Binding of Quinolizidine Alkaloids to Nicotinic and Muscarinic Acetylcholine Receptors

Thorsten Schmeller, Martina Sauerwein, Frank Sporer, Michael Wink, and Walter E. Müller

J. Nat. Prod., **1994**, 57 (9), 1316-1319• DOI: 10.1021/np50111a026 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 9, pp. 1316-1319, September 1994

BINDING OF QUINOLIZIDINE ALKALOIDS TO NICOTINIC AND MUSCARINIC ACETYLCHOLINE RECEPTORS

THORSTEN SCHMELLER, MARTINA SAUERWEIN, FRANK SPORER, MICHAEL WINK,*

Universität Heidelberg, Institut für Pharmazeutische Biologie, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany

and WALTER E. MÜLLER

Abteilung Psychopharmakologie, Zentralinstitut für Seelische Gesundheit, I 5, 68167 Mannheim, Germany

ABSTRACT.—Fourteen quinolizidine alkaloids, isolated from *Lupinus albus, L. mutabilis,* and *Anagyris foetida*, were analyzed for their affinity for nicotinic and/or muscarinic acetylcholine receptors. Of the compounds tested, the α-pyridones, *N*-methylcytisine and cytisine, showed the highest affinities at the nicotinic receptor, while several quinolizidine alkaloid types were especially active at the muscarinic receptor.

Quinolizidine (lupine) alkaloids are characteristic secondary metabolites in many taxa of the subfamily Papilionoideae (family Leguminosae) and over 150 structures have been described (1,2). Quinolizidine alkaloids are important for the well-being of the plants producing them (2–5), since they protect against herbivores, such as insects and grazing mammals. Other functions involve interactions with bacteria, fungi, viruses, and competing plants (2–8).

Various pharmacological and toxicological properties have been attributed to particular lupine alkaloids such as antipyretic, antiinflammatory, CNS depressant, anti-arrhythmic, respiratory depressant and stimulant, uterotonic, diuretic, hypoglycemic, hypotensive, hallucinogenic, antidiabetic and mutagenic properties (1). Quinolizidine alkaloids exhibit both general vertebrate and insect toxicity (1,2,6,7). Modulation of acetylcholine receptors and ion channels (Na^+, K^+) by quinolizidine alkaloids has been suggested as a mechanism and explanation for toxicity and some of their pharmacological properties (1–8).

As most quinolizidine alkaloids are not commercially available, only a few like sparteine, cytisine, and lupinine have been investigated biochemically in any detail. Because plants contain a complex mixture of alkaloids, it would be interesting to know how much each single alkaloidal component contributes to the toxicity and pharmacology of the mixture and, furthermore, which molecular targets are affected.

We have isolated or purchased fourteen quinolizidine alkaloids, which are the major constituents of *Lupinus albus, L. mutabilis, L. luteus, L. angustifolius, Anagyris foetida,* and *Laburnum anagyroides*. In this communication, we describe the interaction of these alkaloids with two acetylcholine receptors (ACh) known to be stimulated by cytisine and sparteine (1,2).

The activity of quinolizidine alkaloids at the nicotinic and muscarinic ACh-receptors was assayed by measuring the displacement of radiolabeled ligands, ^{3}H-nicotine and ^{3}H-quinuclidinyl benzilate (^{3}H-QNB), respectively, by employing a rapid filtration technique (9). All 14 pure quinolizidine alkaloids were assayed in concentrations between 0.5 nM and 10 mM. Typical displacement curves are illustrated in Figures 1A and 1B. From these graphs, IC_{50} values were calculated as the concentrations which displace 50% of the specifically bound ligands (Table 1).

At the nicotinic receptor, *N*-methylcytisine and cytisine showed the highest affinity of all quinolizidine alkaloids studied, confirming earlier work on the mode of action of cytisine (1,7). Lupanine, which is widely distributed in legumes as a

major alkaloid (2), displayed an IC_{50} of 5 μM and is thus 100 times more active than hydroxylated lupanines or alkaloids of the multiflorine series.

Quinolizidine alkaloids are also active at the muscarinic ACh-receptors, but often in a complementary fashion. Quinolizidine alkaloids with high affinities to the nicotinic receptor are less active at the muscarinic site and vice versa (Table 1). Especially active at the muscarinic receptor are 13α-tigloyloxylupanine, sparteine, angustifoline, albine, multiflorine, and 3β-hydroxylupanine.

It has been established that cytisine acts as an agonist at the nicotinic receptor (1). Whether the other quinolizidine alkaloids have similar properties is not known at present since our assay only measured the affinity of alkaloids for the receptors but not agonistic or antagonistic activity.

A typical alkaloid mixture from lupines contains alkaloids that bind to both ACh-receptors. Because nicotinic and muscarinic receptors are widely distributed within the body, a number of tissues and organs will be affected, to a certain degree, if an animal consumes these plants. In addition, inhibition of Na^+ or K^+ channels, which has been found for sparteine and lupanine (1,7, M. Wink and R. Fink, unpublished data) might potentiate the toxicity caused by binding of the alkaloids to ACh-receptors. It has been proposed that most secondary metabolites do not have random structures, but that they have been shaped during evolution ("evolutionary molecular modeling") to interact with cellular targets (2,7). Our study shows that quinolizidine alkaloids must have been optimized to more than one target since some quinolizi-

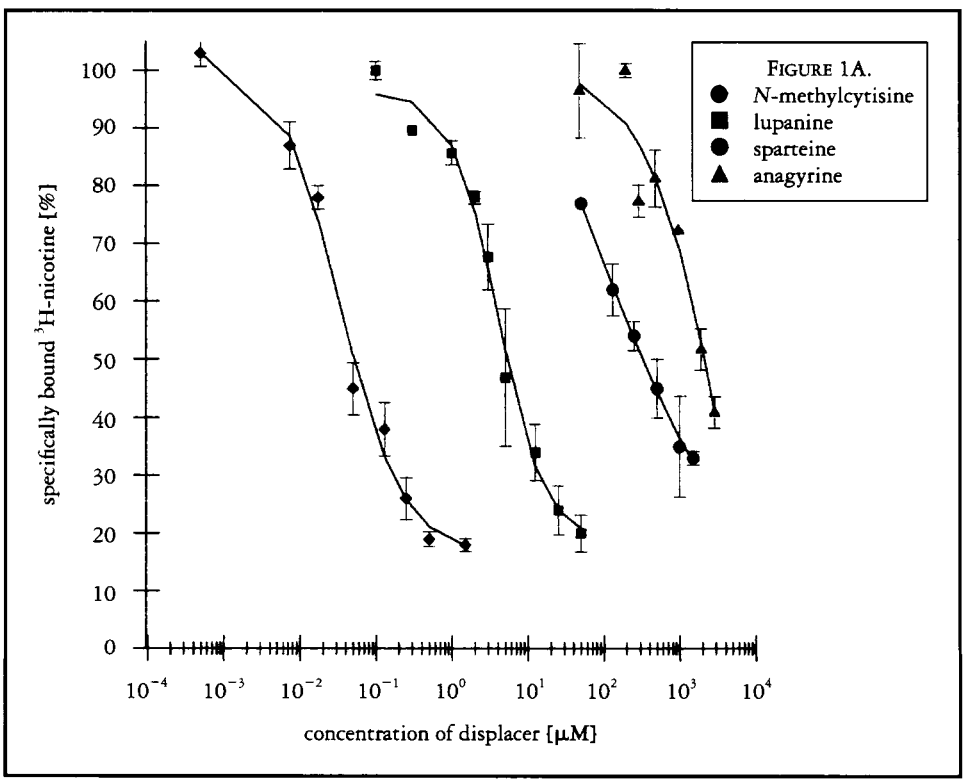

FIGURE 1. Dose-response curves for the binding of some quinolizidine alkaloids to the nicotinic and muscarinic ACh receptors as measured by the displacement of specifically bound radioligands. (Data represent means ± standard deviation [$n=3$]). Figure 1A. Nicotinic receptor; ligand ^{3}H-nicotine.

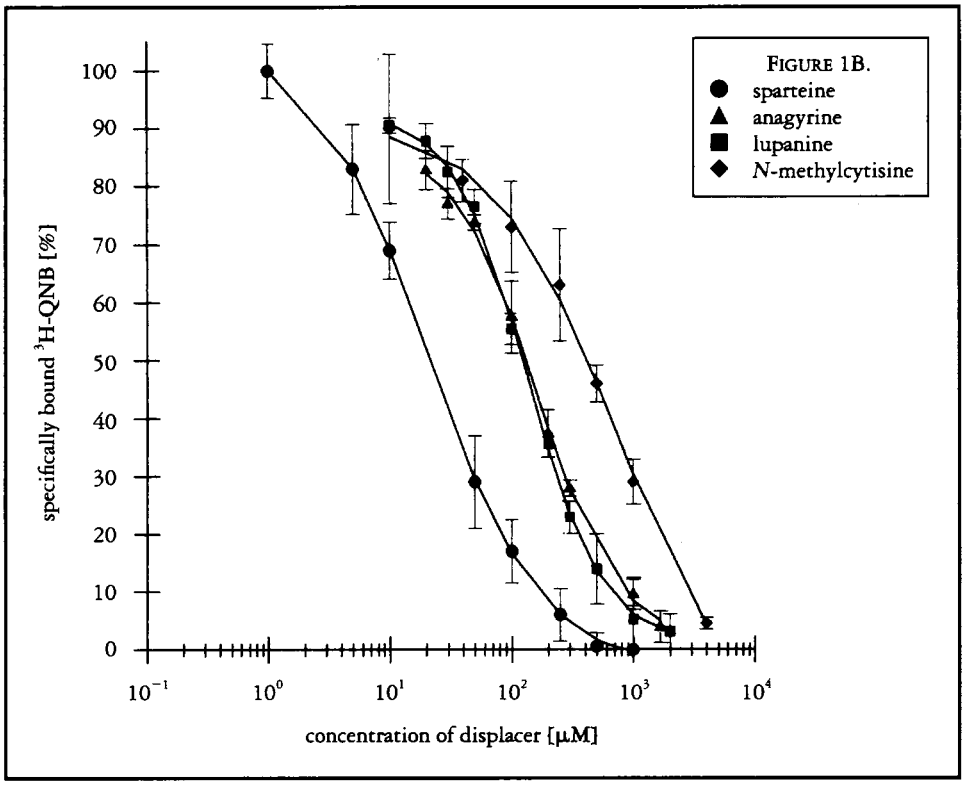

FIGURE 1. Continued. Figure 1B. Muscarinic receptor; ligand ^{3}H-QNB.

dine alkaloids can affect both nicotinic and muscarinic ACh-receptors whereas others bind preferentially to one or the other.

EXPERIMENTAL

TEST COMPOUNDS.—Quinolizidine alkaloids were extracted from seeds of *Lupinus albus, L. mutabilis,* and *Anagyris foetida* by previously de-

TABLE 1. Binding of Quinolizidine Alkaloids (IC_{50}) to Muscarinic and Nicotinic Acetylcholine Receptors.

Compound	Nicotinic receptor[a]	Muscarinic receptor[a]
Albine	193 μM	33 μM
Anagyrine	2096 μM	132 μM
Angustifoline	>500 μM	25 μM
Cytisine	0.14 μM	400 μM
3α-Hydroxylupanine	190 μM	74 μM
13β-Hydroxylupanine	490 μM	140 μM
Lupanine	5 μM	114 μM
Lupinine	>500 μM	190 μM
N-Methylcytisine	0.05 μM	417 μM
Multiflorine	>500 μM	47 μM
17-Oxosparteine	155 μM	118 μM
Sparteine	331 μM	21 μM
Tetrahydrorhombifoline	310 μM	129 μM
13α-Tigloyloxylupanine	160 μM	11 μM

[a]IC_{50} values indicate the concentration of a particular quinolizidine alkaloid that displaces 50% of the specifically bound radiolabeled ligand.

scribed methods (2). The purification and isolation of pure compounds was carried out by vlc using a $CHCl_3/CH_3OH/NH_4OH$ gradient (11). When purified, the crude alkaloids (12 g) from *L. albus* afforded 6 g lupanine, 800 mg multiflorine, 300 mg 13α-hydroxylupanine, 200 mg 13α-tigloyl- and 13α-angeloyloxylupanine, 150 mg albine, and 50 mg angustifoline. Tetrahydrorhombifoline and 3β-hydroxylupanine were isolated from *L. mutabilis* and cytisine, *N*-methylcytisine, and anagyrine from *Anagyris foetida*. 17-Oxosparteine was prepared from sparteine (2), while sparteine and lupinine were purchased from Sigma. The identity of all quinolizidine alkaloids, which are analyzed in our laboratory routinely (2,12), was confirmed by capillary gc, gc-ms and ^{13}C nmr.

MEMBRANE PREPARATION FOR ACETYLCHOLINE RECEPTOR STUDIES.—Porcine brains, which were obtained within 30 min after the death of the animals from a local slaughterhouse, were used to prepare receptor-rich membranes. The brains were immediately frozen in N_2; 50 g brain per 200 ml ice-cold buffer (0.32 M sucrose, 10 mM K^+-phosphate buffer, pH 7.0; 1 mM EDTA) were homogenized twice for 15 sec in a blender and then for 1 min with an ultraturrax (13). The homogenate was centrifuged three times for 15 min at 1400 g and 4° to separate cellular debris. The supernatant was spun down at 100,000 g for 60 min. The resulting pellet was resuspended in buffer (as above but without sucrose). Aliquots were stored frozen at −80°. Protein content was determined by the Biuret method, using bovine serum albumin as a standard.

BINDING ASSAYS.—Binding assays (in triplicate) were performed using a rapid filtration technique essentially as described by Yamamura and Snyder (9).

Muscarinic receptor.—Membrane preparations adjusted to 500 μg protein in a final volume of 500 μl buffer were incubated with ^3H-quinuclidinyl benzilate (QNB) (44.0 Ci/mmol; Dupont NEN) for 1 h at 20° in the absence and presence of quinolizidine alkaloids, employing 2 μmol atropine as a blank. The incubation was stopped with 3 ml ice-cold 0.9% NaCl solution and filtered (by suction) through Whatman GF/C glass fiber filters. The filters were washed three times with 3 ml 0.9% NaCl, placed in vials, and dried at 60° for 30 min. Their radioactivity was measured in a liquid scintillation counter (RackBeta, Pharmacia) using "Ultima Gold" (Packard) as scintillation cocktail.

Nicotinic receptor.—^3H-Nicotine (64 Ci/mmol; Dupont, NEN) was used to assay the specific binding of quinolizidine alkaloids to the nicotinic ACh receptor. The membrane preparation (as above) was incubated for 40 min at 20° with differing concentrations of quinolizidine alkaloids or 1 mM nicotine as a control. The GF/C filters were presoaked in BSA (1 mg/ml) to reduce non-specific binding of ^3H-nicotine. Further procedures were the same as described above.

ACKNOWLEDGMENTS

We thank H. Staudter and H. Wurm for technical assistance, Mittex Anlagenbau (Ravensburg) for financial support, and K. Scheuer for helpful discussion.

LITERATURE CITED

1. A.D. Kinghorn and M.F. Balandrin, in: "Alkaloids: Chemical and Biological Perspectives," Ed. by S.W. Pelletier, John Wiley & Sons, New York, 1984, Vol. 2, pp. 105–148.
2. M. Wink, in: "Methods in Plant Biochemistry. Alkaloids and Sulphur Compounds," Ed. by P. Waterman, Academic Press, London, 1993, Vol. 8, pp. 197–239.
3. M. Wink, *Theor. Appl. Gen.*, **75**, 225 (1988).
4. M. Wink, in: "Allelochemicals. Role in Agriculture and Forestry," Ed. by G.R. Waller, ACS Symposium Series, Washington, DC, No. 330, 1987, pp. 524–533.
5. M. Wink, *Planta Med.*, **53**, 509 (1987).
6. M. Wink, in: "Insect-Plant Interactions," Ed. by E.A. Bernays, CRC Press, Boca Raton, FL, 1992, Vol. IV, pp. 133–166.
7. M. Wink, in: "The Alkaloids," Ed. by G.A. Cordell, Academic Press, San Diego, 1993, Vol. 43, Chapter 1, pp. 1–118.
8. M. Wink and T. Twardowski, in: "Allelopathy: Basic and Applied Aspects," Ed. by S.J.H. Rizvi and V. Rizvi, Chapman & Hall, London, 1992, pp. 129–150.
9. H.I. Yamamura and S.H. Snyder, *Proc. Natl. Acad. Sci., USA*, **71**, 1725 (1974).
10. M. Wink, in: "Hagers Handbuch der Pharmazeutischen Praxis," Ed. by R. Hänsel, W. Keller, H. Rimpler, and G. Schneider, Springer-Verlag, Heidelberg, 1993, Vol. 5, pp. 623–630.
11. S.W. Pelletier, H.P. Chokshi, and H.K. Desai, *J. Nat. Prod.*, **49**, 892 (1986).
12. C. Meißner and M. Wink, in: "Lupinen 1991-Forschung, Anbau und Verwertung," Ed. by M. Wink, University of Heidelberg Press, Heidelberg, 1992, pp. 91–129.
13. T. Haga and K. Haga, in: "Receptor Biochemistry. A Practical Approach," Ed. by E.C. Hulme, IRL Press, Oxford, UK, 1990, Chapter 2, pp. 51–78.

Received 17 March 1994