LIGAND-BINDING PROPERTIES OF A MUSCARINIC ACETYLCHOLINE RECEPTOR FROM *TORPEDO* ELECTRIC ORGAN

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1. Introduction

Muscarinic acetylcholine receptors have been detected in many tissues using the radio-ligand binding technique [1]. The electric organ of *Torpedo* species has been used extensively for studying the cholinergic synapse [2] and particularly for characterizing the nicotinic acetylcholine receptor [3] and in [4–6] a muscarinic acetylcholine receptor in this tissue has been shown using the radio-ligand binding technique. The muscarinic acetylcholine receptors in *Torpedo* electric organ are probably entirely presynaptic [5,6] and so may provide a useful system for studying any differences between presynaptic and postsynaptic muscarinic receptors. Here we describe the ligand-binding characteristics of the *Torpedo* receptor and the effect of guanine nucleotides on ligand-binding to the receptor.

2. Materials and methods

Torpedo marmorata were obtained from the Station Biologique d'Arcachon, France and were kept in circulating sea water tanks in Nottingham at 12–15°C. d-Tubocurarine chloride, decamethonium bromide, phenylmethane sulphonyl fluoride and guanine nucleotides were obtained from Sigma (London) Chemical Co., Poole, Dorset. All other chemicals were obtained from suppliers given in [7,8].

Electric organ membrane fragments were prepared from fresh tissue or tissue stored at −80°C. An homogenate (20%, w/v, in ice-cold 0.25 M sucrose, 0.1 mM EDTA, 0.02 mM phenylmethane sulphonyl fluoride) was prepared using a MSE top-drive homogenizer at full speed for 2 min. The homogenate was sonicated (3 min, 4°C) and centrifuged for 20 min at 2000 × g_{av}. The supernatant was removed and centrifuged for 120 min at 143 000 × g_{av} to give a pellet which was either resuspended in an ice cold Hepes–phosphate–saline buffer (pH 7.4) [8] at ~10 mg protein/ml (determined as in [9]) (P preparation) or resuspended in 0.25 M sucrose (at ~5 mg protein/ml) and applied to the top of a stepwise sucrose gradient containing layers of equal volumes of 0.4 M sucrose and 1 M sucrose. After centrifugation (100 000 × g_{av}, 4 h) the band between the 0.4 M and 1 M sucrose layers was removed, diluted 3-fold in buffer and centrifuged (140 000 × g_{av}, 3 h). The pellet was resuspended in buffer at ~5 mg protein/ml (P_B preparation).

Muscarinic acetylcholine receptor binding was determined as follows: electric organ membrane fragments (0.2–1.0 mg/ml) were incubated in a final volume of 4.25 ml Hepes–phosphate–saline buffer [8] containing [3-^{3}H]quinuclidinyl benzilate ([3-^{3}H]-QNB) (50 pM- 8 nM) and other drugs where appropriate at 30°C for 30 min or 60 min. At the end of the incubation, four 1 ml aliquots were removed and filtered under vacuum through separate presoaked GF/F glass fibre filters (Whatman) which were washed with 2 ml buffer at 20°C. Radioactivity trapped on the filters was determined as in [8].

For atropine and [3-^{3}H]QNB racemic mixtures of the compounds were used but it was assumed that only the (−)-isomers were pharmacologically active [7,8]. Specific (−)-[3-^{3}H]QNB binding (~80% of total binding) was defined as that displaceable by 20 μM atropine [7,8]. Binding parameters were determined from Scatchard plots and by use of the equation:

$$\text{Log (fractional occupancy/1-fractional occupancy)} = n \text{ Log [ligand]} - n \text{ Log } IC_{50}$$

IC_{50} = ([ligand] giving 50% occupancy); n = slope factor (equivalent to a Hill coefficient for saturation binding experiments [10]).

3. Results and discussion

In preliminary experiments using an homogenate of *Torpedo marmorata* electric organ a significant but low number of muscarinic acetylcholine receptors was detected (~50 fmol/mg protein). For more detailed work the homogenate was subfractionated in order to increase the specific activity of binding either by differential centrifugation (P preparation) or by differential and sucrose density gradient centrifugation (P_B preparation). Enrichments of binding were obtained in both preparations: P 175 ± 34 (6) fmol/mg protein; P_B 337 ± 122 (4) fmol/mg protein (mean ± SD (no. expt.)). The binding of [3-^{3}H]QNB to either preparation was saturable (fig.1): dissociation constants for (−)-[3-^{3}H]QNB were 235 ± 5 pM (P) and 38 ± 4 pM (P_B) (mean ± range, 2 expt.). These values are similar to those obtained using other tissues [7,11]. Subcellular fractionation studies (not shown) have suggested

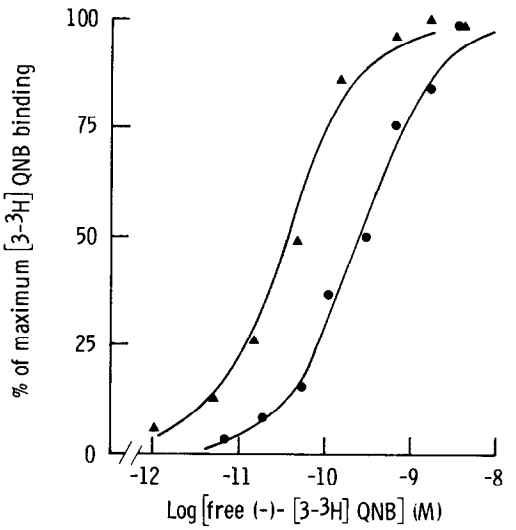

Fig.1. Specific binding of [3-^{3}H]QNB to *Torpedo* electric organ membrane preparations. Specific binding of [3-^{3}H]-QNB was determined as described to P (●) and P_B (▲) preparations. Data are expressed as % of maximum binding (determined by Scatchard analysis) and the lines are theoretical binding curves for binding to a single class of sites (K_d 0.24 nM (P); K_d 0.035 nM (P_B)). Each point is the mean of a quadruplicate determination.

Table 1
Ligand-binding properties of muscarinic acetylcholine receptors

Ligand	*Torpedo*		Rat cerebral cortex [11,13]	
Antagonists	K_d (M)	Slope factor	K_d (M)	Slope factor
(−)-Atropine	1.2×10^{-9} (a)	1.31	5.5×10^{-10}	0.95
(−)-Quinuclidinyl benzilate	3.8×10^{-11} (a) 2.4×10^{-10} (b)	0.82 0.83	1.4×10^{-10}	1.09
(−)-Scopolamine	1.3×10^{-9} (a)	1.03	8.1×10^{-10}	1.01
Agonists	Corr. IC_{50} (M)	Slope factor	IC_{50} (M)	Slope factor
Arecoline	2.6×10^{-6} (b)	0.65	5.0×10^{-6}	0.75
Carbamoylcholine	1.5×10^{-6} (a) 7.5×10^{-6} (b)	0.60 0.61	1.5×10^{-5}	0.33
Oxotremorine-M (c)	3.3×10^{-7} (b)	0.47	1.6×10^{-6}	0.25
Nicotinic acetylcholine receptor ligands				
Decamethonium	7.3×10^{-6} (a)	–	–	–
d-Tubocurarine	2.3×10^{-5} (a)	–	–	–

Ligand-binding properties were determined from displacement or saturation binding experiments as described using P_B preparation (a) or P preparation (b). IC_{50} values from displacement experiments were corrected for receptor occupancy [7,8] to give K_d or corrected IC_{50} values. (c) [13]

the presence of a soluble inhibitor of [3-^{3}H]QNB-binding in *Torpedo* electric organ. The increased affinity of [3-^{3}H]QNB-binding accompanying further purification of the P preparation might therefore reflect the removal of this endogenous inhibitor. Although the identity of this inhibitor has not been established a likely candidate is choline which has been shown to inhibit binding of [3-^{3}H]QNB to muscarinic receptors in mammalian brain [12].

The binding properties of other ligands were studied by displacement of [3-^{3}H]QNB-binding (table 1, fig.2). The selective nicotinic acetylcholine receptor ligands decamethonium and tubocurarine were relatively poor inhibitors whereas the specific muscarinic acetylcholine receptor ligands atropine and scopolamine were potent inhibitors of [3-^{3}H]QNB binding confirming the muscarinic nature of the binding sites (table 1).

The binding of the antagonists tested (atropine, QNB, scopolamine) was characterized by slope factors close to one indicating that binding was at a single class of sites. The values of the dissociation constants for these ligands were comparable with values obtained in other tissues [11]. Agonist binding was characterized by slope factors of <1 indicating some complexity in behaviour. The *Torpedo* receptor, therefore, conforms to the general pattern observed for ligand binding at muscarinic receptors in other tissues [11,13]: antagonist binding is at a single class of sites whereas agonist binding is more complex. When examined in detail, however, the *Torpedo* receptor shows differences from receptors in other tissues. Compared to the rat cerebral cortical receptor [11,13], although affinities for antagonist binding are similar, the *Torpedo* receptor shows higher agonist affinities and the slope factors for agonist binding are greater (table 1).

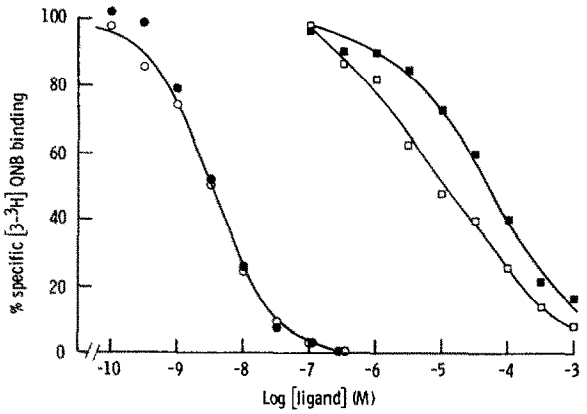

Fig.2. Effect of GTP on ligand-binding to the *Torpedo* muscarinic acetylcholine receptor. The binding of (−)-atropine and carbamoylcholine was assayed by displacement of [3-^{3}H]QNB binding from a P preparation in the presence ((●) (−)-atropine; (■) carbamoylcholine) and absence ((○) (−)-atropine; (□) carbamoylcholine) of 0.1 mM GTP. Each point is the mean of a quadruplicate determination. For atropine the line is a theoretical binding curve for IC_{50} = 3.2 nM.

These differences may reflect the different species used for the experiments (*Torpedo* vs rat) but they may also reflect the location of the receptors: the *Torpedo* receptors are presynaptic whereas the cerebral cortical receptors are likely to be both presynaptic and postsynaptic.

In order to investigate these possibilities further we have tested the effect of guanine nucleotides on the *Torpedo* muscarinic receptor binding properties (fig.2). Guanosine triphosphate (GTP) (0.1 mM) did not affect the binding of atropine but it reduced the ability of carbamoylcholine to inhibit [3-^{3}H]QNB binding (−GTP, corrected IC_{50} 7.5 μM, slope factor 0.61; + GTP, corrected IC_{50} 25.4 μM, slope factor 0.57). Similar effects were observed with guanosine diphosphate and guanylylimidodiphosphate but other guanine nucleotides were inactive.

Similar agonist-specific effects of guanosine nucleotides have been reported for muscarinic receptors in brain and myocardium [14,15]. In the case of myocardium the effect of GTP has been linked with muscarinic inhibition of adenylate cyclase [15]. This raises the possibility that the *Torpedo* muscarinic receptor is also associated with inhibition of adenylate cyclase.

In conclusion, therefore, the *Torpedo* muscarinic acetylcholine receptor shows qualitative similarities with muscarinic receptors in rat cerebral cortex both in terms of agonist and antagonist binding properties and the effects of guanine nucleotides. There are, however, quantitative differences in agonist binding properties which may reflect a species difference or a difference between presynaptic and postsynaptic receptors.

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