A Peptide Enhancement Strategy in Alzheimer's Disease: Pilot Study with TRH-Physostigmine Infusions

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Introduction

Although disruption in several neurochemical systems has been described in Alzheimer's disease (AD), the most robust evidence relates the core cognitive impairment to significant deficits in brain acetylcholine function (Coyle et al 1983). The cholinergic hypothesis of AD has led to treatment strategies aimed at enhancing central cholinergic neurotransmission (Bartus et al 1982). There is modest improvement in cognition after treatment with centrally active acetylcholinesterase inhibitors, such as physostigmine (Davis and Mohs 1982; Thal et al 1989). Enhancing the efficacy of cholinergic agents would provide a novel therapeutic approach based on the cholinergic hypothesis. One possibility is coadministration of a cholinomimetic drug with a neuromodulatory agent known to augment cholinergic activity. The important role for neuropeptides in the modulation of brain neurotransmitter function is well established (Hokfelt et al 1989); thus, a "procholinergic" modulatory neuropeptide might serve as a basis for such an approach.

In addition to its role in the regulation of thyroid function, thyrotropin-releasing hormone (TRH) has long been recognized as a modulatory neuropeptide in the central nervous system (Horita et al 1986). The procholinergic effects of TRH have been demonstrated in animal studies at several levels of organization in the central nervous system (Yarbrough 1983). We have previously shown (Mellow et al 1989) that TRH can be given safely in high doses (required for central nervous system effects) to AD patients and produces behavioral activation and a small improvement in semantic memory. We have also shown it to reverse scopolamine-induced cognitive impairment in normal volunteers (Molchan et al 1990). We are currently testing the safety and feasibility of a peptide enhancement strategy, examining the cognitive and neuroendocrine effects of high-dose TRH in combination with physostigmine in AD patients. We herein report our initial experience in the first six subjects, in which we have tested the safety of this combination, as well as the hypothesis that TRH might modulate the effects of physostigmine.

Methods

Six patients (four men and two women; mean age, 69 ± 7 years), taking no psychotropic medications and in good general health, who met NINCDS-ADRDA criteria for probable Alzheimer's disease (McKhann et al 1984) underwent two separate study days (separated by at least 48 hours) while they were inpatients at the University of Michigan Hospital Clinical Research Center. Patients all received pretreatment with glycopyrrolate (0.1–0.2 mg), a peripheral cholinergic antagonist. Patients (blinded to drug treatment conditions) then received (on randomized days) a 10-min intravenous infusion of TRH (0.1 or 0.3 mg/kg) or placebo, followed by a 30-min infusion of physostigmine (0.5 or 1.0 mg). Patients had continuous electrocardiogram and vital sign monitoring during and at least 1 hour after the drug infusions. Cognitive testing was performed each study day, prior to drug infusions and during the last 10–15 minutes of the physostigmine infusions, by a technician blinded to drug treatment condition. The psychometric battery included the Buschke Selective Reminding Task, a letter retrieval task (a measure of verbal fluency), a picture memory test (a measure of automatic learning), and a digit span task. Patients were also asked to perform self-ratings (1–5 scale) on measures of arousal and mood. Serial blood samples for neuroendocrine measures of central cholinergic activity were obtained prior to, during, and after drug infusions. Samples were collected in chilled ethylene glycol tetraacetic acid (EGTA)-treated tubes, centrifuged at 4°C, and plasma was stored at −80°C until processing. Epinephrine was measured by modification of the method of Bouloux et al (1985), using high-pressure liquid chromatography with electrochemical detection. β-Endorphin was measured by immunoradiometric assay (Voellmy et al 1988) (kits purchased from Nickels Institute, San Juan Capistrano, CA).

Results of the cognitive and behavioral testing, as well as peak hormonal response and maximum vital sign changes, were all expressed as change scores (postinfusion minus preinfusion) for each day and analyzed by paired *t*-test, comparing the phy-

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sostigmine day with the physostigmine plus TRH day for each patient. Three patients received physostigmine, 0.5 mg, plus TRH, 0.1 mg/kg, (or placebo) and three patients received physostigmine, 1.0 mg, plus TRH, 0.3 mg/kg, (or placebo). Due to the small number of subjects, combined data were analyzed from all six subjects. In this preliminary study, corrections for multiple *t*-tests were not used.

Results

All patients tolerated the infusions without adverse effects on their vital signs or electrocardiogram. The only side effects experienced were some nausea (in two patients) and transient shivering (in three patients, those receiving the higher TRH dose). Patients' self-ratings of mood and arousal did not change between the physostigmine day and physostigmine plus TRH day. Systolic blood pressure increases were significantly greater on the physostigmine plus TRH day, compared with the physostigmine day (26.8 ± 4.9 mm Hg versus 14.3 ± 3.0 mm Hg). There were no significant changes in any other vital signs. Patients performed significantly better on measures of verbal fluency on the physostigmine plus TRH day than on the physostigmine day. In addition, patients had significantly fewer verbal intrusion errors on the physostigmine plus TRH day. On measures of delayed recall (word list learning), immediate recall (digit span), and automatic learning, there was no significant difference in performance between days. Although TRH improved performance on two cognitive measures, the overall response to physostigmine in this patient sample was nonsignificant (data not shown). Peak plasma epinephrine response during and after physostigmine infusion was significantly increased on the physostigmine plus TRH days. The β-endorphin response showed no significant difference between days and was quite variable in this sample; only three of six patients showed any rise in β-endorphin after drug infusions. The results are summarized in Table 1.

Discussion

This pilot study represents the first attempt at a peptide enhancement strategy in AD, using the procholinergic neuropeptide TRH in combination with the cholinesterase inhibitor physostigmine. We have found this combination to be safe and well tolerated in AD patients. The increases in blood pressure seen after both physostigmine and the combination of TRH plus physostigmine are consistent with both our own previous work (Mellow et al 1989) and that of others (Janowsky et al 1985). Although we did not include a day in this study when TRH was administered alone, the magnitude of the blood pressure change on the physostigmine plus TRH day suggests that the pressor effects of the two drugs are not synergistic, thereby reinforcing the safety of this strategy. On two measures of cognitive function, TRH improved performance when coadministered with physostigmine. These results are encouraging, because TRH appeared to have some enhancing effect even when the overall response to physostigmine was nonsignificant (which suggests nonoptimal physostigmine dosing in this patient sample). Because of the apparent biphasic dose-response curve for the effects of physostigmine (Davis and Mohs 1982; Mohs and Davis 1982), a rigorous test of any cholinergic enhancement strategy will require adequate dose-finding for each subject, to determine a best dose of physostigmine to be coadministered with high-dose TRH. Under these conditions, coadministration of TRH with physostigmine might yield more robust findings. Another limitation of the current study is the lack of test days in which patients received either placebo alone or TRH alone. Such a design would allow a more precise dissection of potential enhancing effects of TRH. Lacking this, however, the cognitive effects in patients receiving physostigmine plus TRH still suggest an enhancement, because the pattern of improvement (decrease in intrusion errors) differs from that seen with TRH alone in our previous work (Mellow et al 1989).

It has been suggested that the plasma epinephrine response to physostigmine is mediated via a central cholinergic mechanism (Kennedy et al 1984). This response, as well as the β-endorphin response has been shown to be blunted in AD (Raskind et al 1989); the increase in peak epinephrine after high-dose TRH coadministration might reflect a central marker of cholinergic enhancement, which could be correlated in future studies with results of behavioral and cognitive measurements. The lack of a test day with TRH alone makes these results somewhat complex to interpret. Although peripherally administered TRH does not

Table 1. Acute Effects of TRH–Physostigmine Infusions in Six Alzheimer's Disease (AD) Patients[a]

Variable	Physostigmine	Physostigmine + TRH	*p*
Verbal fluency (words)	−0.7 ± 0.4	0.9 ± 0.8	<0.04
Intrusions (words)	3.3 ± 1.5	−9.2 ± 3.5	<0.04
Recall (words)	1.9 ± 3.0	−2.8 ± 3.8	NS
Digit span (digits)	−0.4 ± 0.4	−0.3 ± 0.5	NS
Peak Δ systolic BP (mm Hg)	14.3 ± 3.0	26.8 ± 4.9	<0.04
Peak Δ diastolic BP (mm Hg)	15.8 ± 2.4	17.8 ± 2.6	NS
Peak Δ pulse rate (bpm)	10.7 ± 3.8	17.2 ± 6.3	NS
Peak Δ epinephrine (pg/ml)	23.9 ± 15.7	64.7 ± 25.6	<0.03
Peak Δ β-endorphin (pg/ml)	30.2 ± 16.5	23.5 ± 26.2	NS

[a]All variables are expressed as change scores (postdrug minus predrug) ± SEM, and compared by paired *t*-test between days when patients received physostigmine alone and when they received physostigmine plus TRH. See text for details of drug administration and testing. TRH, thyrotropin-releasing hormone; NS, not significant.

reliably produce plasma epinephrine increases (Morley et al 1981; Zaloga et al 1984), there is evidence that centrally administered TRH and its analogs increases epinephrine levels (Kabayama et al 1985; Ishikawa et al 1990). Once again, a rigorous test of the utility of epinephrine response as a marker of TRH enhancement awaits a larger study in which the hormonal effects of TRH alone are studied in this paradigm. The lack of a significant β-endorphin response (which also may be cholinergically mediated) may be because the physostigmine dosing in this pilot study was not optimized.

Further studies are under way to characterize more fully the dose requirements for this enhancement paradigm in AD. Future research might extend the time frame of treatment to long-term, combined drug administration, and the use of longer-acting analogs of both acetylcholinesterase inhibitors and TRH. The recent interest in the use of the oral cholinesterase inhibitor tacrine in AD (Farlow et al 1992; Davis et al 1992) renders this paradigm more timely in its clinical implications. Such work could lead to a novel treatment approach in the experimental pharmacology of AD.

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