Manuscript discussion section

Manuscript: Discussion Section A broad range of endogenous and exogenous molecules interact with neuronal nicotinic acetylcholine receptors (nAChRs)through different binding sites. The agonist binding sites are well characterized and are potential loci of drug design. These nAChRs also contain additional binding sites where allosteric non-competitive antagonists (NCA) located primarily within the membrane receptors. The noncompetitive antagonism of a number of clinically useful drugs has been attributed to binding within the central lumen of the nAChRs. NCA activity has been routinely determined by measurement of concentration-dependent effects on whole-cell currents or nicotine-induced 86RB+ influx, yielding IC50 values. In this study affinity chromatographic technique has been used to derive binding affinities. With the application of affinity chromatography to direct determination of functional activities, there is problem with determination of functional activities, indicating the fact that affinity in binding may not be directly correlated with the pharmacological action of the drug on the pharmacological target. In case of competitive agonists and antagonists, these properties are related using Cheng-Prusoff relationship,

Ki = IC50/1 + (S/Km),

where Ki is the binding affinity of the inhibitor, IC50 is the functional strength of the inhibitor, S is substrate concentration, and Km is the affinity of the substrate for the enzyme. The Cheng-Prusoff relationship cannot be used with NCAs due to occurrence of allosteric interactions. The relative antagonistic activities of a series of noncompetitive antagonists, namely, imipramine, ethidiuum, phencyclidine, dextromethorphan, and mecamylamine expressed as IC50 values towards nAChRs can be measured using data from affinity chromatography on an immobilized nAChR stationary

phase using nondirect method of multivariate analysis for assessment of IC50 values. Using this technique and non-linear modeling of chromatographic process, the interaction between the NCAs and the immobilized 34 nAChR subtype can be characterized. This also has determined the thermodynamic retention factors, k, association and dissociation rate constants ka and kd respectively. Applied to Ibogaione and Ibogaine analogues, 18-methoxycoronaridine, 18-methylaminocoronaridine, 2-methoxyethyl 18-MC, Albifloranin, and 19 -OH Ibogaine, this relationship translates into

ki = IC50 / {1 + ([Ibogaine] / KdIbogaine)},

where [Ibogaine] is the initial concentration of [3H]Ibogaine and KdIbogaine is the dissociation constant for [3H]Ibogaine, determined by Scatchard-type analyses (Scatchard, 1949).

In order to characterize the ibogaine binding sites within the human a3b4 AChR, the same approach was used to determine the binding affinity of several noncompetitive antagonists including imipramine, ethidiuum, phencyclidine, dextromethorphan, and mecamylamine.

From the data curve analysis, the Hill coefficients (nH) were calculated, and the values are represented in the tables below. The ki values were calculated in each of the groups from the above equation.

NCA

ki

nH

Imipramine

2. 40. 2

0. 650. 04

Ethidiuum

585263

0. 610. 14

Dextromethrophan

122

0. 720. 06

Mecamylamine

476

0. 710. 07

Phencyclidine

172

0. 820. 05

Table 1: Showing ki and nH values of NCAs studied

Similar values for ibogaine and ibogaine analogues were tabulated below in table 2.

Ibogaine and Its Analogues

ki

nH

18-methoxycoronaridine (MC)

Desensitized (D)

630 340

0. 410. 08

18-methoxycoronaridine

Resting (R)

18578

0. 670. 18

18-methylaminocoronaridine

2. 50. 3

0. 650. 06

2-methoxyethyl 18-MC

13431

0. 790. 14

Albifloranin

19122

0. 790. 09

19 -OH Ibogaine

315

0. 660. 07

Ibogaine Resting (R)

1. 00. 1

0. 820. 08

Ibogaine (D) Desensitized

0. 40. 1

1. 010. 11

Table 2: Showing ki and nH values of ibogaine and ibogaine analogues

From these data, it is evident that ibogaine binds to the receptor with a specific binding. There is a specific single binding site of the ibogaine in the resting state (1. 00. 1) that has a very high affinity (0. 820. 08). In the desensitized state however, these receptors do not demonstrate identical affinity, and the binding of ibogaine happens with a 2. 5 times less specificity. The other ibogaine derivatives demonstrate affinity to these

receptors 18-methylaminocoronaridine> 19 -OH Ibogaine> 2-methoxyethyl 18-MC> 18-methoxycoronaridine (R)> Albifloranin> 18-methoxycoronaridine (MC) (D) in this order. When competitive antagonism is considered, impiramine shows highest affinity to these receptors with almost identical binding and affinity with 18-methylaminocoronaridine. Drugs Dextromethrophan and Phencyclidine will have higher binding affinity than 19 -OH Ibogaine, but far less than 18-methylaminocoronaridine and ibogaine resting or desensitized, allowing them predominance when competition is concerned. Mecamylamine will have lower affinity and binding than 19 -OH Ibogaine. However, all these competitive antagonists would take precedence over 19 -OH Ibogaine> 2-methoxyethyl 18-MC> 18-methoxycoronaridine R > Albifloranin. Ethidiuum would demonstrate low affinity to the binding sites but comparatively and competitively its affinity would be more than 18-methoxycoronaridine (MC) (D). Therefore, this data indicates that even in the presence of other drugs with addictive potential, even in patients who are treated with depression by imipramine, ibogaine would competitively bind to the single binding site in the nAChRs.