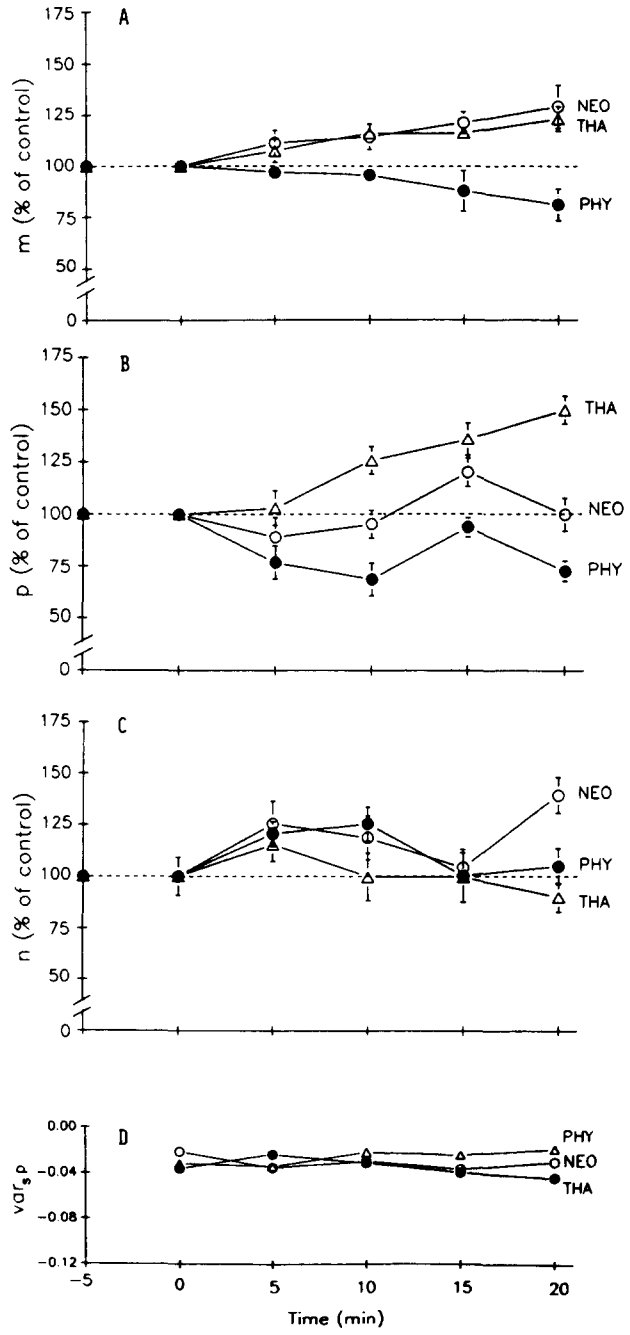

Fig. 1. Comparative effects of equimolar concentrations (3 μM) of THA, neostigmine (NEO) and physostigmine (PHY) on (A) no. of quanta released m, (B) probability of release p, and (C) no. of operational release sites n, as a function of time. Results from continuous single cell recordings were normalized to percents of the control values obtained at time zero. D: effect of drugs on var_s p with time (results not normalized because of negative values). Points represent means of 6 experiments for each drug, and bars (except in D where omitted for clarity) indicate ± 1 S.E.M.

Similar studies were carried out using 12.5 mM K^+ in the control Ringer to examine whether results were affected by $[K^+]$. As expected, the increase in $[K^+]$ produced a higher basal level of quantal release. Application of 3 μM THA again caused an increase in m which was due primarily to an increase in p ($N=6$), and 3 μM physostigmine produced a decrease in m, due in part to decreases in n and p ($N=6$).

The dose–response effect of [THA] on p is shown in Fig. 2. Data were obtained from 6 junctions exposed

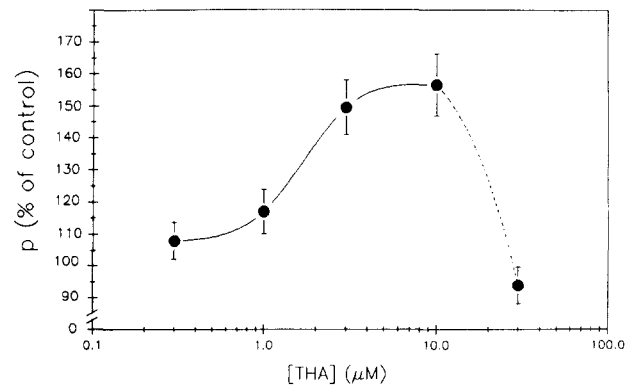

Fig. 2. Dose–response curve of [THA] vs p. Data were obtained in 10 mM K^+ Ringer during sequential exposure of single junctions to increasing [THA]. Results were normalized to percents of the values obtained in the absence of THA (points indicate means ± 1 S.E.M. for $N=6$). Dotted line to 30 μM shows decrease in p to below control levels, believed to represent a separate, inhibitory effect.

sequentially to 0.3, 1, 3, 10 and 30 μM THA. Up to 10 μM, THA produced a dose-dependent increase in p but, contrary to the results in Fig. 1A, produced no increase in m. At 30 μM, THA caused a marked decrease in m and p, indicating an actual inhibition of transmitter release. This may have been due to the long duration of each experiment (20 min for each concentration) and the inability of the system to maintain quantal output under these conditions. Alternatively, it may have been due to a hemicholinium-like action of THA to block high-affinity choline uptake [3]. Further studies, including the addition of choline to the Ringer, will be needed to clarify this point.

Finally, MEPP amplitudes were measured in the presence of equimolar concentrations (3 μM) of THA, physostigmine and neostigmine to compare their postsynaptic effects, i.e., their relative abilities to enhance cholinergic transmission due to antiChE activity. Measurements were made in 10 mM K^+ Ringer and 20 min after adding each antiChE. The results (Fig. 3) after appropriate corrections [7, 21] showed that neostigmine produced the greatest increase in MEPP amplitude (79.3%), whereas physostigmine increased MEPP amplitudes by 45.6%, and THA increased amplitudes only 17.5%.

The primary finding of this study is that THA produces an increase in m which is due to an increase in p and that physostigmine produces a decrease in m which is due to a decrease in p. Using Mg^{2+}-paralyzed junctions, Alderdice [1, 2] found that physostigmine decreased the m of endplate potentials but had no effect on resting MEPP frequency. The present results (Fig. 1A) show that the effect of physostigmine on MEPP frequency can be 'unmasked' using 10 mM K^+ depolarization to mimic nerve stimulation. The actual [K^+] used is not

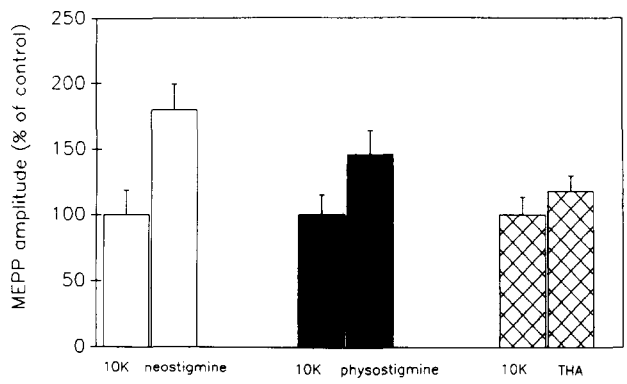

Fig. 3. Comparative effect of equimolar concentrations (3 μM) of neostigmine, physostigmine, and THA on MEPP amplitude. Recordings of MEPPs were obtained in 10 mM K^+ Ringer before and 20 min after adding each antiChE agent. Results normalized for comparative purposes. Controls (100%) are equal to 0.847 mV for neostigmine, 0.848 mV for physostigmine, and 0.677 for THA (means $\pm$ 1 S.E.M., $N=3$).

of critical importance, since the effects are also seen with 12.5 mM K^+. After 20 min, the effects of physostigmine on m and p diverge from the effects of THA by 41.8% (Fig. 1A) and 87.5% (Fig. 1B), respectively. The differences should be even greater with longer term exposures, since neither process appears close to peaking (Fig. 1A,B). The increase in transmitter release with THA (Fig. 1A), coupled with its weak postsynaptic action (Fig. 3), suggests that its neurochemical and behavioral effects may be due to something other than, or in addition to, ChE inhibition (cf. Nielsen et al. [17]).

The increase in m with neostigmine (Fig. 1A) supports the idea that the drug has a direct or indirect (feedback of ACh) action at the nerve terminal [12]. The failure of others to note this effect may be due to their use of blocking agents such as Mg^{2+} (cf. ref. 1). Although Nilsson et al. [18] have suggested that ACh feedback may be involved in the actions of THA and physostigmine, it is unlikely that presynaptic autoreceptors are involved in the present effects on p. Otherwise, neostigmine, which had the greatest antiChE action (Fig. 3), should have produced the most ACh feedback and the greatest effect on p. We instead favor an intracellular site of action for THA and physostigmine, since p is generally associated with intraterminal Ca^{2+}, and since neostigmine (which is charged and does not cross the membrane) had no consistent effect on p. However, the exact sites and mechanisms of action remain to be elucidated.

The results in Fig. 2 suggest that the increase in m with THA is finitely limited, due possibly to an inability to replenish transmitter stores. THA also appears to inhibit transmitter release (Fig. 2) at doses ($>$ 10 μM) which are known to depress nodal action potentials [9]. Possible mechanisms may include inhibition of choline uptake [3] or block of K^+ or Ca^{2+} currents [9]. Accordingly, the ability to obtain enhancement or inhibition may underlie a large part of the variation in response to THA [10].

Acute administration of physostigmine produces an improvement in cognitive function which is short lasting, presumably due to rapid elimination of the compound [5, 16]. However, high doses or chronic administration produces a 'rebound deterioration' [16], which is not explained by drug elimination. One possibility may be that the initial improvement is due to the anti-ChE effect, whereas the deterioration is due to the reduction in transmitter output (which is delayed by the need for physostigmine to penetrate the nerve membrane). If this is so, then physostigmine, even in sustained-release formulation, may not be suitable for long term treatment of Alzheimer's disease. Despite its weaker postsynaptic effect, THA may be a more viable candidate because of its enhancement of transmitter release plus its longer duration of action.

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1 Alderdice, M.T., Physostigmine, but not neostigmine, inhibits acetylcholine release, Brain Res., 178 (1979) 596–599.
2 Alderdice, M.T., Further comparison of the effects of physostigmine and neostigmine on frog neuromuscular transmission, Clin. and Exp. Pharmacol. & Physiol., 9 (1982) 35–43.
3 Buyukuysal, R.L. and Wurtman, R.J., Tetrahydroaminoacridine but not 4-aminopyridine inhibits high-affinity choline uptake in striatal and hippocampal synaptosomes, Brain Res. 482 (1989) 371–375.
4 Chatellier, G. and Lacomblez, L., Tacrine (tetrahydroaminoacridine; THA) and lecithin in senile dementia of the Alzheimer type: a multicentre trial, Br. Med. J., 300 (1990) 495–498.
5 Christie, J.E., Physostigmine and arecoline infusions in Alzheimer's disease. In S. Corkin, K.L. Davis, J.H. Growdon, E. Usdin and R.J. Wurtman (Eds.), Alzheimer's Disease: A Report of Progress in Research, Vol. 19, Raven, New York, 1982, pp. 413–419.
6 Gauthier, S., Bouchard, R., Lamontagne, A., Bailey, P., Bergman, H., Ratner, J., Tesfaye, Y., Saint-Martin, M., Bacher, Y., Carrier, L., Charbonneau, R., Clarfield, A.M., Collier, B., Dastoor, D., Gautier, L., Germain, M., Kissel, C., Krieger, M., Kushnir, S., Masson, H., Morin, J., Nair, V., Neirinck, L. and Suissa, S., Tetrahydroaminoacridine-lecithin combination treatment in patients with intermediate-stage Alzheimer's disease, New Engl. J. Med., 322 (1990) 1272–1276.
7 Katz, B. and Thesleff, S., On the factors which determine the amplitude of the 'miniature end-plate potential', J. Physiol., 137 (1957) 267–278.
8 Katzman, R., Alzheimer's disease, New Engl. J. Med., 314 (1986) 964–973.
9 Kotake, H., Noguchi, N., Doi, T., Matsuoka, S., Hisatome, I., Hasegawa, J. and Mashiba, H., An electrophysiological study of 9-amino-1,2,3,4-tetrahydroacridine (THA) on sino-atrial node preparations of rabbits, Gen. Pharmacol., 21 (1990) 489–492.
10 Kumar, V. and Becker, R.E., Clinical pharmacology of tetrahydroaminoacridine: a possible therapeutic agent for Alzheimer's disease, Int. J. Clin. Pharmacol. Ther. Toxicol., 27 (1989) 478–485.
11 McGeer, P.L., Aging, Alzheimer's disease, and the cholinergic system, Can. J. Physiol. Pharmacol., 62 (1984) 741–754.
12 Miyamoto, M.D., The actions of cholinergic drugs on motor nerve terminals, Pharmacol. Rev., 29 (1978) 221–247.
13 Miyamoto, M.D., Probability of quantal transmitter release from nerve terminals: theoretical considerations in the determination of spatial variation, J. Theor. Biol., 123 (1986) 289–304.
14 Miyamoto, M.D., Determination of unbiased quantal release parameters, including spatial variance in p, using miniature endplate potential (mepp) frequencies, Physiologist, 31 (1988) A84.
15 Moffatt, E.J. and Miyamoto, M.D., Effect of sodium and calcium channel blockade on the increase in spontaneous transmitter release produced by the mitochondrial inhibitor, dinitrophenol, J. Pharmacol. Exp. Ther., 244 (1988) 613–618.
16 Muramoto, O., Sugishita, M. and Ando, K., Cholinergic system and constructional praxis: a further study of physostigmine in Alzheimer's disease, J. Neurol. Neurosurg. Psychiatry, 47 (1984) 485–491.
17 Nielsen, J.A., Mena, E.E., Williams, I.H., Nocerini, M.R. and Liston, D., Correlation of brain levels of 9-amino-1,2,3,4-tetrahydroacridine (THA) with neurochemical and behavioral changes, Eur. J. Pharmacol., 173 (1989) 53–64.
18 Nilsson, L., Adem, A., Hardy, J., Winblad, B. and Nordberg, A., Do tetrahydroaminoacridine (THA) and physostigmine restore acetylcholine release in Alzheimer brains via nicotinic receptors?, J. Neural Transm., 70 (1987) 357–368.
19 Peters, B.H. and Levin, H.S., Effects of physostigmine and lecithin on memory in Alzheimer disease, Ann. Neurol., 6 (1979) 219–221.
20 Summers, W.K., Majovski, L.V., Marsh, G.M., Tachiki, K. and Kling, A., Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type, New Engl. J. Med., 315 (1986) 1241–1245.
21 Takeuchi, N., Some properties of conductance changes at the endplate membrane during the action of acetylcholine, J. Physiol., 167 (1963) 128–140.
22 Thal, L.J. and Fuld, P.A., Memory enhancement with oral physostigmine in Alzheimer's disease, New Engl. J. Med., 308 (1983) 720.