J. Pharm. Pharmacol. 1986, 38: 476–478
Communicated December 9, 1985

Interactions of muscarinic drugs with their receptors in single cells of guinea-pig taenia caecum

ISSEI TAKAYANAGI*, KATSUO KOIKE, TSUYOSHI HIRUTA, *Department of Chemical Pharmacology, Toho University School of Pharmaceutical Sciences, Miyama, Funabashi, Chiba 274, Japan*

Single cells were prepared from the guinea-pig taenia caecum and used for the study of drug-receptor interactions. The cells showed a graded response to carbachol, the dose response curve being shifted in a parallel fashion by atropine, indicating a competitive antagonism. The pA_2-value of atropine was in agreement with that estimated using the intact tissue. [^{3}H]QNB (quinuclidinyl benzilate) combined with the single cells, the Scatchard plot yielding a straight line. The dissociation constant of QNB was similar to values estimated in other membrane preparations. The apparent dissociation constants of cholinergic drugs estimated from inhibition of the specific binding of [^{3}H]QNB (0·20 nM) to the single cells by the cholinergic drugs were also in agreement with the values in other membrane preparations. The results indicate that the single smooth muscle cells are useful for the study of drug-receptor interactions.

To study the interaction of a drug with its receptor in smooth muscle, estimation of the mechanical response of intact tissue to the drug and drug binding to membrane preparations derived from various tissues are used. Recently, isolation and contraction of single cells from the stomach of *Bufo marinus*, the aorta of rabbit, vas deferens of rat and taenia caecum of guinea-pig were reported (Fay & Delise 1973; Small 1974; Murray et al 1975; Momose & Gomi 1977, 1978; Johns & Riehl 1982; Obara 1984). Single cells have little extracellular space and contain no tissues except muscle cells. Therefore, they are thought to be useful for studies of drug-receptor interactions. We have observed mechanical responses of single cells to a muscarinic drug and also tested the binding of a ^{3}H-labelled drug to the single cells to study muscarinic drug-receptor interactions.

Materials and methods

Preparation of single cells. Single smooth muscle cells from guinea-pig taenia caecum were prepared by a modified method of Momose & Gomi (1978). Male guinea-pigs, 300–350 g, were killed and the taenia caecum quickly removed and suspended in an incubation medium (mM) NaCl 137, KCl 2·7, $MgCl_2$ 1·0, $CaCl_2$ 0·18, glucose 5·6, HEPES 4·2, pH 7·4, kept at 35 °C for 90 min. Then, slices of the tissue were incubated in a medium containing 0·2% collagenase, 0·4% trypsin inhibitor and 1·0% bovine serum albumin at 35 °C for 30 min followed by centrifugation at 1000*g* for 5 min. The pellets were resuspended in the incubation medium at 35 °C for 15 min. Thereafter, single cells could be readily separated from muscle by gently pipetting the muscle strips in the same solution through a wide-pour Pasteur pipette. The suspension of single cells was filtered through nylon mesh.

Contraction of single cells. Contraction of the single cells was examined as reported previously (Momose & Gomi 1978). The cells were perfused continuously with the incubation medium on a dimethyldichlorosilane-coated slide glass and agonists were applied with the perfusate. The incubation medium was kept at 32 °C and gassed with carbogen. Contraction of the cells was observed with a phase contrast microscope and the degree of the contraction of each was determined by measuring the cells' size on photographic paper. Fig. 1 shows an example of a measurement of cell size. The total length of a cell was divided into straight parts and each straight part was measured; total cell size was then estimated to be the sum of the lengths of all straight parts. Contraction percents in the presence of various doses of carbachol were estimated as

$$[(S_o - S)/(S_0 - S_m)] \times 100$$

where S_0 is the size in the absence and S_m the size in the presence of carbachol, 10^{-5} M, which was the highest dose used, and S the size in the presence of carbachol in the actual dose used. Thus, a dose-response curve was obtained.

The activity of single cells was assessed by the trypan blue exclusion test (Bagby et al 1971; Johns & Riehl 1982). This involved the addition of 20 μl of 0·4% trypan blue solution which was delivered to a Burker–Turk haemocytometer. Then the total number of stained and viable single smooth muscle cells was determined.

Binding assay. Single cells were incubated with various doses of [^{3}H]quinuclidinyl benzilate (QNB) in a total volume of 1·2 ml of incubation medium at 32 °C for 60 min. The incubation mixture was then rapidly filtered through a Whatman GF/B glass filter and the filters washed 3 times with 3 ml of ice-cold incubation medium. After the passage, the filters were dried and radioactivity was determined in a toluene base scintillator with a liquid scintillation spectrometer (Aloka LSC-900).

Non-specific binding was determined as the radioactivity bound to the single cells which was not displaced by 1 μM atropine. To determine the concentration of

* Correspondence.

each test drug that induced 50% inhibition of [^{3}H]QNB binding (IC50 value), competitive antagonism between [^{3}H]QNB (0·20 nM) and the test drug was assessed.

Mechanical response of isolated taenia caecum. To compare mechanical responses of single cells to the muscarinic agonist with those of the intact tissue, a piece of taenia caecum was isolated from a male guinea-pig (300–350 g). This 3–4 cm piece was suspended in a 20 ml organ bath filled with a low Ca-physiological solution of composition the same as that of the incubation medium kept at 32 °C and gassed with carbogen. Responses to drugs were recorded under a tension of 0·7 g.

To test antagonism between an agonist and an antagonist, the isolated taenia caecum and single cells were preincubated with the antagonist for 5 min. Competitive antagonistic activity by a drug against carbachol was expressed as a pA_2 value which was calculated from parallel shifts of the dose response curve of carbachol by the antagonist using the table of van Rossum (1963), and agonistic activity of carbachol as a pD_2 value which was the molar concentration producing a 50% maximal response.

Results and discussion

The viability of the single cells assessed by trypan blue exclusions showed 81·6 ± 2·7% viability (mean with s.e. of 8 groups).

Carbachol contracted the single cells dose-dependently (Figs 1, 2A). The dose-response curve obtained was similar to that in the intact tissue (Fig. 2B); however, the slope of the latter curve was slightly steeper than that of the former curve. This small discrepancy might be due to the presence or absence of the tension (0·7 g), when the contractile responses to carbachol were estimated. Contraction of single cells isolated from the stomach of *Bufo marinus* (Bagby et al 1971; Small 1974) and from guinea-pig taenia caecum (Momose & Gomi 1978) was reported to be a graded response. The present results were consistent with those mentioned above. Atropine (Fig. 2) and pirenzepine, dose-dependently shifted the dose-response curves in both preparations. The pA_2 and pD_2 values estimated in two preparations were in agreement (Table 1).

The specific binding of [^{3}H]QNB to the single smooth muscle cells was saturable (Fig. 3A). The Scatchard plot (Scatchard 1949) of the specific binding of [^{3}H]QNB to the single cells yielded a straight line, and the dissocia-

Table 1. The pD_2 values of carbachol and the pA_2 values of atropine and pirenzepine against carbachol estimated from the mechanical responses of the intact tissue and single smooth muscle cells.

	Carbachol (pD_2)	Atropine (pA_2)	Pirenzepine (pA_2)
Single cells	7·29 ± 0·06 (18)	8·45 ± 0·13 (18)	6·82 ± 0·12 (18)
Intact tissue	7·83 ± 0·08 (4)	8·40 ± 0·10 (4)	6·87 ± 0·15 (4)

Each value is presented as a mean ± s.e. ( ): number of experiments.

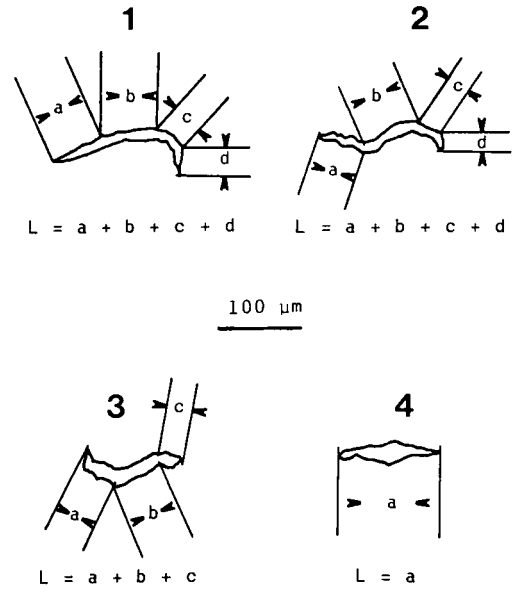

FIG. 1. Measurement of cell size in the absence and presence of carbachol. The total length of a cell was divided into straight parts and the length of each part was measured. The cell size was estimated as the sum of lengths of all straight parts. L: Total cell size. a, b, c or d: length of a straight part. Horizontal bar = 100 μm. 1: The total size (L) is estimated as a + b + c + d, in the absence of carbachol. 2, 3 and 4: The total sizes are estimated as a + b + c + d, a + b + c and a alone in the presence of 10^{-7} M, 10^{-6} M and 10^{-5} M of carbachol, respectively.

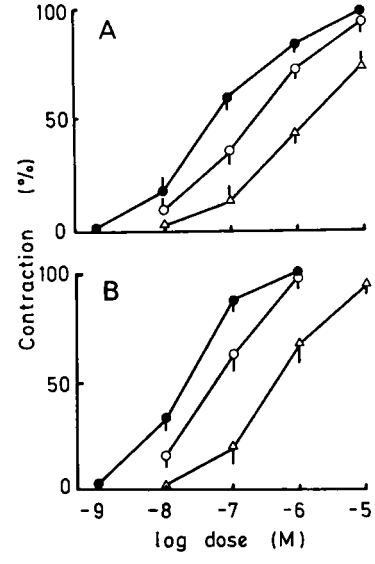

FIG. 2. Dose-response curves of carbachol in the absence and presence of atropine. A: single cells. B: intact tissue. Ordinate: contraction %. Abscissa: log dose (M) of carbachol. ●: carbachol alone, ○: with atropine 10^{-8} M, △: with atropine 10^{-7} M. Each value is presented as a mean ± s.e. (bar). Number of experiments: 18 for single cells and 4 for intact tissue.

tion constant (K_D) and the maximum binding sites (B_{max}) were 0·40 nM and 59·8 fmol 10^{-4} cells, which are a mean of three independent experiments. The K_D value (0·40 nM) is in agreement with that (0·58 nM) estimated in the microsomal fraction from the longitudinal muscle of the guinea-pig ileum (Takayanagi et al 1984) and also similar to K_D values for [^{3}H]QNB binding in membrane preparations from a variety of smooth muscles (Yamamura & Snyder 1979; Bergermeister et al 1978; Morisset et al 1981; Ehlert et al 1980; Johns & Riehl 1982).

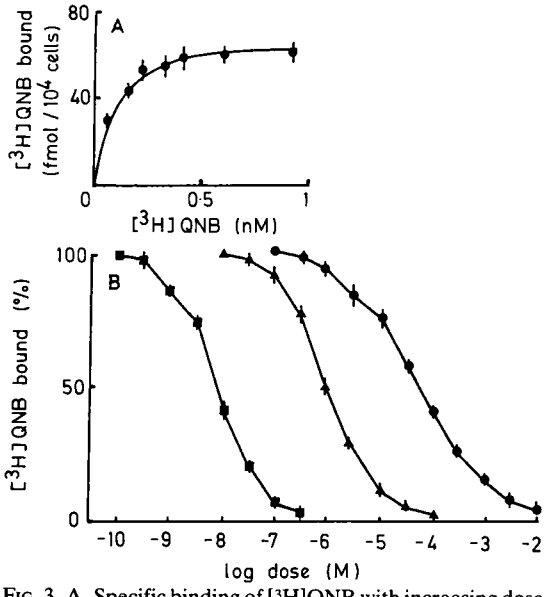

FIG. 3. A. Specific binding of [^{3}H]QNB with increasing dose of [^{3}H]QNB to the single smooth muscle cells. Ordinate: [^{3}H]QNB bound, fmol 10^{-4} cells. Abscissa: dose (nM) of [^{3}H]QNB. Note that specific binding of [^{3}H]QNB to the single cells is saturable. B: Inhibition of [^{3}H]QNB binding to the single cells by carbachol (●), atropine (■) and pirenzepine (▲). Ordinate: [^{3}H]QNB binding (%). The specific binding of [^{3}H]QNB (0·20 nM) was expressed as 100%. Abscissa: log dose (M). Each value is expressed as a mean with s.e. of 4 experiments.

Competitive inhibition curves for specific binding of [^{3}H]QNB by various doses of the test drugs were obtained as shown in Fig. 3B. The 50% inhibition concentrations (IC50 values) were estimated by non-linear analysis (Lemoine & Kauman 1983). The apparent dissociation constants, K_i, were calculated by the following equation: $K_i = IC50/(1 + L/K_D)$, where L and K_D are the dose and dissociation constant of [^{3}H]QNB, respectively. The pK_i of atropine (8·52 ± 0·05) was in agreement with that (8·41) calculated from the IC50 estimated in the microsomal fractions derived from the longitudinal muscle of the guinea pig ileum (Takayanagi et al 1984, 1985). The pK_i values of atropine (8·52 ± 0·05) and pirenzepine (6·39 ± 0·05) were equal to their pA_2 values estimated from the mechanical responses of the single cells and intact tissue (Table 1). The slope (0·62 ± 0·03) of the competitive inhibition curve for carbachol only, was significantly less than unity. A modified Scatchard plot was drawn by a dose (M) of carbachol and inhibition (%) of specific binding of [^{3}H]QNB (0·20 nM) by various doses of carbachol according to Rugg et al (1978). The modified Scatchard plots for competitive antagonists, atropine and pirenzepine yielded straight lines and the slope (nH) of Hill plots for them were not different from unity. The modified Scatchard plot for carbachol was concave, suggesting that there are two binding sites: high and low affinity sites. The Hill coefficient (nH) was lower than unity. The modified Scatchard analysis of the specific QNB binding is in agreement with studies in membrane preparations from other tissues (Takayanagi et al 1985; Ehlert et al 1980).

Comparison between the specific bindings of some β-adrenergic drugs to the single smooth muscle cells and to the microsomal fraction was reported by Koike & Takayanagi (1984), who found that the results in single cells were in agreement with those in the microsomal fraction. The present results indicate that the single smooth muscle cells are useful for studies of the drug-receptor interactions.

REFERENCES

Bagby, R. M., Young, A. M., Dotson, R. S., Fisher, B. A., McKinnon, K. (1971) Nature 234: 351–352

Bergermeister, W., Klein, W. L., Nirenberg, M., Witkop, B. (1978) Mol. Pharmacol. 14: 751–767

Ehlert, F. J., Roeske, W. R., Yamamura, H. I. (1980) J. Supramol. Struct. 14: 149–162

Fay, F. S., Delise, C. M. (1973) Proc. Nat. Acad. Sci. 70: 641–645

Johns, A., Riehl, R. M. (1982) J. Pharmacol. Methods 7: 153–159

Koike, K., Takayanagi, I. (1984) Gen. Pharmacol. 15: 47–50

Lemoine, H., Kauman, A. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 322: 111–120

Momose, K., Gomi, Y. (1977) Chem. Pharm. Bull. 25: 2449–2451

Momose, K., Gomi, Y. (1978) J. Pharmacobiodyn. 1: 184–191

Morisset, J., Geoffnion, L., Losose, L., Lanöe, J., Poirier, G. G. (1981) Pharmacology 22: 189–195

Murray, J. J., Reed, P. W., Fay, F. S. (1975) Proc. Nat. Acad. Sci. 72: 4459–4463

Obara, K. (1984) Jap. J. Pharmacol. 34: 41–54

Rugg, E. L., Barnett, D. B., Nahorski, S. R. (1978) Mol. Pharmacol. 14: 96–1005

Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51: 660–672

Small, J. V. (1974) J. Cell Sci. 24: 327–349

Takayanagi, I., Koike, K., Okumura, K. (1984) Eur. J. Pharmacol. 99: 107–110

Takayanagi, I., Okumura, K., Koike, K. (1985) Gen. Pharmacol. 16: 269–272

van Rossum, J. M. (1963) Arch. Int. Pharmacodyn. Ther. 143: 299–330

Yamamura, H. I., Snyder, S. H. (1979) Mol. Pharmacol. 10: 861–867