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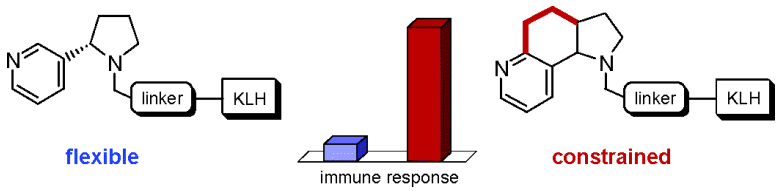

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A New Strategy for Improved Nicotine Vaccines Using Conformationally Constrained Haptens

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Cigarette smoking and the use of other tobacco products are the leading cause of avoidable cancer death, as well as a variety of other diseases, in many developed nations worldwide. Numerous studies indicate that smoking and chewing tobacco probably account for more than 500 000 deaths each year in the United States alone, including approximately 30% of all cancer-related mortality.[1] A great deal of evidence supports the view that people continue to smoke and use tobacco because of the addictive effects of nicotine.[2] Interventions aimed at treating tobacco dependence, particularly smoking, include behavioral counseling, group support programs, and pharmacotherapies, such as the popular "nicotine replacement" products.[3] However, none of these strategies have proven effective in significantly reducing tobacco use and increasing abstinence.

Immunopharmacotherapy provides a novel way for potentially treating various drug addictions.[4] Previous work from our laboratory demonstrated the efficacy of cocaine hapten immunization protocols for blocking the psychoactive and reinforcing effects of cocaine in animal studies.[5] Other groups have recently published positive results for the attenuation of the effects of nicotine in animals using nicotine vaccines.[6] We described the synthesis of a nicotine hapten and preliminary antibody binding data.[7] Our continuing investigations indicate that the immune response in mice and rats generated by our nicotine vaccine has been much less robust than that generated by the cocaine vaccine. Moreover, the data from the published nicotine vaccines suggest a similar phenomenon. The differences in the immunogenicity observed between the cocaine and nicotine haptens is puzzling and poses questions for the future clinical development of a therapeutically useful nicotine vaccine.

Cocaine and nicotine are anticipated to be similar in terms of immune recognition. Both are small molecules of similar molecular weight and display as primary haptenic determinants an aromatic moiety and a tertiary *N*-methylamine that will be protonated at physiological pH (Figure 1).[8,9] The key difference between the two structures is the constrained tropane framework of the cocaine molecule. Notably, the ability to "freeze out" the conformational dynamics of a ligand can improve the affinity and specificity for a receptor primarily as a result of reducing the entropic loss upon binding. This concept has been perhaps most widely exploited with regard to the conformational constraint of peptides for various applications, including vaccines.[10,11] However, constraining non-peptide drug molecules to improve immunogenicity has not been reported. We present the foundation for our investigations into the relationship between the conformational constraint of nicotine and its immunogenicity, and therefore the development of new nicotine vaccines.

To date, we, as well as others, have focused solely on functionalizing nicotine itself in designing haptens without taking into consideration the favored solution conformations of the molecule (Figure 2).[6,7]

Figure 1. Comparison of nicotine and cocaine structures. The *N*-methylamine moiety is protonated under physiological conditions; pK_a (cocaine) ≈ 8.6, pK_a (nicotine) ≈ 8.0.

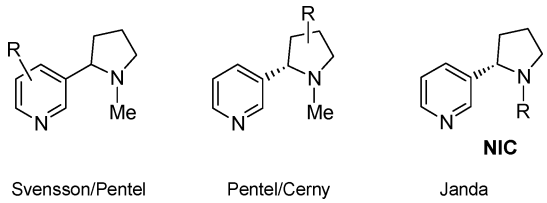

Figure 2. Haptens previously used in nicotine vaccines.

Recently, Elmore et al. carried out computational studies on nicotine conformations in various media.[12] For the monoprotonated species in aqueous solution, there are four major conformations present, of which the two approximately isoenergetic trans rotamers (Scheme 1) are preferred over their cis counterparts by >10:1.

The immune response will essentially sample all possible isomers of a hapten in the bloodstream and can deviate from a "normal distribution" of antibody affinities.[13] Intrinsically, a small, flexible nicotine hapten is expected to afford a very heterogeneous antibody population of primarily moderate affinity directed against both trans isomers, and even the minor cis isomers. As compared to cocaine, these factors contribute to a reduced antibody "titer", a measure of the average antibody affinity and concentration, and the critical parameter of an immune response.

To study and alleviate this problem, the haptens **CNA** and **CNI** were designed and synthesized on the basis of reported conformationally constrained nornicotine analogues **1** and **2** (Scheme 2).[14] The *N*-Me nicotine-like derivative of **1** had analgesic effects, but the receptor remains unidentified.[14a,15] Recently, other constrained analogues were developed[16] and shown to bind with low nanomolar affinity to the nicotinic acetylcholine receptor.[16a] We settled upon **1** and **2**, because they are readily prepared and provide a reasonable mimic of the two trans conformers of nicotine that is supported by crystal-structure data.[14a] The aim was for **CNA** and **CNI** to elicit high antibody titers that could bind (*S*)-nicotine in solution.

The constrained haptens utilized a linker for coupling to the carrier protein keyhole limpet hemocyanin (KLH) that was identical to that of our first-generation nicotine hapten (**NIC**)[7] which would allow a direct comparison. After standard immunization protocols used in our laboratory, the **NIC**-KLH immunoconjugate

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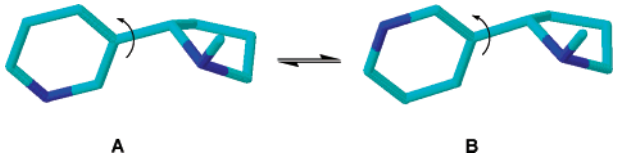
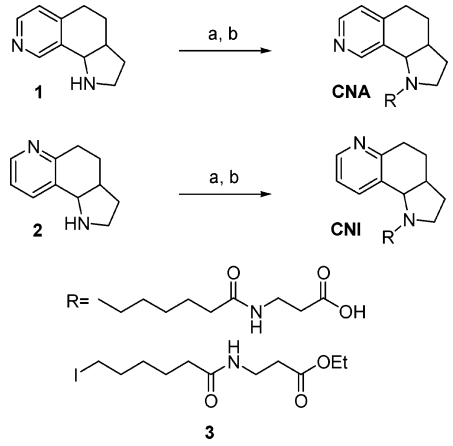
Scheme 1. The Major Conformational Isomers of Nicotine in Aqueous Solution



Scheme 2. Synthesis of Constrained Nicotine Haptens[a]



[a] (a) **3**, CH_3CN, DIEA; (b) NaOH, MeOH.

provided low titers having a mean value of ~3200. Competition ELISA and equilibrium dialysis measurements yielded the serum affinity for (*S*)-nicotine as a K_d-avg $\approx 1.7 \pm 0.20$ μM. Other groups reported titers for nicotine-based haptens of 10 000 or less and affinities in the micromolar range.[6] On the other hand, immunizations using our second-generation immunoconjugates **CNA**-KLH and **CNI**-KLH resulted in antisera with greatly increased titers of ~25 000, comparable to those we observed in our cocaine vaccination studies. Significantly, the K_d-avg $\approx 1.0 \pm 0.10$ μM and 0.60 ± 0.10 μM values, respectively, were nearly 2- and 3-fold improved. Also, the antisera showed >10:1 specificity for (*S*)-nicotine versus the major metabolite (*S*)-cotinine, similar to **NIC**-KLH antiserum. Interestingly, **CNA**-KLH and **CNI**-KLH antisera had low cross-reactivity in binding **CNI**-bovine serum albumin (BSA) (1:15) and **CNA**-BSA (1:6) conjugates used for titering, respectively, suggesting an important role for the pyridyl nitrogen in the antibody−hapten interactions and that recognition of the distinct trans species of nicotine is possible.

Constraining nicotine conformations yields an enhanced immune response as compared to a flexible nicotine hapten. The improved effect is likely caused by a narrowing in the range of antibody heterogeneity skewed toward a population of increased affinity. We believe the titers are in the range of the necessary minimum in mice/rats to model the development of a clinically useful nicotine vaccine. In addition, this example of augmenting immunogenicity upon hapten constraint could provide a general route toward vaccination strategies for other pharmacologically important small molecules. Subsequent work will include further improving nicotine binding by using enantiomerically pure constrained haptens and the study of new nicotine vaccines in rodent models.

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Supporting Information Available: Experimental preparation of **3**, **CNA** and **CNI**, and data of the binding studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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