Binding of optically pure (−)-[^{3}H]nicotine to rat brain membranes

Leo G. Abood, Susan Grassi and Maria Costanza

Center for Brain Research and Department of Biochemistry, University of Rochester Medical Center, Rochester, NY 14641, USA

Received 27 April 1983

With the recent availability of (−)-[^{3}H]nicotine of high specific activity, binding studies were performed on rat brain membranes in the presence of a variety of nicotine analogues and cholinergic drugs. Both a higher affinity (K_d = 2 × 10^{-10} M) and a lower affinity (2 × 10^{-9} M) site were observed; the stereoselectivity of both sites being similar. A good correlation was observed between IC_{50}-values and psychotropic potency of a series of N'-alkyl substituted nicotine analogues.

Nicotine receptor *Rat brain* *Nicotine analogue* *Cholinergic drug* *Nicotine psychopharmacology*

1. INTRODUCTION

Recently, a number of studies have appeared on the binding of ^{3}H-labeled nicotine to rat brain membranes [1–5], but with one exception [3] the studies were not performed with the naturally occurring, optically pure (−)-nicotine. With (−)-[^{3}H]nicotine having a specific activity of 5 Ci/mmol, authors in [3] obtained (with rat brain membranes) a K_d of 5 × 10^{-6} M and a binding density of 0.01 × 10^{-14} mol/mg protein. With (±)-[^{3}H]nicotine of higher specific activity, the K_d-values ranged from 6–100 × 10^{-9} M, while stereoselectivity of the (−)-nicotine over (+)-nicotine ranged from 0–20-times [1–5]. With the recent availability of (−)-[^{3}H]nicotine of very high specific activity (87 Ci/mmol), we sought to re-examine its binding characteristics to rat brain membranes, particularly with regard to its stereoselectivity.

2. MATERIALS AND METHODS

The procedures for preparation of rat brain membranes and for measuring specific [^{3}H]nicotine binding are described in [1]. Binding was determined by a centrifuge assay. To a 2.0 ml polypropylene tube was added 2 mg membrane protein along with various concentrations of (−)-[^{3}H]nicotine (spec. act. 87 Ci/mmol; New England Nuclear) with or without various concentrations of (+)- or (−)-nicotine, nicotine analogues, and cholinergic drugs in a final volume of 1.2 ml 0.05 M Tris, pH 7.5. After incubating in an ice bath (0–4°C) for 15 min, the tubes were centrifuged in an Eppendorf centrifuge for 2 min and the pellet washed twice by filling the tubes with Tris (pH 7.5) and aspirating. The bottom of the tubes was then cut off (animal nail clipper) and counted by liquid scintillation.

The optical purity of the (−)-[^{3}H]nicotine was determined by the use of *P. putida* (gift of A.M. Tometsko) as in [6]. Preparation of N'-alkyl-substituted nicotine derivatives was done by the method in [7] as modified in [8]. Psychotropic potency was measured by administering the agent to rats via cannulae implanted into the lateral ventricles and determining the minimal dose required to induce prostration involving all 4 limbs [1,5].

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

3. RESULTS

A Scatchard plot of specific (−)-[^{3}H]nicotine binding was curvilinear and was resolvable into two slopes; yielding a higher affinity K_d-value of 1.9×10^{-10} M and a lower one of 1.7×10^{-9} M (fig.1). B_m-values were 0.51×10^{-14} and 2.9×10^{-14} mol/mg membrane protein, respectively.

A variety of nicotine analogues and cholinergic agents were tested for their ability to compete with (−)-[^{3}H]nicotine for binding to rat brain membranes (table 1). The data are presented for binding (IC_{50}) at both 2×10^{-10} and 2×10^{-9} M [^{3}H]nicotine to distinguish binding at the higher and lower affinity sites, respectively. Data on the psychotropic potency of the agents are also presented; the value designating the relative potency to (−)-nicotine to induce prostration in rats. The IC_{50}-value for (+)-nicotine was 3-times greater than that for (−)-nicotine at the higher affinity site and 2-times greater at the lower affinity

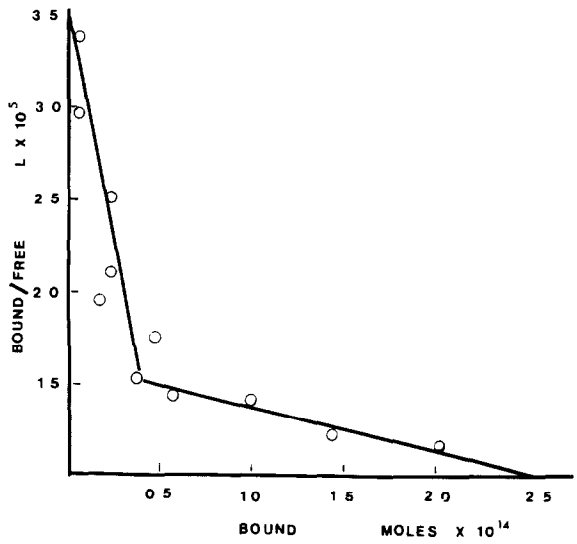

Fig.1. Scatchard plot of specific (−)-[^{3}H]nicotine binding.

Table 1

IC_{50}-values against [^{3}H]nicotine binding and relative psychopharmacologic potency of various nicotine analogues and cholinergic agents

Agent	High affinity IC_{50} (M)	Lower affinity IC_{50} (M)	Prostration relative potency
(−)-Nicotine	1×10^{-10}	3×10^{-10}	1
(+)-Nicotine	3×10^{-10}	1×10^{-9}	1/20
(±)-Nornicotine	2×10^{-10}	1×10^{-9}	1/20
(±)-*N'*-Ethylnornicotine	4×10^{-9}	1×10^{-8}	1/10
(−)-Anabasine	8×10^{-8}	5×10^{-7}	1/20
(±)-*N'*-isopropyl nornicotine	8×10^{-8}	6×10^{-7}	1/40
(±)-*N'*-*n*-butyl nornicotine	2×10^{-7}	1×10^{-6}	IA
(±)-6-β-hydroxyethyl nicotine	5×10^{-9}	3×10^{-8}	1/30
N-benzyl nornicotine	1×10^{-8}	2×10^{-7}	IA
Cotinine	M	$>1 \times 10^{-4}$	IA
Acetylcholine	1×10^{-6}	5×10^{-6}	IA
Carbamylcholine	2×10^{-8}	8×10^{-7}	IA
Hexamethonium	1×10^{-4}	1×10^{-4}	IA
α-Bungarotoxin	$>5 \times 10^{-5}$	$>5 \times 10^{-5}$	IA
Mecamylamine	5×10^{-5}	1×10^{-4}	IA
1-3-Quinuclidinyl benzilate	8×10^{-5}	1×10^{-4}	IA
Oxotremorine	5×10^{-5}	1×10^{-4}	IA

IC_{50}-values for higher affinity site were obtained at 2×10^{-10} M [^{3}H]nicotine and at 2×10^{-9} M at lower affinity site, and are average of 3 separate experiments agreeing within 7%. Prostration was determined as described in [1,5] and is expressed as relative potency to l-nicotine which was effective at 30 nmol (in 5 μl). IA = inactive at 50-times the dose of l-nicotine

site; whereas the psychotropic potency of (−)-nicotine was 20-times that of the (+)-isomer. A 20-fold and 40-fold increase in the IC_{50}-values occurred with replacement of methyl by ethyl or isopropyl, respectively; and the psychotropic potency was 1/10 and 1/40 that of (−)-nicotine, respectively. Replacement of methyl by *n*-butyl increased the IC_{50} still further and abolished psychotropic activity. Substitution of β-hydroxylethyl in the 6 position of nicotine increased the IC_{50} 50-fold, while decreasing psychotropic activity 30-fold. Although the binding affinity of nornicotine was 1/2 that of (−)-nicotine, it was only 1/20 as effective as (−)-nicotine in producing prostration. Hexamethonium and α-bungarotoxin, which are both nicotinic cholinergic antagonists, had IC_{50}-values around 1×10^{-4} M for both the higher and lower affinity sites. Acetylcholine, as well as the remaining cholinergic agonists and antagonists, also exhibited low binding affinity and did not produce prostration even at 50-times the effective dose of (−)-nicotine.

4. DISCUSSION

The present studies were undertaken to determine whether the binding characteristics of (−)-[^{3}H]nicotine to rat brain membranes were significantly different from those obtained with (±)-[^{3}H]nicotine. With (±)-[^{3}H]nicotine, the affinity of (−)-isomer was 3-times greater than that of (+)-nicotine; however, no greater stereoselectivity was found in the present study using (−)-[^{3}H]nicotine. The major new finding was the demonstration of a higher affinity site with a K_m of 1.9×10^{-10} M and a B_m of 0.65×10^{-14} mol/mg membrane protein; while the lower affinity site had a K_m of 1.9×10^{-9} and a B_m of 3.0×10^{-14} mol/mg, which agreed with previous data with the racemic [^{3}H]nicotine [1,5]. Included in this study were some nicotine analogues with modifications in the pyrrolidine *N*. As the alkyl chain length increased from methyl to butyl, there was a dramatic decrease in the binding affinity with a corresponding decrease in psychotropic potency. Replacement of methyl by benzyl decreased binding affinity by over two orders of magnitude; and, although the analogue did not produce prostration, it acted as an antagonist to the nicotine-induced prostration [1,5]. As reported in [1,2,4], mecamylamine, hexamethonium, α-bungarotoxin, and other nicotinic cholinergic antagonists exhibited very low binding affinity for the nicotine site and did not produce prostration, with the exception of carbomylcholine and acetylcholine, which had IC_{50}-values in the μM range. The remainder of cholinergic agents showed very little binding, while none produced prostration at 50-times the dose of nicotine.

Our main conclusions are that the affinity of the (−)-isomer of nicotine to rat brain membranes is no more than 3-times greater than that of the (+)-isomer; the relative binding of the two enantiomers being similar whether (−)-[^{3}H]nicotine or (±)-[^{3}H]nicotine was used as the ligand. An additional finding was the existence of a higher affinity site; however, the stereoselectivity of this site was no different from that of the lower affinity site. Finally, with a series of *N'*-substituted nicotine derivatives, a good correlation was observed between binding affinity and potency in producing prostration in rats following administration of (−)-nicotine intraventricularly.

ACKNOWLEDGEMENT

This research was supported by grant DA 00464.

REFERENCES

[1] Abood, L.G., Reynolds, D.T. and Bidlack, J.M. (1980) Life Sci. 27, 1307–1314.
[2] Romano, C. and Goldstein, A. (1980) Science 210, 647–649.
[3] Vincek, W.D., Martin, B.R., Aceto, M.D. and Bownan, E.R. (1980) J. Med. Chem. 23, 960–962.
[4] Sershen, H., Reith, M.E.A., Lajtha, A. and Gennara, J. jr (1981) J. Receptor Res. 2, 1–15.
[5] Abood, L.G., Reynolds, D.T., Booth, H. and Bidlack, J.M. (1981) Neurosci. Biobehav. Rev. 5, 479–486.
[6] DeTraglia, M.C. and Tometsko, A. (1980) Appl. Environ. Microbiol. 39, 1067–1072.
[7] Smissman, E.E. and Ruenxitz, P.C. (1976) J. Org. Chem. 41, 1593–1597.
[8] Seeman, J.I., Secor, H.V. and Forrest, G. (1979) J. Labelled Compd. Radiopharm. 16, 387–395.