The author is indebted to Miss Ch. Grunenwald, Mr J.-J. Feldtrauer and Mr K. Stoecklin for their skilful technical assistance.

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J. Pharm. Pharmacol. 1985, 37: 60–61
Communicated June 21, 1984

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Muscarinic cholinergic receptors in rabbit retina

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Binding sites with high affinity and specificity for [^{3}H]-quinuclidinyl benzilate are present in rabbit retinal homogenates. Only one set of binding sites was detected with an apparent dissociation constant of 2·13 × 10^{-10} M and a density of 59·2 fmol mg^{-1} of protein. The pharmacological characteristics of specific binding were similar to those in the goldfish, chicken and cow.

Acetylcholine (ACh) is believed to be a neurotransmitter substance in most, if not all, vertebrate retinas (for review see Neal 1983). The evidence is particularly strong in the rabbit retina, where two sub-populations of amacrine cells are believed to be cholinergic (Masland & Mills 1979). Electrophysiological, biochemical and autoradiographical experiments have emphasized the importance of nicotinic cholinergic receptors in the rabbit retina, but there is little information on the presence of muscarinic receptors in this species. For this reason, we have examined the muscarinic receptors in the rabbit retina using [^{3}H]quinuclidinyl benzilate (QNB).

Methods

Rabbit retinas were obtained from Buxted Olac Rabbits, Sussex, and were frozen intact immediately after dissection. Approximately 1 g of retinal tissue was homogenized in 15 ml of 50 mM Na-K phosphate buffer (pH 7·4) and then dispersed using a Polytron homogenizer (setting 6, 30 s). The homogenate was centrifuged at 27 000*g* for 10 min, resuspended and rehomogenized in buffer. It was then centrifuged at 49 000*g* for 10 min, resuspended and rehomogenized in buffer. Protein determinations were performed as described by Lowry et al (1951).

To demonstrate the presence of muscarinic cholinergic receptors in the rabbit retina, DL-[3-^{3}H]quinuclidinyl benzilate ([^{3}H]QNB, 10 Ci mmol^{-1}, Amersham International) was used as described previously (Yamamura & Snyder 1974). [^{3}H]QNB was incubated with retinal tissue with 2 ml of 50 mM Na-K phosphate buffer (pH 7·4) for 60 min at 37 °C. Incubation was terminated by rapid filtration through Whatman GF/C filters under vacuum. The filters were washed 4 times with 5 ml of ice cold buffer and then transferred to scintillation vials. 4 ml ethoxyethanol and 10 ml butyl PBD (0·5%) in toluene were added for liquid scintillation counting.

A duplicate set of tubes was incubated with 1 μM atropine to measure non-specific binding which was routinely subtracted from total binding to give the specific binding. All determinations were in triplicate and were repeated at least 4 times.

Results

The specific binding of [^{3}H]QNB increased linearly with the amount of retinal tissue over the range 0·1–0·8 mg per 2 ml assay volume. Increasing the concentration of [^{3}H]QNB resulted in saturation of the specific binding sites in the homogenate (Fig. 1). The non-specific binding measured after incubation with atropine (1 μM) was less than 17% of the total (Fig. 1).

A Scatchard plot (Scatchard, 1949; Fig. 1) revealed a single high affinity binding site, with an apparent dissociation constant (K_D) of 2·13 × 10^{-10} ± 0·21 × 10^{-10} M (n = 3), while the maximum number of binding sites (B_{max}) was 59·2 ± 2·8 fmol mg^{-1} protein (n = 3).

The pharmacological specificity of [^{3}H]QNB binding to rabbit retinal tissue was determined from inhibition curves of several cholinoceptor agonists and antagonists (Fig. 2A). Hill plots of the inhibition curves obtained with atropine, d-tubocurarine, pilocarpine and carbachol are illustrated in Fig. 2B. Both the antagonists and

* Correspondence.

the agonists, had Hill coefficients of less than 1·0 (about 0·7 and 0·5 respectively) suggesting the possibility of negative co-operativity.

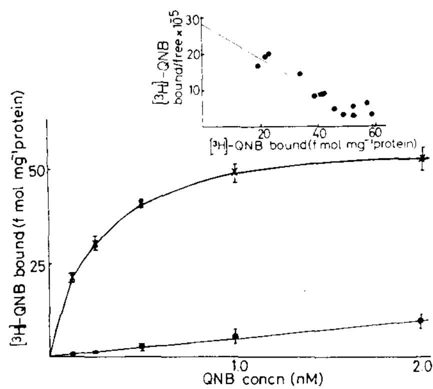

FIG. 1. Saturation curve of [³H]QNB binding to rabbit retina, showing specific binding (×) and non-specific binding (●). Each point is the mean ± standard error of the mean of 4 determinations. Non-specific binding was less than 17% of total binding. Inset: Scatchard plots of [³H]QNB binding to rabbit retina. The apparent K_D (from the slope of the line) is $2·13 \times 10^{-10}$ M and the B_{max} (from the X-intercept) is 59·2 fmol mg^{-1} protein.

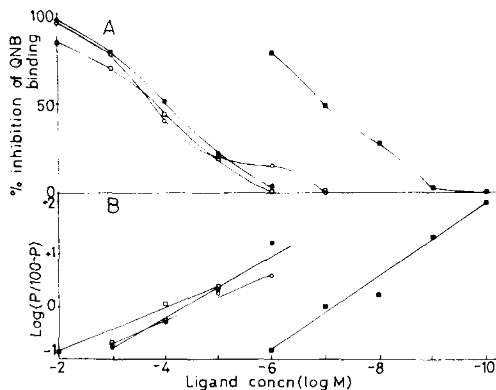

FIG. 2. (A) Inhibition of specific [³H]QNB binding to rabbit retina by cholinergic agents. Symbols: ○, pilocarpine; ●, (+)-tubocurarine; □, carbachol; ■, atropine. 400 μg of protein was incubated for 60 min at 37 °C in medium containing 4 nM [³H]QNB and the concentrations of the agents indicated. Each point represents the mean of 4 determinations and agreed within 15%. The inhibition constants (K_i values, where K_i = IC50/(1 + L.K_d) were: pilocarpine, $9·5 \times 10^{-6}$; carbachol, $9·5 \times 10^{-6}$; (+)-tubocurarine, $4·7 \times 10^{-6}$; atropine, $3·8 \times 10^{-9}$. IC50 is the concentration of inhibitor required to inhibit 50% of the specific binding, L is the ligand concentration and K_d is $2·13 \times 10^{-10}$ M) were determined. (B) Hill plots of the inhibition of [³H]QNB binding by cholinergic agents. Symbols are the same as for (A). P is the percentage bound. Hill coefficients as determined from the slope of the Hill plots were: pilocarpine, 0·51; carbachol, 0·43; (+)-tubocurarine, 0·69; atropine, 0·64.

Discussion

It is generally accepted that the specific binding of [³H]QNB is a good index of muscarinic receptors. Hence the present study provides strong evidence for the presence of muscarinic receptors in the rabbit retina. The characteristics of [³H]QNB binding in the rabbit retina are similar to those in the goldfish, chicken and cow, although the density of receptor sites is 2–3 times higher in the latter two species (Sugiyama et al 1977; Hruska et al 1978; Moreno-Yanes & Mahler 1979; Morgan & Mundy 1982). We do not know the localization of [³H]QNB binding sites in the rabbit retina but in an autoradiographic study in the chicken most of the grains occurred in the inner plexiform layer (Sugiyama et al 1977). Hence the receptors could be present on bipolar, amacrine or ganglion cells. Morgan & Mundy (1982) found that in the chick, intraocular injections of colchicine which selectively destroyed ganglion cells, reduced [³H]α-bungarotoxin binding but had no effect on [³H]QNB binding. These results suggest that the muscarinic receptors are localized in amacrine and/or bipolar cells. Although it would be unwise to assume the same localization of muscarinic receptors in the rabbit retina, the lack of muscarinic receptors on ganglion cells is consistent with most electrophysiological studies of cholinergic drugs on ganglion cell firing (Neal 1983).

In all species, nicotinic receptors (as defined by α-BTX binding) predominate in the retina (Morgan & Mundy 1982; Vogel & Nirenberg 1976; Yazulla 1979) but the muscarinic receptors are by no means negligible. However, their role at present remains unknown.

Supported by the Medical Research Council.

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