European Journal of Pharmacology, 155 (1988) 265–270
Elsevier

EJP 50487

THA increases action potential duration of central histamine neurons in vitro

Peter B. Reiner * and Edith G. McGeer

Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver BC, V6T 1W5 Canada

Received 2 May 1988, revised MS received 11 July 1988, accepted 26 July 1988

Long-term administration of 9-amino-1,2,3,4-tetrahydroacridine (THA) has been reported to produce marked clinical improvement in patients suffering from Alzheimer's disease. The dramatic enhancement of cognitive function seen with THA contrasts sharply with the modest improvements seen with other forms of anticholinesterase therapy, suggesting that additional mechanisms might play a role in its therapeutic efficacy. When applied to hypothalamic histamine neurons maintained in vitro, THA produced a dose-dependent increase in action potential duration. By its effect upon action potential duration, THA may increase release of transmitter from the axon terminals of cortically projecting aminergic neurons which have been shown to degenerate in Alzheimer's disease. Thus, the therapeutic efficacy of THA may derive from a combination of its anticholinesterase activity and its effects upon the duration of action potentials of aminergic neurons.

9-Amino-1,2,3,4-tetrahydroacridine (THA); Histamine neurons; K^+ channels; Alzheimer's disease; (Intracellular recordings)

1. Introduction

Alzheimer's disease is the major cause of dementia in the elderly (Terry and Katzman, 1983). The etiology of the disorder is unknown, and at present there is no cure. Initial studies focused upon the deficit in cortical cholinergic innervation (Coyle et al., 1983), and considerable effort has gone into devising therapeutic strategies which might restore function in this system. However, most 'cholinergic replacement therapies' have resulted in only modest improvements in cognitive function (Thal et al., 1983; Stern et al., 1987).

One promising therapeutic agent in the palliative treatment of Alzheimer's disease is 9-amino-1,2,3,4-tetrahydroacridine (THA), which has been reported to produce marked clinical improvement when administered chronically to mildly demented patients (Summers et al., 1986). THA was originally used for its action as an inhibitor of the acetylcholine degradative enzyme acetylcholinesterase (Heilbronn, 1961; Ho and Freeman, 1965). However, it is becoming increasingly apparent that the pathology of Alzheimer's disease is not limited to the cortical cholinergic innervation. Rather, a number of cortically projecting neuronal systems are affected, including central aminergic neurons (Mann et al., 1980; Boundareff et al., 1982; Iversen et al., 1983; Yamamoto and Hirano, 1985; German et al., 1987; Saper and German, 1987). Furthermore, THA is structurally related to 4-aminopyridine, a compound which blocks potassium channels in a variety of excitable tissues (Thompson, 1977; Gustafsson et al., 1982). These observations prompted us to re-examine the pharmacology of THA, using as our test system the histaminergic neurons of the rat tuberomam-

* To whom all correspondence should be addressed.

millary nucleus (Panula et al., 1984; Steinbusch and Mulder, 1984; Watanabe et al., 1984).

2. Materials and methods

Intracellular recordings were obtained from histaminergic neurons of the tuberomammillary nucleus in hypothalamic slices maintained in vitro using standard electrophysiological techniques. Male Wistar rats (75-150 g) were anesthetized with ether, decapitated, the brain rapidly removed and immersed in ice-cold Ringer solution of the following composition (in mM): Na^+ 152; K^+ 2.5; Ca^{2+} 2.4; Cl^- 136; PO_4^{2-} 1.2; Mg^{2+} 1.3; CO_3^- 25; glucose 11; pH 7.4 when saturated with 95% O_2, 5% CO_2. The hypothalamus was dissected free and coronal slices of 400 μm thickness were prepared using a vibratome. Slices were stored in oxygenated Ringer at room temperature before being transferred singly to a recording chamber where they were submerged and continually superfused with warmed (30 °C) oxygenated Ringer flowing at 1-1.5 ml/min. THA (Aldrich) and tetrodotoxin (Sigma) were applied at known concentrations directly to the perfusate. As recovery from the effects of THA was very slow, application of drugs to a given slice was carried out only once. Tuberomammillary neurons were recorded from both the lateral and ventral subnuclei which were readily identified in the slice by their locations, respectively, at the extreme lateral and ventral edges of the mammillary body at the level of the mammillary recess. Intracellular recordings from tuberomammillary neurons were made using glass micropipettes drawn from 2.0 mm (outside diameter) microfilament glass and filled with either 3 M KCl or 4 M K acetate. Data was photographed directly from the oscilloscope as well as being displayed on a chart recorder.

All 38 tuberomammillary neurons included in the present study exhibited the constellation of membrane properties previously described for positively identified histaminergic neurons (Haas and Reiner, 1988) including resting membrane potential between −50 and −55 mV, half amplitude spike duration at the resting potential around 2 ms, spike afterhyperpolarizations of 5-15 mV amplitude and 50-200 ms duration, apparent input impedance of 150 to 400 MΩ, inward and transient outward rectification. Stable intracellular recordings lasted between 1 and 4 h. As membrane potential has marked effects upon action potential duration of histaminergic neurons (Haas and Reiner, 1988), membrane potential was meticulously maintained at −60 mV during all experiments.

3. Results

Bath application of THA resulted in dose-dependent broadening of the action potential of tuberomammillary neurons (fig. 1A,B). The half-amplitude duration of the action potential was significantly ($P < 0.05$) increased by concentrations of 50 μM and above applied for 10 min ($N = 3$-5 observations at each dosage level). Effects on action potential duration far outlasted the duration of drug administration; in several instances, further broadening of the action potential was observed after 15 min of washing in drug-free solution. Only partial recovery was achieved after 1 h in control media. THA had no effect upon the transient outward rectification (presumably mediated by A-type potassium channels; Haas and Reiner, 1988) seen in histamine neurons.

There appeared to be a critical interplay between the duration of THA application and the dosage required to produce action potential broadening. When applied at 1 μM, action potential duration progressively increased over the course of 1 h ($N = 3$; fig. 1C,D). Although the effect did not appear to saturate after 1 h, the difficulty of maintaining stable intracellular penetrations precluded longer experiments.

The anticholinesterase activity of THA (Heilbronn, 1961; Ho and Freeman, 1965) might increase the effective availability of acetylcholine in the slice and thereby broaden the action potential by suppressing the M-current, a voltage-dependent potassium current which is blocked by muscarinic agonists (Brown and Adams, 1980). However, bath application of 10 μM physostigmine for 1 h (which should completely inhibit acetylcholinesterase, Silver, 1974) had no measura-

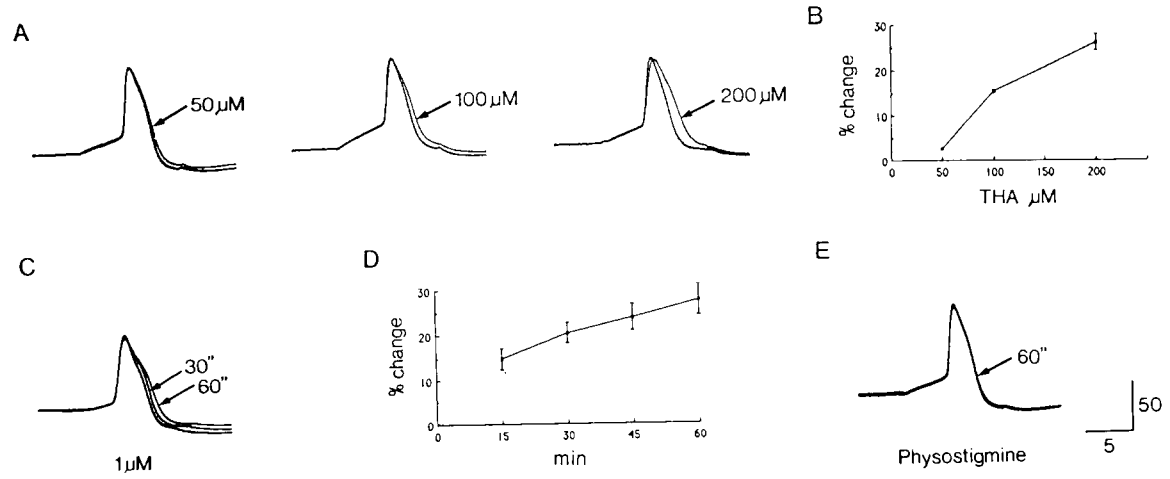

Fig. 1. (A) THA increases action potential duration of tuberomammillary neurons in a dose-dependent manner. The effect of a 10 min application of THA at the indicated doses upon the duration of the action potential of tuberomammillary neurons is illustrated. Each trace displays one control action potential and one superimposed action potential at the end of a 10 min drug application (arrow). Action potentials were evoked by depolarizing current pulses of 10 ms duration and 0.2 nA intensity from a membrane potential of −60 mV; the offset of each pulse appears as a notch in the afterhyperpolarization. Data taken from three different neurons. (B) Dose-response curve showing the percent increase in half-amplitude duration of the action potential of tuberomammillary neurons to 10 min applications of THA. Each data point represents the mean ± S.D. of three to five neurons. (C) Longer duration application of THA reveals effects at lower doses. When applied for 60 min, 1 μM THA significantly increased action potential duration (N = 3). (D) Time-dependent broadening of the action potential with 1 μM THA. The effect did not appear to saturate at 1 h. (E) Superimposed sweeps before and at the end of 60 min of bath application of physostigmine (10 μM). There was no change in action potential duration. All vertical calibrations in mV and horizontal calibrations in ms. Calibration in E also applies to A and C.

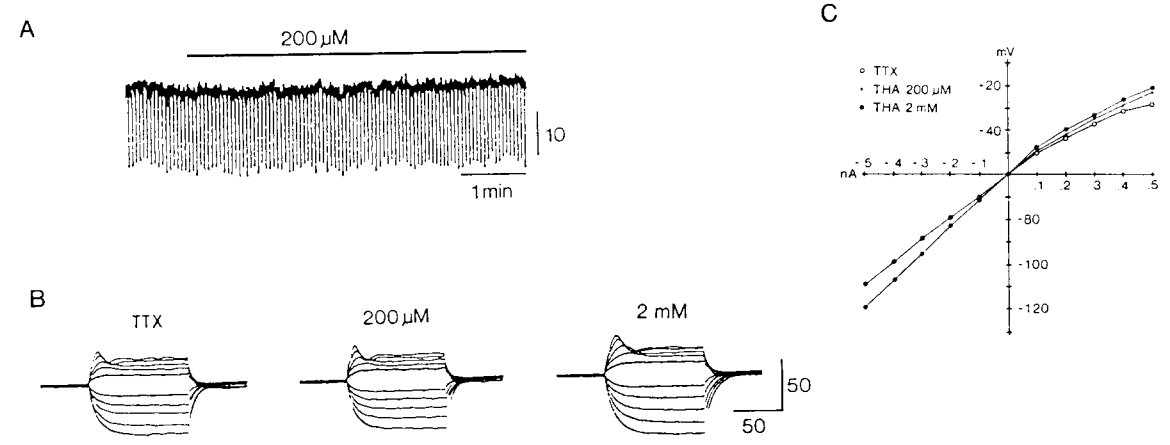

Fig. 2. (A) Chart record demonstrating that 200 μM THA does not affect either resting membrane potential or the apparent input impedance of tuberomammillary neurons as measured by the voltage responses (downward deflections) to hyperpolarizing current pulses of 0.2 nA amplitude and 100 ms duration. The increase in action potential duration for this neuron is shown in fig. 1A. (B) The effects of THA on a tuberomammillary neuron in the presence of tetrodotoxin (TTX, 3 μM) is shown. A family of depolarizing and hyperpolarizing current pulses (±0.1-0.5 nA, 100 ms duration) were applied in TTX, following 10 min in 200 μM THA, and following a further 5 min in 2 mM THA. (C) I-V curves from the experiment shown in B. Voltage responses to depolarization were measured at the peak of the early depolarizing wave, while hyperpolarizing responses are steady state. Addition of 200 μM THA increased the slope of the I-V curve in the dipolarizing direction, but had no effect on the slope of the I-V curve in the hyperpolarizing direction. Increasing the concentration to 2 mM caused a further increase in the slope of the I-V curve in the depolarizing direction and additionally produced an increase in the slope of the curve in response to hyperpolarizing current pulses.

ble effect on the duration of the action potential (fig. 1E). Thus, the effect of THA on action potential duration appears to be mediated by a mechanism distinct from its anticholinesterase activity.

THA at doses up to 200 μM had no appreciable effect upon the apparent input resistance of tuberomammillary neurons as measured by application of hyperpolarizing current pulses (fig. 2A), yet the same dose broadened the action potential by upwards of 25%. These observations suggest that the ionic conductance(s) affected by THA are selectively activated upon depolarization. In the presence of tetrodotoxin to block sodium spikes, 200 μM THA increased the slope of the I-V curve in the depolarizing direction while hyperpolarizing responses were unchanged (N = 3, fig. 2B,C). Raising the concentration to 2 mM resulted in a further increase in the slope of the I-V curve with depolarization, and also caused an increase in the slope of the hyperpolarizing plot, the latter being due largely to blockade of inward rectification (Haas and Reiner, 1988). Thus, THA appears to reduce selectively a conductance activated upon depolarization.

4. Discussion

The present data are, to our knowledge, the first to demonstrate effects of THA on the membrane properties of mammalian central neurons in doses as low as 1 μM. One hypothesis for the mechanism by which THA broadens action potentials of tuberomammillary neurons is that the drug suppresses one or more potassium conductances involved in spike repolarization. That THA reduced a conductance activated on depolarization in the presence of tetrodotoxin is consistent with that view, insofar as such potassium conductances should be activated by depolarizing stimuli. THA increases action potential duration of both guinea pig cardiac myocytes and peptidergic neurons in the snail *Lymnia stagnalis*, and voltage clamp experiments demonstrate that these effects are paralleled by blockade of a slow potassium current (Drukarch et al., 1987; Osterrieder, 1987). While these data demonstrate that THA is able to block potassium channels in excitable tissues, other mechanisms for increasing action potential duration such as slowing of sodium inactivation (Schauf and Sattin, 1987) or increasing the calcium conductance which accompanies spike generation in tuberomammillary neurons (Haas and Reiner, 1988) cannot be excluded.

THA does not appear to broaden the action potentials of all mammalian central neurons. Action potential duration of hippocampal pyramidal cells is not markedly altered by 200 μM THA (Stevens and Cotman, 1987), and we confirmed this observation using our experimental paradigm (P.B. Reiner and E.G. McGeer, unpublished observations). Rather, THA has been suggested to block leakage (Stevens and Cotman, 1987) and A-type (Rogawski, 1987) potassium channels in hippocampal pyramidal cells.

The slow onset of THA's action may be explained by assuming that THA acts intracellularly and, being only moderately lipophilic, penetrates neuronal membranes slowly. Indeed, THA blocks ionic channels in *Myxicola* giant axons more rapidly when applied from the inside than from the outside (Schauf and Sattin, 1987). As the effects of 1 μM THA did not appear to saturate after 1 h, chronic administration of THA in the clinical setting (where therapeutic serum concentrations are as low as 30 nM; Summers et al., 1986) may be sufficient to produce an increase in action potential duration of tuberomammillary neurons.

There is considerable histological evidence for pathological changes in the mammillary bodies of patients with Alzheimer's disease (Hirano and Zimmerman, 1962; Ishii, 1966), and the pathology appears to be specific to the magnocellular neurons of the tuberomammillary nucleus (Saper and German, 1987). Consistent with these observations, biochemical data indicate that cortical histamine levels are reduced in Alzheimer's disease (Mazurkiewicz-Kwilecki and Nsowah, 1987). Assuming that THA has similar effects upon both the somata and axon terminals of tuberomammillary neurons, THA would increase synaptic release of histamine, thereby partly alleviating the deficit in cortical histaminergic innervation.

Histamine acting at H_2 receptors increases the excitability of hippocampal pyramidal neurons by

blocking a calcium-dependent potassium conductance involved in the generation of spike afterhyperpolarizations (Haas and Konnerth, 1983; Haas and Greene, 1986). Via a distinct mechanism, acetylcholine blocks the same calcium-dependent potassium conductance (Cole and Nicoll, 1984; Madison et al., 1987). Reductions in both transmitter systems in Alzheimer's disease would therefore be expected to have profound effects on the excitability of hippocampal pyramidal neurons. By increasing release of histamine upon its cortical and hippocampal targets, as well as increasing the duration of action of acetylcholine via its anticholinesterase activity, THA may partly restore cortical excitability.

While the effects of THA on central histaminergic and cholinergic systems may be sufficient to account for its clinical efficacy, it is intriguing to note that the membrane properties of histaminergic neurons (Haas and Reiner, 1988) are strikingly similar to those of brainstem noradrenergic and serotonergic neurons (Aghajanian and VanderMaelen, 1982; Williams et al., 1985), suggesting that THA may broaden the action potential in these systems as well. Indeed, THA has been shown to induce a long-lasting increase in the release of pre-loaded [^{3}H]noradrenaline and serotonin in vitro (Drukarch et al., 1988). As these amines also increase excitability of cortical neurons (Madison and Nicoll, 1982; Andrade and Nicoll, 1987; Colino and Halliwell, 1987) and the parent cell bodies degenerate in Alzheimer's disease (German et al., 1987), the resultant increase in release of cortical noradrenaline and serotonin might also be of therapeutic value in the treatment of Alzheimer's disease.

Transmitter replacement therapies aimed solely at increasing the availability of acetylcholine in the cerebral cortex have resulted in only modest improvements in cognitive function (Thal et al., 1983; Stern et al., 1987), while the effects of THA treatment have been reported to be rather dramatic (Summers et al., 1986). Based upon our current understanding of the neuropathology of Alzheimer's disease, it seems reasonable to predict that efficacious therapy requires restoration of function in multiple transmitter systems. The present study suggests that THA, acting via distinct mechanisms, may ameliorate both cholinergic and aminergic deficits in Alzheimer's disease.

Acknowledgements

We thank Dr. Kazue Semba for helpful discussions during the course of the experiments. Supported by the Medical Research Council of Canada.

References

Aghajanian, G.K. and C.P. VanderMaelen, 1982, Intracellular recording from serotonergic dorsal raphe neurons: pacemaker potentials and the effect of LSD, Brain Res. 238, 463.

Andrade, R. and R.A. Nicoll, 1987, Pharmacologically distinct actions of serotonin on single pyramidal neurones of the rat hippocampus recorded in vitro, J. Physiol. 394, 99.

Boundareff, W., C.Q. Mountjoy and M. Roth, 1982, Loss of neurons of origin of the adrenergic projection to cerebral cortex (nucleus locus ceruleus) in senile dementia, Neurology 32, 164.

Brown, D.A. and P.R. Adams, 1980, Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neurone, Nature 283, 673.

Cole, A. and R.A. Nicoll, 1984, Characterization of a slow cholinergic postsynaptic potential recorded in vitro from rat hippocampal pyramidal cells, J. Physiol. (London) 352, 173.

Colino, A. and J.V. Halliwell, 1987, Differential modulation of three separate K-conductances in hippocampal CA1 neurons by serotonin, Nature 328, 73.

Coyle, J.T., D.L. Price and M.R. DeLong, 1983, Alzheimer's disease: a disorder of cortical cholinergic innervation, Science 219, 1184.

Drukarch, B., K.S. Kits, E.G. Van der Meer, J.C. Lodder and J.C. Stoof, 1987, 9-Amino-1,2,3,4-tetrahydroacridine (THA), an alleged drug for the treatment of Alzheimer's disease, inhibits acetylcholinesterase activity and slow outward K^+ current, European J. Pharmacol. 141, 153.

Drukarch, B., J.E. Leysen and J.C. Stoof, 1988, Further analysis of the neuropharmacological profile of 9-amino-1,2,3,4-tetrahydroacridine (THA), an alleged drug for the treatment of Alzheimer's disease, Life Sci. 42, 1011.

German, D.C., C.L. White and D.R. Sparkman, 1987, Alzheimer's disease: neurofibrillary tangles in nuclei that project to the cerebral cortex, Neuroscience 21, 305.

Gustafsson, B., M. Galvan, P. Grafe and H. Wigstrom, 1982, A transient outward current in a mammalian central neurone blocked by 4-aminopyridine, Nature 299, 252.

Haas, H.L. and R.W. Greene, 1986, Effects of histamine on hippocampal pyramidal cells of the rat in vitro, Exp. Brain Res. 62, 123.

Haas, H.L. and A. Konnerth, 1983, Histamine and noradrenaline decrease calcium-activated potassium conductance in hippocampal pyramidal cells, Nature 302, 432.

Haas, H.L. and P.B. Reiner, 1988, Membrane properties of histaminergic tuberomammillary neurones of the rat hypothalamus in vitro, J. Physiol. (London) 399, 633.

Heilbronn, E., 1961, Inhibition of cholinesterases by tetrahydroaminacrin, Acta Chem. Scand. 15, 1386.

Hirano, A. and H.M. Zimmerman, 1962, Alzheimer's neurofibrillary changes, Arch. Neurol. 7, 227.

Ishii, T., 1966, Distribution of Alzheimer's neurofibrillary changes in the brain stem and hypothalamus of senile dementia, Acta Neuropathol. (Berlin) 6, 181.

Ho, A.K.S. and S.E. Freeman, 1965, Anticholinesterase activity of tetrahydroaminoacrine and succinyl choline hydrolysis, Nature 205, 1118.

Iversen, L.L., M.N. Rossor, G.P. Reynolds, R. Hills, M. Roth, C.Q. Mountjoy, S.L. Foote, J.H. Morrison and F.E. Bloom, 1983, Loss of pigmented dopamine-β-hydroxylase positive cells from locus coeruleus in senile dementia of Alzheimer's type, Neurosci. Lett. 39, 95.

Madison, D.V., B. Lancaster and R.A. Nicoll, 1987, Voltage clamp analysis of cholinergic action in the hippocampus, J. Neurosci. 7, 733.

Madison, D.V. and R.A. Nicoll, 1982, Noradrenaline blocks accommodation of pyramidal cell discharge in the hippocampus, Nature 299, 636.

Mann, D.M.A., J. Lincoln, P.O. Yates, J.E. Stamp and S. Toper, 1980, Changes in the monoamine containing neurons of the human CNS in senile dementia, Br. J. Psychiat. 136, 533.

Mazurkiewicz-Kwilecki, I. and S. Nsowah, 1987, Distribution of histamine in post mortem brain regions of Alzheimer patients, Abstr. Can. Coll. Neuropsychopharmacol. 10, Th-25.

Osterrieder, W., 1987, 9-Amino-1,2,3,4-tetrahydroacridine (THA) is a potent blocker of cardiac potassium channels, Br. J. Pharmacol. 92, 521.

Panula, P., H.-Y.T. Yang and E. Costa, 1984, Histamine containing neurons in the rat hypothalamus, Proc. Natl. Acad. Sci. U.S.A. 81, 2572.

Rogawski, M.A., 1987, Tetrahydroaminoacridine blocks voltage-dependent ion channels in hippocampal neurons, European J. Pharmacol. 142, 169.

Saper, C.B. and D.C. German, 1987, Hypothalamic pathology in Alzheimer's disease, Neurosci. Lett. 74, 364.

Schauf, C.L. and A. Sattin, 1987, Tetrahydroaminoacridine blocks potassium channels and inhibits sodium inactivation in *Myxicola*, J. Pharmacol. Exp. Ther. 243, 609.

Silver, A., 1974, The Biology of Cholinesterases (North Holland Publishing Company, Amsterdam) p. 457.

Steinbusch, H.W.M. and A.H. Mulder, 1984, Immunohistochemical localization of histamine in neurons and mast cells in the rat brain, in: Handbook of Chemical Neuroanatomy, Vol. 3, Classical Transmitters and Receptors in the CNS, Part II, eds. A. Björklund, T. Hökfelt and M.J. Kuhar (Elsevier, Amsterdam) p. 126.

Stern, Y., M. Sano and R. Mayeux, 1987, Effects of oral physostigmine in Alzheimer's disease, Ann. Neurol. 22, 306.

Stevens, D.R. and C.W. Cotman, 1987, Excitatory actions of tetrahydro-9-aminoacridine (THA) on hippocampal pyramidal neurons, Neurosci. Lett. 79, 301.

Summers, W.K., V. Majovski, G.M. Marsh, K. Tackiki and A. Kling, 1986, Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type, N. Engl. J. Med. 315, 1241.

Terry, R.D. and R. Katzman, 1983, Senile dementia of the Alzheimer type, Ann. Neurol. 14, 497.

Thal, L.J., P.A. Fuld, D.M. Masur and N.S. Sharpless, 1983, Oral physostigmine and lecithin improve memory in Alzheimer disease, Ann. Neurol. 13, 491.

Thompson, S.H., 1977, Three pharmacologically distinct potassium channels in molluscan neurones, J. Physiol. (London) 265, 465.

Watanabe, T., Y. Taguchi, S. Shiosaka, J. Tanaka, H. Kubota, Y. Terano, M. Tohyama and H. Wada, 1984, Distribution of the histaminergic neuron system in the central nervous system of rats; a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker, Brain Res. 295, 13.

Williams, J.T., R.A. North, S.A. Shefner, S. Nishi and T. Egan, 1985, Membrane properties of rat locus coeruleus neurones, Neuroscience 14, 95.

Yamamoto, T. and A. Hirano, 1985, Nucleus raphe dorsalis in Alzheimer's disease: Neurofibrillary tangles and loss of large neurons, Ann. Neurol. 17, 573.