Binding of tropane alkaloids to nicotinic and muscarinic acetylcholine receptors

T. SCHMELLER, F. SPORER, MARTINA SAUERWEIN and M. WINK

Fourteen tropane and related alkaloids were analyzed for their affinity for nicotinic and/or muscarinic acetylcholine receptors. The biogenetic intermediates littorine, 6β-hydroxyhyoscyamine, 7β-hydroxyhyoscyamine exhibit similar affinities at the muscarinic receptor as scopolamine and atropine. The quaternary derivatives N-methylatropine and N-methylocscopolamine show the highest binding with IC<sub>50</sub> values of less than 100 pM and 300 pM, respectively. The tropane alkaloids (including cocaine) also bind to the nicotinic acetylcholine receptor, albeit with much lower affinities.

Affinität von Tropanalkaloiden zu nicotinischen und muscarinischen Acetylcholin-Rezeptoren


1. Introduction

Tropane alkaloids, such as hyoscyamine and derivatives, constitute the characteristic secondary metabolites of many solanaceous plants, especially in the genera Atropa, Datura, Brugmansia, Hyoscyamus, Mandragora and Duboisia. Cocaine is a chemically closely related compound, which is exclusively produced by members of the genus Erythroxylum. Both groups of compounds have distinct pharmacological and physiological properties and are toxic and lethal to animals at higher doses [1–3]. As the main mechanism of the diverse effects of atropine and scopolamine, their binding and inhibition of muscarinic acetylcholine receptors have been well established [2]. Cocaine, although biogenetically related, primarily affects dopamine biochemistry (uptake and metabolism) [3].

A number of naturally occurring tropane alkaloids have recently been isolated (such as littorine, 6β-hydroxyhyoscyamine, 7β-hydroxyhyoscyamine), which are thought to be intermediate metabolites of hyoscyamine and scopolamine biosynthesis [4]. Other secondary compounds are apocapomamine, hyalbidone, noratropine, scopine, tropine and tropic acid [5, 6]. Chemical derivatives are eucatropine, N-methylatropine and N-methylocscopolamine. We have recently established sensitive in vitro assays to measure the binding of alkaloids to both acetylcholine receptor types [7]. These assays were used to study the binding of 14 alkaloids including atropine and scopolamine, biosynthetic intermediates as well as a few chemical derivatives. Since cocaine is chemically related, this Erythroxylum alkaloid was included in our study.

2. Investigations, results and discussion

As expected, atropine (i.e., hyoscyamine) and scopolamine bind to muscarinic acetylcholine receptors from porcine brains with high affinity (IC<sub>50</sub> 0.0047 μM and 0.0022 μM, respectively) (Fig., Table). The biogenetic intermediates littorine, 6β-hydroxyhyoscyamine and 7β-hydroxyhyoscyamine show comparable binding activities (0.0027 μM, 0.0395 μM, and 0.0075 μM, respectively), whereas the precursors tropine and tropic acid were inactive in our assays (Table). Surprisingly, scopine binds with an IC<sub>50</sub> of 3 μM. Whereas the removal of the N-methylgroup of atropine reduces the binding of nor-
tropine by a factor of 50 as compared to hyoscymine, the N-methylhydroylation of hyoscymine and scopolamine, which gives a stable quaternary N as in acetylcholine, increases the binding by a factor of 50 to 70 (IC50 < 0.0001 μM < 0.0003 μM, respectively). Although not determined in our assay, it can be assumed that the tropine alkaloids studied are also inhibitory and lead to parasympatholytic effects, as described for atropine and scopolamine [1–3].

In textbooks of pharmacology and toxicology it is usually suggested that hyoscymine and scopolamine bind specifically to the muscarinic acetylcholine receptors. We have tested this assumption and have checked, whether the tropine alkaloids active in muscarinic acetylcholine receptor assays, can also bind to nicotinic receptors. As can be seen from the Table none of the tropine alkaloids bind to these receptors in the nanomolar range as observed for the muscarinic receptors. However, a number of tropanes show IC50 values between 165 and 284 μM. Although these activities are probably without any significance for the medicinal use of these compounds, they might affect their toxicology, since during intoxication concentrations of tropine alkaloids may be high enough to affect both types of receptors. All other tropine alkaloids displayed activities higher than 500 μM, which we would classify as almost inactive. We don’t know, whether the modulation of the nicotinic receptor is inhibitory or stimulatory.

Although cocaine primarily affects dopamine biochemistry [3], a few of its toxicological and pharmacological activities might be caused through interaction with ACh-receptors (IC50 57 μM at muscarinic and 371 μM at nicotinic receptor) (Table). The introduction of the carboxymethylglycol group to the tropine moiety and the β-configuration at C3 seems to reduce its affinity for acetylcholine receptors.

As a conclusion, a requisite for a close binding to the muscarinic acetylcholine receptor is an ester bond and charged nitrogen in the tropine moiety [1]. Variations at C6 and C7 of tropine and of the troic acid moiety (littorine) are of less importance. Under physiological conditions the nitrogen of tropine alkaloids is protonated. As can be seen from the Table, the permanent charge of the nitrogen in N-methyl atropine and N-methyl scopolamine is more efficient and can increase the binding activity by a factor of 50 to 70. The question, which immediately arises is, why natural selection has not favoured these N-methyl alkaloids. Although the advantages of higher binding seem obvious, the quaternary alkaloids are unable to pass biomembranes by simple diffusion. Whereas the producing plant could overcome this problem by evolving carrier proteins for these compounds, it would not be the case in animals against which the allelochemicals have been selected in nature. In contrast to the quaternary alkaloids, hyoscymine and scopolamine can diffuse through biomembranes as free bases, and can thus be resorbed by animals. To have a tertiary nitrogen which can be protonated under physiological hydrogen concentrations seems to be a compromise between membrane permeability and binding activity.

### 3. Experimental

#### 3.1. Test compounds

6β-Hydroxyhyoscymine and 7β-hydroxyhyoscymine were isolated according to the literature [4]. Littorine and norhyoscymine were synthesized in our laboratory [5, 8]. Hyalidine was isolated from hairy root cultures of Hyoscyamus niger. All other substances were purchased from Sigma. The purity of all alkaloids was at least 97%, as determined by HPLC [6, 9].

#### 3.2. Membrane preparation for acetylcholine receptor studies

Membranes were prepared from frozen porcine brains [7]. 50 g of brain per 200 ml icecold buffer (0.32 M sucrose, 1 mM EDTA, 10 mM potassium phosphate buffer, pH 7.0) were homogenized. The resulting pellet was resuspended in buffer (as above, without sucrose). The protein content was determined by the Biuret-method, with bovine serum albumin as standard. Aliquots were frozen and stored at −80°C until use.

#### 3.3 Binding assays

Binding assays (in triplicates) were performed using a rapid filtration technique, essentially as described in [10].

#### 3.3.1. Muscarinic receptor

Membrane preparation containing 500 μg protein in a final volume of 500 μl was incubated with 3H quinuclidinyl benzilate (QNB) (440 Ci/mmoll, NEN) for 60 min at 20 °C in absence and presence of 2 μM atropine as blank. The incubation was stopped with 3 ml icecold 0.9% NaCl solution and filtered through Whatman GF/C glass fiber filters with suction. The filters were washed three times with 3 ml icecold 0.9% NaCl solution, placed in vials, dried for 30 min at 60 °C and extracted with Ultima Gold scintillation cocktail for 1 h. Radioactivity was measured in a Pharmacia RackBeta scintillation counter.

#### 3.3.2. Nicotinic receptor

3H nicotine (640 Ci/mmoll, NEN) was used to assay specific binding on the nicotinic acetylcholine receptor. The receptor preparation was incubated for 40 min at 20 °C in absence and presence of 1 μM (-)-nicotine as blank. The GF/C filters were presoaked in a BSA-solution (1 mg/ml BSA) to reduce nonspecific binding. Further procedures were the same as described above.

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**Table: IC50 values for binding of tropane alkaloids at muscarinic and nicotinic acetylcholine receptors**

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Binding (IC50)</th>
<th>Nicotinic receptor</th>
<th>Muscarinic receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>284 μM</td>
<td>0.0047 μM</td>
<td></td>
</tr>
<tr>
<td>Aposcopalamine</td>
<td>188 μM</td>
<td>0.0192 μM</td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>371 μM</td>
<td>0.0000 μM</td>
<td></td>
</tr>
<tr>
<td>Eucatropine</td>
<td>&gt; 500 μM</td>
<td>0.5830 μM</td>
<td></td>
</tr>
<tr>
<td>Hyalidine</td>
<td>176 μM</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>6β-Hydroxyhyoscymine</td>
<td>&gt; 500 μM</td>
<td>0.0395 μM</td>
<td></td>
</tr>
<tr>
<td>7β-Hydroxyhyoscymine</td>
<td>&gt; 500 μM</td>
<td>0.0075 μM</td>
<td></td>
</tr>
<tr>
<td>Littorine</td>
<td>910 μM</td>
<td>0.0027 μM</td>
<td></td>
</tr>
<tr>
<td>N-methylatropine</td>
<td>165 μM</td>
<td>&lt; 0.0001 μM</td>
<td></td>
</tr>
<tr>
<td>N-methylscopalamine</td>
<td>187 μM</td>
<td>&lt; 0.0003 μM</td>
<td></td>
</tr>
<tr>
<td>Noratropine</td>
<td>494 μM</td>
<td>0.2010 μM</td>
<td></td>
</tr>
<tr>
<td>Scopine</td>
<td>&gt; 500 μM</td>
<td>3.0000 μM</td>
<td></td>
</tr>
<tr>
<td>Scopolamine</td>
<td>928 μM</td>
<td>0.0020 μM</td>
<td></td>
</tr>
<tr>
<td>Tropic acid</td>
<td>&gt; 500 μM</td>
<td>&gt; 500.0000 μM</td>
<td></td>
</tr>
<tr>
<td>Tropine</td>
<td>&gt; 500 μM</td>
<td>&gt; 500.0000 μM</td>
<td></td>
</tr>
</tbody>
</table>

n.d. = not determined; IC50 values indicate the concentration of a particular alkaloid that displaces 50% of the specifically bound radiolabeled ligand

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References


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