BINDING OF QUINOLIZIDINE ALKALOIDS TO NICOTINIC AND MUSCARINIC ACETYLCHOLINE RECEPTORS

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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, antibacterial 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 lupanine 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 alka-

loidal 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,3H-nicotine and 3H-quinuclidinyl benzilate (3H-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, IC50 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 IC₅₀ 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-tigloyloxy lupanine, sparteine, angustifoline, albine, multiflorine, and 3β-hydroxy lupanine.

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-
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\textsubscript{50}) to Muscarinic and Nicotinic Acetylcholine Receptors.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Nicotinic receptor(^a)</th>
<th>Muscarinic receptor(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albine</td>
<td>193 (\mu)M</td>
<td>33 (\mu)M</td>
</tr>
<tr>
<td>Anagyrine</td>
<td>2096 (\mu)M</td>
<td>132 (\mu)M</td>
</tr>
<tr>
<td>Angustifoline</td>
<td>&gt;500 (\mu)M</td>
<td>25 (\mu)M</td>
</tr>
<tr>
<td>Cytisine</td>
<td>0.14 (\mu)M</td>
<td>400 (\mu)M</td>
</tr>
<tr>
<td>3(\alpha)-Hydroxylupanine</td>
<td>190 (\mu)M</td>
<td>74 (\mu)M</td>
</tr>
<tr>
<td>13(\beta)-Hydroxylupanine</td>
<td>490 (\mu)M</td>
<td>140 (\mu)M</td>
</tr>
<tr>
<td>Lupanine</td>
<td>5 (\mu)M</td>
<td>114 (\mu)M</td>
</tr>
<tr>
<td>Lupinine</td>
<td>&gt;500 (\mu)M</td>
<td>190 (\mu)M</td>
</tr>
<tr>
<td>N-Methylcytisine</td>
<td>0.05 (\mu)M</td>
<td>417 (\mu)M</td>
</tr>
<tr>
<td>Multiflorine</td>
<td>&gt;500 (\mu)M</td>
<td>47 (\mu)M</td>
</tr>
<tr>
<td>17-Oxospar teine</td>
<td>155 (\mu)M</td>
<td>118 (\mu)M</td>
</tr>
<tr>
<td>Spar teine</td>
<td>331 (\mu)M</td>
<td>21 (\mu)M</td>
</tr>
<tr>
<td>Tetrahydroxomibofoline</td>
<td>310 (\mu)M</td>
<td>129 (\mu)M</td>
</tr>
<tr>
<td>13(\alpha)-Tigloyloxylupanine</td>
<td>160 (\mu)M</td>
<td>11 (\mu)M</td>
</tr>
</tbody>
</table>

\(^a\)IC\textsubscript{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 CHCl₃/CH₂OH/NH₄OH 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. Tetrahydrohombifoline and 3β-hydroxylupanine were isolated from L. mutabilis and cytisine, N-methylcytisine, and anagyrine from Anagyrus foetida. 17-Oxoparteine was prepared from parteine (2), while parteine and lupinidine 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 13C 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 N₂; 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°C 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°C. 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).

Muscarnic 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°C 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°C 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°C with differing concentrations of quinolizidine alkaloids or 1 mM nicotine as a control. The GF/C filters were pre-soaked in BSA (1 mg/ml) to reduce non-specific binding of 3H-nicotine. Further procedures were the same as described above.

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Literature cited


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