

Biochemical studies on muscarinic receptors in the salamander olfactory epithelium

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Received 16 August 1983

Muscarinic cholinergic receptors in the olfactory epithelium of the salamander, *Ambystoma tigrinum*, were studied via binding of 3-[³H]quinuclidinyl benzilate. The receptors are present on the olfactory receptor cells in the epithelium to an amount of 0.08 pmol/mg homogenate protein. Both choline acetyltransferase and acetylcholine esterase are present in the salamander olfactory epithelium.

Muscarinic receptor Olfactory epithelium Salamander

1. INTRODUCTION

There have been several attempts to approach the problem of how odorant molecules interact with olfactory receptors to induce a response in the receptor cells of the olfactory epithelium. Cells of the epithelium have been dissociated and attempts have been made to characterize their biochemical and histological properties [1], with different markers for different cell types, such as the olfactory marker protein for olfactory receptor cells [2]. The majority of investigations have however concentrated on the physiology and electrical responses of the receptor cells (cf. [3,4]) and on intracellular analyses [5,6].

The presence of muscarinic cholinergic receptors on olfactory receptor cells in the mouse olfactory epithelium was suggested in [7]. We have extended these findings and characterized muscarinic receptors in the olfactory epithelium of the salamander, *Ambystoma tigrinum*. The results presented here indicate that muscarinic receptors are present on the receptor cells and that enzymes for synthesis and degradation of the transmitter, acetylcholine, are also present in the olfactory epithelium.

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2. MATERIALS AND METHODS

3-[³H]Quinuclidinyl benzilate (3-[³H]QNB) (33 Ci/mmol) and [³H]acetyl coenzyme A (0.5 Ci/mmol) were obtained from New England Nuclear (Boston MA). All other chemicals were of reagent grade and purchased from Sigma (St Louis MO).

Tiger salamanders, *Ambystoma tigrinum*, were obtained from Charles D. Sullivan's Farm (Tennessee). The olfactory epithelium was dissected out, homogenized on ice in Krebs-Ringer's buffer in a glass-Teflon homogenizer, 395 rev./min with 10 up and down strokes, and used without further treatment.

Binding of 3-[³H]QNB to muscarinic receptors was measured at room temperature as in [8] using Whatman GF/F glassfiber filters and a Krebs-Ringer's buffer containing 104.4 mM NaCl, 1.82 mM KCl, 3.6 mM CaCl₂, 0.71 mM MgCl₂, 25 mM Tris-HCl, 5.6 mM glucose, pH 7.4. Specific binding was defined as the difference in binding in the absence and presence of atropine (0.01 mM). Acetylcholine esterase (AChE) activity was determined as in [9] and choline acetyltransferase (ChAT) as in [10]. Protein was determined as in [11] using bovine serum albumin as standard. Denervations were made by transecting the olfac-

tory nerve close to the olfactory bulb after anesthetizing the skin with xylocaine [12]. The animals were then kept for another 7 days before sacrifice. Values were compared using two-tailed Student's *t*-test.

3. RESULTS

3-[³H]QNB (2 nM) bound reversibly to a homogenate of the olfactory epithelium (fig.1). The association rate constant was calculated to be $0.21 \text{ nM}^{-1} \cdot \text{min}^{-1}$ and the dissociation rate constant 0.17 min^{-1} . Dissociation of 3-[³H]QNB from the receptor was started by addition of atropine ($10 \mu\text{M}$). This gives an estimated K_d for 3-[³H]QNB of 0.8 nM.

Total and non-specific binding of 3-[³H]QNB are shown in fig.2. The inset to fig.2 depicts the specific binding of 3-[³H]QNB; 0.1 pmol/mg protein could be specifically bound. The specific binding of 3-[³H]QNB (2 nM) can be abolished by trypsin treatment or heat denaturation (20 min at 60°C) (not shown), indicating that 3-[³H]QNB is

specifically bound to a protein in the olfactory epithelium.

Seven days after olfactory nerve transection the olfactory epithelium is characterized by little or no mature receptor cells present in the epithelium [12,13]. Transection of the olfactory nerve, which is followed by degeneration of olfactory receptor cells, almost completely abolished specific binding of 3-[³H]QNB (2 nM), as shown in table 1, decreasing it from 0.1–0.02 pmol/mg protein specifically bound 3-[³H]QNB. AChE-activity and ChAT-activity were also somewhat decreased (table 1) but not absent.

The pharmacological specificity of the muscarinic receptors was investigated through competition with 1 nM 3-[³H]QNB (table 2). It can be observed that muscarinic antagonists, such as atropine, and muscarinic agonists (acetylcholine (+ $10 \mu\text{M}$ eserine), oxotremorine, pilocarpine, carbamylcholine) were effective in nanomolar (nM) and micromolar (μM) concentrations. Other agents, such as the nicotinic antagonist d-tubocurarine, were 10^3 – 10^4 -times less potent in inhibiting 3-[³H]QNB binding.

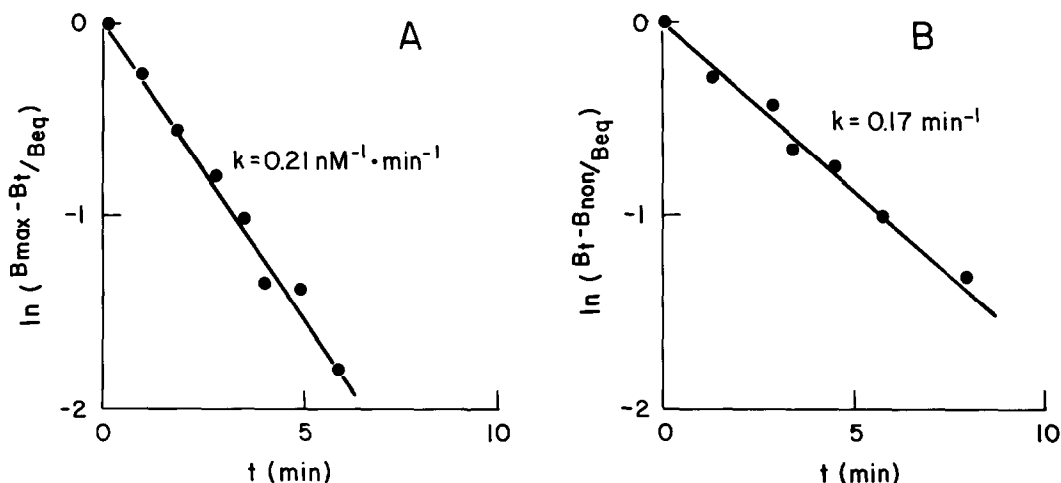


Fig.1. (A) Semilogarithmic plot of the association rate of 3-[³H]QNB (2 nM) for binding to muscarinic receptors using a homogenate of the olfactory epithelium at room temperature measured under pseudo-first order conditions. (B) Semilogarithmic plot of the dissociation rate of 3-[³H]QNB from muscarinic receptors under pseudo-first order conditions using a homogenate of the olfactory epithelium at room temperature. Dissociation of 3-[³H]QNB from the receptor was started by addition of atropine ($10 \mu\text{M}$). B_t equals amount bound at time t , B_{non} stands for non-specific binding (saturated within seconds), B_{max} stands for maximal total binding of 3-[³H]QNB and B_{eq} , the specific binding at equilibrium, equals $B_{\text{max}} - B_{\text{non}}$.

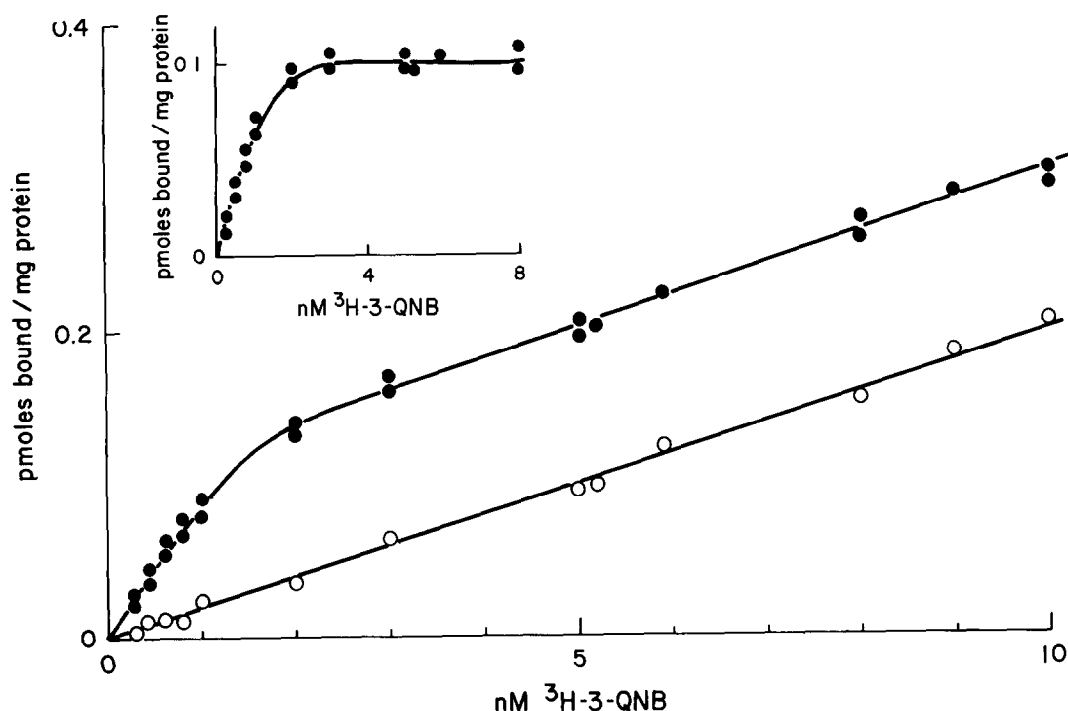


Fig.2. Total (●—●) and non-specific (○—○) binding of 3-[³H]QNB to a homogenate of the salamander olfactory epithelium. Non-specific binding was determined in the presence of 10 μ M atropine. 3-[³H]QNB bound with a K_d of 0.8 nM. The inset shows the specific binding of 3-[³H]QNB.

Table 1

Cholinergic parameters measured in a homogenate of the olfactory epithelium from control animals and in animals 7 days after olfactory nerve transection

Treatment	Specific binding of 3-[³ H]QNB (pmol/mg protein)	AChE-activity (units/mg protein)	ChAT-activity (cpm [³ H]ACh. min ⁻¹ .mg protein ⁻¹)
Control	0.1 $\pm$ 0.02 (n = 23)	4.7 $\pm$ 0.7 (n = 8)	30923 $\pm$ 3850 (n = 8)
Transected	0.02 $\pm$ 0.02 (n = 24) ^a	3.2 $\pm$ 0.7 (n = 7) ^b	26372 $\pm$ 2322 (n = 8) ^c

^a $p < 0.001$ as compared to control

^b $p < 0.01$ as compared to control

^c $p < 0.05$ as compared to control

Values are given as mean $\pm$ SD

Numbers in parentheses indicate number of determinations

4. DISCUSSION

The results presented here clearly indicate that the main criteria, summarized in [14], for ligand-muscarinic receptor interactions are fulfilled in the salamander olfactory epithelium. The

binding is reversible (fig.1) and there is a finite amount of receptors present (fig.2). The receptor has the pharmacological specificity of a muscarinic receptor (table 2). Results also indicate that 3-[³H]QNB binds specifically to a protein.

These results raise the question of the

Table 2

IC_{50} -values for inhibition of 3-[3H]QNB (1 nM) by different neurotransmitter substances

Substance	IC_{50} (M)
Muscarinic:	
Atropine	1×10^{-9}
Acetylcholine	1.5×10^{-5}
Carbamylcholine	1.5×10^{-5}
Oxotremorine	5×10^{-6}
Pilocarpine	6×10^{-6}
Other:	
D-tubocurarine	$>1 \times 10^{-2}$
Dopamine	$>1 \times 10^{-2}$
Noradrenaline	$>1 \times 10^{-2}$
Glutamate	1×10^{-2}
Aspartate	1×10^{-2}

significance of the presence of muscarinic receptors, since there are no known cholinergic inputs. The fact that olfactory nerve transection abolishes 3-[3H]QNB binding (table 1) indicates that muscarinic receptors are present on olfactory receptor cells, and one possibility is that these receptors may be in some way involved in modulating olfactory receptor activation. Both AChE and ChAT are present but with a somewhat different cellular location than the muscarinic receptors, indicated by the fact that these enzymes are less affected by olfactory nerve transection (table 1). The decrease in AChE activity is most likely due to its presence to a small extent in non-cholinergic neurons [15]. The slight decrease in ChAT activity on the other hand may be due to general degeneration of the tissue [13].

Results in [16] indicate that stimulation of olfactory receptors by odorant molecules may induce an increased secretory response from the supporting cells of the epithelium. This site could very well be the site of release of acetylcholine, which then could act as a feedback signal to modulate the responsiveness of the olfactory receptor cells to further stimuli.

ACKNOWLEDGEMENTS

The authors are grateful to Professor Jack R. Cooper, Yale University, for providing facilities for these experiments. This study was supported by a grant (MS-07609) from the NINCDS to G.M.S.

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