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Binding of tropane alkaloids to nicotinic and muscarinic acetylcholine receptors

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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 quarternary derivatives N-methylatropine and N-methylscopolamine show the highest binding with IC_{50} 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.

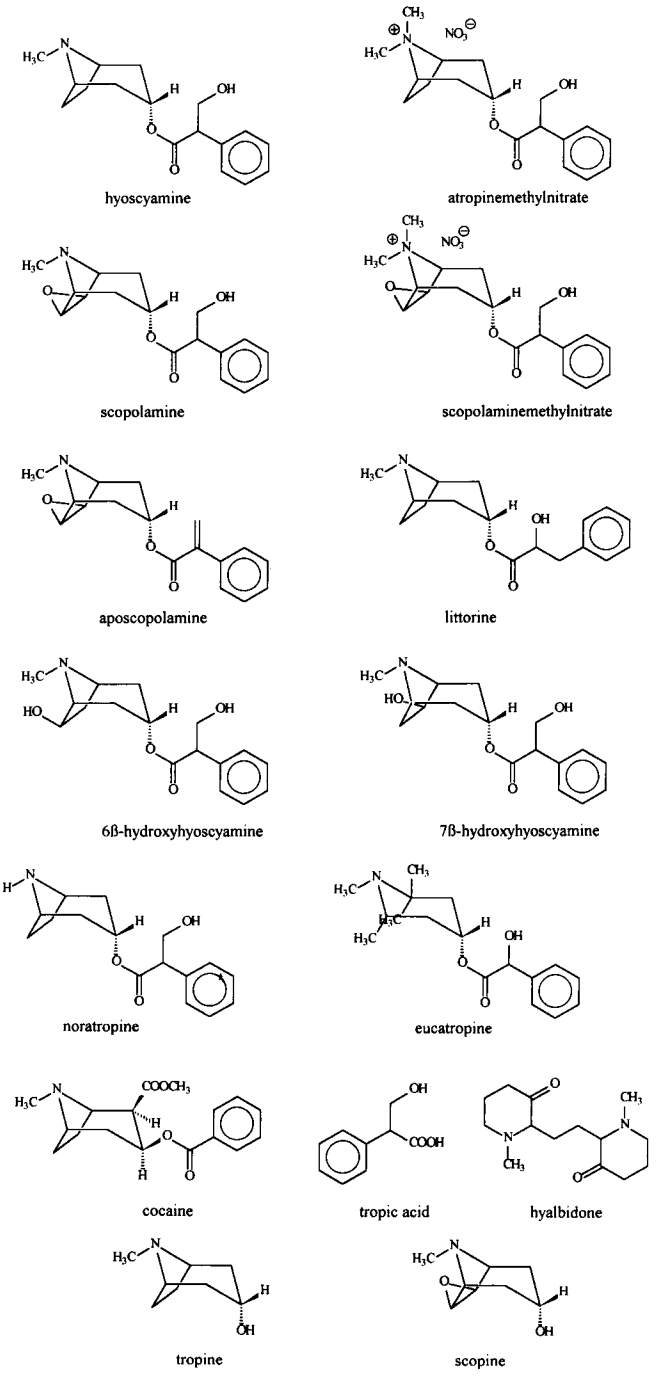
Affinität von Tropanalkaloiden zu nicotinischen und muscarinischen Acetylcholin-Rezeptoren

Vierzehn Tropan- und verwandte Alkaloide wurden auf ihre Affinität für muscarinische und/oder nicotinische Acetylcholin-Rezeptoren untersucht. Die biogenetischen Intermediate Littorin, 6β-Hydroxyhyoscyamin, 7β-Hydroxyhyoscyamin zeigten ähnliche Affinitäten für den muscarinergen Rezeptor wie Scopolamin und Atropin. Die quartären Derivate N-Methylatropin und N-Methylscopolamin zeigten die höchste Bindung mit IC_{50} Werten von weniger als 100 pM bzw. 300 pM. Die Tropanalkaloide (einschließlich Cocain) binden auch an den nicotinergen Acetylcholin-Rezeptor, wenn auch mit wesentlich geringeren Affinitäten.

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 occuring 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 aposcopolamine, hyalbidone, noratropine, scopine, tropine and tropic acid [5, 6]. Chemical derivatives are eucatropine, N-methylatropine and N-methylscopolamine. 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_{50} 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_{50} of 3 μM. Whereas the removal of the N-methylgroup of atropine reduces the binding of nora-

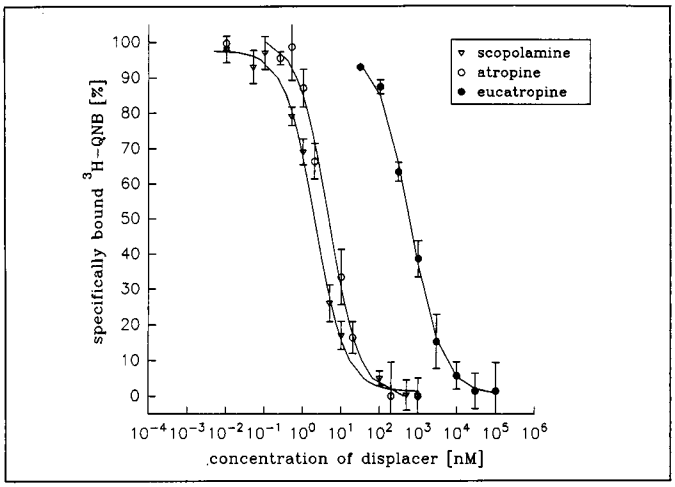


Fig.: Dose-response curves for the binding of some tropane alkaloids to the muscarinic ACh receptor as measured by the displacement of specifically bound radioligand (^{3}H-QNB). Data represent means (± standard deviation (n = 3)).

tropine by a factor of 50 as compared to hyoscyamine, the N-methylation of hyoscyamine and scopolamine, which gives a stable quarternary 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 tropane 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 hyoscyamine and scopolamine bind specifically to the muscarinic acetylcholine receptors. We have tested this assumption and have checked, whether the tropane alkaloids active in muscarinic acetylcholine receptor assays, can also bind to nicotinic receptors. As can be seen from the Table none of the tropane alkaloids bind to these receptors in the nanomolar range as observed for the muscarinic receptors. However, a number of tropanes show IC_{50} 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 tropane alkaloids may be high enough to affect both types of receptors. All other tropane 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.

Table: IC_{50} values for binding of tropane alkaloids at muscarinic and nicotinic acetylcholine receptors

Compd.	Binding (IC_{50})	
	Nicotinic receptor	Muscarinic receptor
Atropine	284 µM	0.0047 µM
Aposcopolamine	188 µM	0.0192 µM
Cocaine	371 µM	57.0000 µM
Eucatropine	> 500 µM	0.5830 µM
Hyalbidone	176 µM	n.d.
6β-Hydroxyhyoscyamine	> 500 µM	0.0395 µM
7β-Hydroxyhyoscyamine	> 500 µM	0.0075 µM
Littorine	910 µM	0.0027 µM
N-methylatropine	165 µM	< 0.0001 µM
N-methylscopolamine	187 µM	< 0.0003 µM
Noratropine	494 µM	0.2010 µM
Scopine	> 500 µM	3.0000 µM
Scopolamine	928 µM	0.0020 µM
Tropic acid	> 500 µM	> 500.0000 µM
Tropine	> 500 µM	> 500.0000 µM

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

Although cocaine primarily affects dopamine biochemistry [3], a few of its toxicological and pharmacological activities might be caused through interaction with ACh-receptors (IC_{50} 57 µM at muscarinic and 371 µM at nicotinic receptor) (Table). The introduction of the carboxylmethylester 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 a charged nitrogen in the tropine moiety [1]. Variations at C6 and C7 of tropine and of the tropic acid moiety (littorine) are of less importance. Under physiological conditions the nitrogen of tropane 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 quarternary 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 quarternary alkaloids, hyoscyamine 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-Hydroxyhyoscyamine and 7β-hydroxyhyoscyamine were isolated according to the literature [4]. Littorine and norhyoscyamine were synthesized in our laboratory [5, 8]. Hyalbidone was isolated from hairy root cultures of *Hyoscyamnus albus*. 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 ^{3}H quinuclidinyl benzilate (QNB) (44.0 Ci/mmol, NEN) for 60 min at 20 °C in absence and presence of 2 µmol 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

^{3}H nicotine (64.0 Ci/mmol, 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 mM (−) 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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