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DECREASED LEVELS OF MUSCARINIC RECEPTORS IN BLADDERS FROM THE ALCOHOL PREFERRING RAT LINE

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Abstract

The $B_{\max}$ for [³H]QNB binding in the bladders of alcohol preferring (AA) rats was only approximately 60% of that in the alcohol non-preferring (ANA) rats. No significant change in $B_{\max}$ for [³H]QNB binding in bladder was observed between alcohol insensitive (AT) and alcohol sensitive (ANT) rats. No significant change in K_d for [³H]QNB binding in bladder was observed between the four different rat lines studied. Therefore, alcohol preference but not sensitivity is associated with a decrease in muscarinic receptor density in the rat bladder. Because all of the rats used in this study were ethanol-naïve, the decrease in muscarinic receptor density in the bladders of alcohol preferring rats is associated with genetic factors inherent to this rat line. Further studies are needed to determine if these observations are tissue specific or specific to the m2 subtype, which predominates in the rat bladder.

Introduction

Through selective outbreeding, avoiding sib-mating, rat lines for high and low voluntary alcohol consumption and differential alcohol sensitivity have been developed (1,2). The alcohol preferring (AA) line of rats prefer 10% alcohol to water. The alcohol non-preferring (ANA) line of rats choose water to the virtual exclusion of alcohol. The alcohol insensitive (AT) rat line is characterized by decreased motor impairment on a tilting plane than the alcohol sensitive (ANT) line at the same blood level of alcohol. Differences in neurotransmitters and their respective receptor proteins in these animals may be associated with alcohol preference or sensitivity. Previous studies have focused on differences in various regions of the brain (3,4,5). In this study we have focused on the urinary bladder as a model end organ since its innervation is fairly well characterized. Differences between bladders from different rat lines could reveal contributing mechanisms to alcohol preference or sensitivity.

In this study, the levels of muscarinic receptors in the bladders of the four different rat lines were examined. The rat lines were grouped AA (Alko Alcohol) or ANA (Alko Non-Alcohol) and AT (Alcohol Tolerant) or ANT (Alcohol Non-Tolerant). Previous studies in this area have demonstrated that the rat bladder consists primarily of the m2 subtype (P. Wang, S.E. Hicks, G.R. Luthin, M.R. Ruggieri, *J Pharmacol Exp Ther*, submitted for publication). Therefore, any changes in muscarinic receptor density may be associated

primarily with a change in the m2 subtype. By focusing on the m2 subtype, comparisons may be made to other tissues such as the heart, where this subtype is predominant.

Materials

[³H]QNB (*l*-[benzyl-4,4'-³H]-quinuclidinyl benzilate, 45.4 Ci/mmol) was obtained from DuPont-New England Nuclear Research Products (Wilmington, DE). Atropine sulfate was obtained from Sigma Chemical Company (St. Louis, MO). Scintiverse II scintillation cocktail was obtained from Fisher Scientific (Fair Lawn, NJ). All other reagents were of the highest commercial grade available. No. 30 glass fiber filters were obtained from Schleicher & Schuell (Keene, NH). AT, ANT, AA & ANA rat bladders were a generous gift from *Alko Ltd.* (Helsinki, Finland). All rats were ethanol-naive.

Membrane Preparation

Rats were killed by decapitation and the bladders were rapidly removed and frozen at -80°C until preparation of membranes. The bladders were weighed, minced and homogenized for 15 seconds using a Brinkman Polytron (model 10/35) on setting 10 at 4°C in a buffer containing 10 mM Tris and 1 mM EDTA at pH 7.5. The resulting homogenate was centrifuged at 20,000 × G for 20 minutes. The resulting pellet was resuspended in a volume of 50 mg tissue/ml buffer. Protein was determined by the method of Lowry et al. (6).

Binding Assay

[³H]QNB (10, 25, 50, 100, 250, 500 pM) and 50 µg of protein were incubated in triplicate in a final volume of 1 ml of buffer containing 10 mM Tris and 1 mM EDTA. Atropine (1 µM) was used to define non-specific binding at each radioligand concentration. After a 2–3 hour incubation period at 32°C, samples were quenched with 5 ml of an ice-cold buffer containing 10 mM Tris, 1 mM EDTA and 150 mM NaCl. Samples were then filtered over no. 30 glass fiber filters and washed with 5 ml of buffer. Filters were added to 4 ml of Scintiverse II scintillation cocktail and counted at 55% efficiency using a Beckman LS5000TA scintillation counter.

Analysis of Binding Data

Saturation isotherms were plotted according to the method of Scatchard (7). All fitted curves for determination of B_{max} (maximal number of binding sites) and K_d (equilibrium dissociation rate constant) had an r² > 0.95. All values are expressed as the mean of eight determinations ± standard error of the mean. Differences between groups were determined using analysis of variance followed by a Scheffe test.

Results

The muscarinic receptor density of the bladders from the AA rats were significantly lower than those of the ANA rats (Figure 1). The B_{max} for [³H]QNB binding in the bladders of AA rats was only 60% of that in the ANA rats. No significant change in the K_d for [³H]QNB binding in the bladder was observed between the two groups. No significant change in muscarinic receptor density or affinity in the bladder was observed between the AT and ANT rats. The average K_d for [³H]QNB binding to the bladders from the AA, ANA, AT & ANT rats was determined to be 49±15 pM.

Preliminary work in bladders from Sprague-Dawley rats revealed that ethanol had no effect on muscarinic agonist or antagonist binding at concentrations up to 1.0 M (data not shown). This ethanol concentration is greater than fifty-times the blood-alcohol concentration which legally defines alcohol intoxication. Furthermore, this concentration of ethanol exceeds the lethal blood-alcohol concentration in humans and rats by more than five-times.

Discussion

The findings reported here indicate that genetic selection for alcohol preference but not tolerance is related to reduced muscarinic receptor density in the rat bladder. The AA rats had a significant decrease in muscarinic receptor density in the bladder when compared to the ANA rats. The AT and ANT rats, however, displayed no significant differences in muscarinic receptor density in the bladder. Neither ethanol preference or sensitivity altered the affinity of muscarinic receptors for [³H]QNB, because no significant change in K_d was observed between groups. The decrease in muscarinic receptor density observed in the AA rats can be correlated to a decrease in the receptor density of the m2 subtype, since the rat bladder consists primarily of this subtype. Further studies need to be done with the AA rats to determine if this change occurs in other tissue where the m2 subtype is present. The heart would be an ideal tissue to study since it too consists primarily of the m2 subtype (8). It would be interesting to determine whether this phenomenon is tissue specific or perhaps selective for a specific muscarinic subtype.

Because the rats used in this study were ethanol-naïve, differences observed are not due to the presence of ethanol. Preliminary studies in this lab confirmed that the presence of ethanol has no direct effect on muscarinic agonist or antagonist binding in the rat bladder. Therefore, the alterations in muscarinic receptor density in the bladders of AA rats are most likely due to genetic factors associated with alcohol preference. It should be noted, however, that differences observed in this study could be coincidental, an artifact of the genetic selection procedure. Ethanol has been shown to alter the properties of the GABA/benzodiazepine complex (9,10). The decrease in muscarinic receptor density in the bladders of the AA rats may be related to the primary effects of ethanol at the GABA/benzodiazepine receptor complex. Further work needs to be done in this area to determine if these changes are related.

To summarize, genetic selection of rats for alcohol preference and not tolerance produced a decrease in muscarinic receptor density in the rat bladder. This decrease in receptor density can be associated with a decrease in the m2 subtype, since the rat bladder consists primarily of the m2 subtype. The decrease in muscarinic receptor density in the bladders of alcohol preferring rats is not due to the effect of ethanol on muscarinic ligand binding but instead is associated with genetic factors inherent to this rat line.

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MUSCARINIC RECEPTOR DENSITY IN AA, ANA, AT & ANT RAT BLADDERS **Figure 1.**

Average B_{max} (n= 8) for [³H]QNB binding to rat bladders from AA, ANA, AT & ANT rates
B_{max} in fmol/mg protein: AA- 141±11, ANA- 238±11, AT- 249±17, ANT- 287±23.
(*p<0.01).