

Endogenous Anticholinergic Substances May Exist During Acute Illness in Elderly Medical Patients

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Background. The purpose of this study was to determine if serum anticholinergic activity (SACA) arises from endogenous substances produced during illness.

Methods. Elderly medical inpatients ($N = 612$) were screened for anticholinergic medication use in the week prior to the study by interviews of subjects and proxies and review of emergency room, hospital, and nursing home medication administration records. Of 24 subjects without a recent anticholinergic medication history, 15 were recruited and 10 completed the study. Serum samples were obtained on Day 2 of hospital admission. SACA was measured using a radio-nuclide displacement assay. Medications taken by subjects were assayed for central muscarinic receptor binding at therapeutic concentrations.

Results. Eight of the ten subjects had SACA detectable in the serum. No medication used by these subjects had anticholinergic activity at usual therapeutic concentrations.

Conclusions. Endogenous anticholinergic substances may exist during acute illness. Characterization of such substances may increase the depth of our understanding of delirium and lead to useful intervention strategies.

THE neuropathophysiology of delirium in older persons is incompletely understood. One leading hypothesis is that delirium results from an impairment of central cholinergic transmission (1,2). The assay for serum anticholinergic activity (SACA), first introduced by Tune and Coyle (3) almost 2 decades ago, has given support to the cholinergic deficiency hypothesis. This assay measures the ability of a subject's serum to block central muscarinic receptors using a rat forebrain preparation. Higher levels of SACA have been associated with an increased likelihood of delirium in both surgical (4,5) and medical (6,7) inpatients. A dose-response relationship between symptoms of delirium and SACA has also been suggested (7).

The origin of SACA has generally been assumed to be with medications or their metabolites (3). Supporting this hypothesis is the recognition that many commonly prescribed medications, such as furosemide, digoxin, and methylprednisilone, have been shown to block cholinergic receptors using this assay (8). If SACA is of pathophysiologic importance in delirium and the origin of SACA is solely from medications, then potential clinical interventions for SACA reduction are limited to medication discontinuations, exchanges, and dose adjustments.

An alternative hypothesis is that, in addition to medications, elevated SACA may arise from endogenous substances. If this hypothesis is correct, then an understanding of the origins and nature of the endogenous source(s) of SACA could give new insights into potential treatments for delirium. Accordingly, we sought to measure SACA during acute illness in elderly medical inpatients carefully screened to ensure that they had received no anticholinergic medications.

METHODS

This study was conducted at the Beth Israel Deaconess Medical Center, a tertiary-care academic hospital in Boston, Massachusetts, and was approved by the Clinical Investigations Committee of that facility. Between April 1 and October 31, 1997, all persons aged 70 years or older and who were admitted to the medical service on a weekday were screened for inclusion in the present study. Subjects were excluded if there was any evidence, by history or medical record documentation, of any anticholinergic medication use in the week prior to admission. No subjects had participated in previous studies of SACA. Medication use was determined by review of outpatient records, emergency room and inpatient medication administration records, and interviews with subjects and family members that included questions regarding the use of over-the-counter medications and/or alternative medicinal products. Nursing home medication administration sheets were also reviewed for all nursing home residents. Subjects were also excluded if they were on dialysis, had received a blood transfusion in the week prior to admission, or were on an unknown study medication. To minimize the possibility of cumulative drug effects, subjects on six or more medications were excluded. Because this study was designed to assess whether SACA is detectable in persons with no anticholinergic drug exposure, rather than the relationship between SACA and delirium, formal data on the mental status of subjects were not collected.

For the purposes of this study, a medication was categorized as potentially anticholinergic if that medication had (i) well-documented in vivo anticholinergic effects, (ii) demonstrated in vitro anticholinergic activity, or (iii) belonged to a class of drugs with a member that has demonstrated in

vitro anticholinergic activity. If there was any question as to whether a medication might have anticholinergic activity, the medication was categorized as anticholinergic. Medications used by subjects that were categorized as anticholinergic in this study were as follows: codeine, compazine, digoxin, diphenhydramine, dexamethasone, doxepin, famotidine, fentanyl, fludrocortisone, furosemide, hydrocortisone, hydroxyzine, hyoscyamine, ipratropium, isosorbide, meclizine, meperidine, methylprednisolone, morphine, nifedipine, nortriptyline, oxybutynin, oxycodone, prednisone, procainamide, prochlorperazine, promethazine, quinidine, trazadone, trilafon, triamcinolone, and triamterene.

Informed consent was obtained from subjects and/or their proxies on the first day after admission. Six hundred and twelve individuals were screened. Of these potential subjects, 24 had no history of any anticholinergic medication during the hospital admission or for the week prior to admission. Of these 24 subjects, 9 subjects declined participation and 15 subjects were recruited. The analysis, however, excludes 5 subjects who consented but did not have a serum sample drawn due to hospital discharge (2 subjects), missed blood draw (2 subjects), and refusal (1 subject). Thus, complete data were available for 10 subjects.

Data Collection

On the second morning following admission, a blood sample was drawn in a 10-cc heparinized tube. The blood was centrifuged, and the serum was separated and stored at -80°C . Our previous experience has shown that SACA levels remain stable up to 1 year following collection when stored in this fashion. SACA was measured using the competitive binding radionuclide assay described by Tune and Coyle (2). In this assay, a radio-labeled reversible binder of the forebrain muscarinic receptor, 3H-quinuclidinyl benzilate (H3-QNB; New England Nuclear, Boston, MA), was added to a Sprague-Dawley rat forebrain membrane preparation. Separate samples of increasing dilutions of atropine were assayed, and displacement of the H3-QNB was measured by counting residual radioactivity after each preparation was washed with buffer. The assay was then repeated, substituting the subject's serum for atropine to determine the equivalents of atropine that would be as anticholinergic as the subject's serum. All controls and samples were run in duplicate. The rat forebrain preparation was custom prepared and stored in phosphate buffer at -80°C (Analytic Biological Services, Wilmington, DE). All medications that were used by subjects who were found to have detectable SACA levels were diluted to therapeutic serum concentrations (9) in the phosphate buffer system and tested for anticholinergic activity. The SACA testing on all subjects' serum was performed at the same time to reduce the effect of interassay variability. The intraassay variability was 6.7%. The SACA testing on all medications also was performed together, but on a different day than the assay of the subjects' serum.

RESULTS

SACA was present in 8 of the 10 subjects who had taken no anticholinergic medications. SACA in these 8 subjects ranged from 0.23–1.72-nmol/l atropine equivalents per 200-

μl sample (mean = 0.69 [$SD \pm 0.52$] nmol/l atropine equivalents/200- μl sample). Medications taken by these individuals are listed in Table 1. None of the medications that were used by these 8 subjects had demonstrable anticholinergic activity as measured by the anticholinergic activity assay.

DISCUSSION

Although the present study does not address the pathophysiological relationship between SACA and delirium, it does present evidence that SACA is detectable in ill elderly persons who are receiving no anticholinergic medications. This finding challenges the hypothesis that medications are the sole source of SACA and adds to several lines of evidence that other sources of SACA exist. One study of six hospitalized older persons showed a decline in SACA with a resolution of delirium that was seemingly unrelated to medication changes (6). Another study has demonstrated that SACA declined significantly following resolution of febrile illness in elderly nursing home residents, and this decline also was seemingly unrelated to medication changes (10). Interestingly, despite the accepted belief and common observation that anticholinergic medications may contribute to delirium, some large epidemiological studies of delirious patients found no association between anticholinergic medication use and delirium in either elderly medical (11,12) or postoperative surgical patients (13). Although this finding may be due in part to improper classification of medications, the presence of endogenous anticholinergic substances in those patients classified as not being exposed to anticholinergic substances might also obscure this relationship.

Some naturally occurring substances have been shown to have antimuscarinic activity in vitro. For example, dynorphin A and myelin basic protein have been shown to inhibit binding at muscarinic receptors by altering the receptor conformation (14). Protamine may also inhibit binding at muscarinic receptors through a similar mechanism (15). Furthermore, a naturally occurring inhibitor of antagonist binding to cholinergic receptors has been described in human brains (16). It seems unlikely that such a protective system would have evolved in the absence of any anticholinergic substance to inhibit. Thus, our conclusion that clinically important endogenous anticholinergic substances exist seems reasonable.

Table 1. Medication Use in Subjects With Serum Anticholinergic Activity (SACA)

Age (years)	Gender	Diagnosis	Medications	SACA [†]
87	F	Pneumonia	Cefuroxime, insulin, glyburide	1.72
75	F	Pneumonia	Erythromycin, cefuroxime	1.20
94	F	Pneumonia	Cefuroxime	0.73
78	F	Urosepsis	Ampicillin, aspirin, gentamicin, metoprolol	0.60
81	F	<i>C. Difficile</i>	Lorazepam, potassium, vancomycin	0.41
77	F	Delirium	Ampicillin, ducosate, lorazepam, L-thyroxine, valproate	0.38
94	F	Dehydration	None	0.24
85	F	<i>C. Difficile</i>	Aspirin, metronidazole, multivitamin	0.23

Note: SACA = serum anticholinergic activity; F = female.

[†]Units of SACA are nmol/l atropine equivalents per 200- μl sample.

This study has several limitations. First, the serum anticholinergic activity assay itself is not standardized. Previous studies have not reported inter- and intraassay variability, but in our laboratory, this has been up to 20% and 10%, respectively, despite our efforts at standardization. In addition, we cannot completely exclude the possibility that a metabolite of the medication(s) used by these subjects was the source of anticholinergic activity. However, this is very unlikely given the fact that the medications used by these subjects were demonstrated not to have any anticholinergic activity. Furthermore, one subject who received no medications at all still had detectable SACA. It is also important to note that although there was no gender exclusion for this study, all the subjects were women. Finally, while in-hospital medication use was obtained from medical records, self and/or proxy-reported medication use was used in many cases to identify the medications that were taken prior to admission. However, as blood samples were taken on Day 2 of admission to the hospital, a minimum of 36 hours had passed since the most recent possible ingestion of any potentially anticholinergic medication.

It is also important to point out that "normal" levels of SACA have not been defined, but it is generally assumed that under conditions of health and no medication use, humans should have no detectable SACA. Systematic assessments of SACA in otherwise healthy individuals not receiving medications have not been performed. Although the presence of circulating endogenous anticholinergic substances in healthy young or older individuals would be a significant finding on its own, no data exist to support such a hypothesis. One study that measured SACA during relative good health 1 month following a febrile illness in older persons found a mean SACA of 0.09- nmol/l atropine equivalents per 200- μ l sample (substantially lower than the SACA levels found in the present study), and no SACA in 14 of 22 subjects (63%), although this study did not control for medication use (9).

Although the SACA assay was initially developed to evaluate the anticholinergic effects of medications, its application to the syndrome of delirium has led to important insights regarding that syndrome's pathophysiology. Although medications are undoubtedly a source of anticholinergic activity in the serum in some cases, the present study suggests that clinically important endogenous anticholinergic substances may also exist during acute illness. Further characterization of such substances may increase the depth of our understanding of delirium and lead to new treatment options for those suffering from this common, morbid, and costly complication of illness.

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REFERENCES

1. Trzepacz P. The neuropathogenesis of delirium. *Psychosomatics*. 1994;4:374-391.
2. Flacker JM, Lipsitz LA. Neural mechanisms of delirium: current hypotheses and evolving concepts. *J Gerontol Biol Sci*. 1999;54A:B239-B246.
3. Tune L, Coyle JT. Serum levels of anticholinergic drugs in treatment of acute extrapyramidal side-effects. *Arch Gen Psychiatry*. 1980;37:293-297.
4. Tune L, Holland A, Folstein MF, Damlouji NF, Gardner TJ, Coyle JT. Association of postoperative delirium with raised serum levels of anticholinergic drugs. *Lancet*. 1981;2:651-652.
5. Golinger RC, Peet T, Tune LE. Association of elevated plasma anticholinergic activity with delirium in surgical patients. *Am J Psychiatry*. 1987;144:1218-1220.
6. Mach JR, Dysken MW, Kuskowski M, Richelson E, Holden L, Jilk KM. Serum anticholinergic activity in hospitalized older persons with delirium: a preliminary study. *J Am Geriatr Soc*. 1995;43:491-495.
7. Flacker JM, Cummings V, Mach J, Bettin K, Kiely DK, Wei J. Serum anticholinergic activity is associated with delirium in ill elderly persons. *Am J Geriatr Psychiatry*. 1998;6:31-41.
8. Tune L, Carr S, Hoag E, Cooper T. Anticholinergic effects of drugs commonly prescribed for the elderly: potential means for assessing risk of delirium. *Am J Psychiatry*. 1992;149:1393-1394.
9. McEvoy GK, ed. *American Hospital Formulary Service 1997 Drug Information*. Bethesda, MD: American Society of Health-System Pharmacists; 1997.
10. Flacker JM, Lipsitz LA. Serum anticholinergic activity changes with acute illness in elderly medical patients. *J Gerontol Med Sci*. 1999;54A:M12-M16.
11. Schor JD, Levkoff SE, Lipsitz LA, et al. Risk factors for delirium in hospitalized elderly. *JAMA*. 1992;267:827-831.
12. Francis J, Martin D, Kapoor WN. A prospective study of delirium in hospitalized elderly. *JAMA*. 1990;263:1097-1101.
13. Marcantonio ER, Juarez G, Goldman L, et al. The relationship of postoperative delirium with psychoactive medications. *JAMA*. 1994;272:1518-1522.
14. Hu J, El-Fakahany EE. Allosteric interaction of dynorphin and myelin basic protein with muscarinic receptors. *Pharmacology*. 1993;47:351-359.
15. Hu J, Wang S, Foray C, El-Fakahany EE. Complex allosteric modulation of cardiac muscarinic receptors by protamine: potential model for putative endogenous ligands. *Mol Pharmacol*. 1992;42:311-324.
16. Frey WH, Emory CR, Wiebenga ME, et al. Inhibitor of binding to muscarinic receptor is elevated in Alzheimer's brain. *Brain Res*. 1994;655:153-160.

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