6. M. Goyette, C. J. Petropoulos, P. R. Shank and N. Fausto, *Science* **219**, 510 (1983).
7. C. Petropoulos, G. Andrews, T. Tamaoki and N. Fausto, *J. biol. Chem.* **258**, 4901 (1983).
8. K. Taketa, A. Watanabe and K. Kosaka, *Ann. N.Y. Acad. Sci.* **259**, 80 (1975).
9. C. Aussel, C. Stora and B. Krebs, *Biochem. biophys. Res. Commun.* **95**, 796 (1980).
10. M. Mourelle and B. Rubalcava, *J. biol. Chem.* **256**, 1656 (1981).
11. I. Matsui, L. Wiegand and A. E. Pegg, *J. biol. Chem.* **256**, 2454 (1981).
12. R. Labow, G. F. Maley and F. Maley, *Cancer Res.* **29**, 366 (1969).
13. P. Groebner and P. Loidl, *Biochim. biophys. Acta* **697**, 83 (1982).
14. R. L. Blakley, *The Biochemistry of Folic Acid and Related Pteridines*, p. 231. Elsevier, New York (1969).
15. M. Friedkin, *Adv. Enzymol. Relat. Areas molec. Biol.* **38**, 235 (1973).
16. A. Kornberg, *DNA Replication*, p. 39. Freeman, San Francisco (1980).
17. F. M. Huennekens, C. K. Mathews and K. G. Scrimgeour, *Meth. Enzym.* **VI**, 802 (1966).
18. A. Karmen, F. Wroblewski and J. S. La Due, *J. clin. Invest.* **34**, 126 (1955).
19. F. Wroblewski and J. S. La Due, *Proc. Soc. exp. Biol. Med.* **91**, 569 (1956).
20. D. Roberts, *Biochemistry* **5**, 3546 (1966).
21. E. Bresnick, U. B. Thompson, H. P. Morris and A. G. Liebelt, *Biochem. biophys. Res. Commun.* **16**, 278 (1964).
22. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
23. H. J. Zimmerman, Y. Kodera and M. West, *J. Lab. clin. Med.* **66**, 315 (1965).
24. F. Maley and G. F. Maley, *J. biol. Chem.* **235**, 2968 (1960).
25. J. A. Ferdinandus, H. P. Morris and G. Weber, *Cancer Res.* **31**, 550 (1971).
26. R. C. Garner and A. E. M. McLean, *Biochem. Pharmac.* **18**, 645 (1961).
27. M. F. Sorrell, D. T. Tuma, J. K. Noffsinger and A. J. Barak, *Proc. Soc. exp. Biol. Med.* **143**, 839 (1973).
28. G. R. Cameron and W. A. E. Karunaratne, *J. Path. Bact.* **42**, 1 (1936).

Biochemical Pharmacology, Vol. 34, No. 4, pp. 588–590, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

Trihexyphenidyl—Further evidence for muscarinic receptor subclassification

(*Received* 5 *March* 1984; *accepted* 3 *August* 1984)

Lately, an increased interest has been focused on the study of subtypes of muscarinic receptors. Recent reports [1–6] have shown that pirenzepine, a new synthetic muscarinic antagonist, binds with high affinity to some tissues and low affinity to others. Cerebral cortex, sympathetic ganglia, salivary glands and epithelial cells of the intestine* show high affinity pirenzipine binding and are thought to contain muscarinic M-1 receptors. Cerebellum, heart and muscle layers of the gastrointestinal tract* show low affinity pirenzepine binding and are thought to contain muscarinic M-2 receptors [1, 2]. New synthetic anticholinergic analogues have been identified which also have a tissue selectivity like that of pirenzepine and differentiate between M-1 and M-2 subtypes of muscarinic receptors [7]. Since a large number of compounds with antimuscarinic properties are available, it seems probable that some of those might show selectivity similar to that of pirenzepine. Many compounds with muscarinic receptor antagonist properties have been compared for binding affinity to muscarinic receptors of rat brain and guinea pig ileum [8]. Most antagonists show equal affinity for the two tissues, but trihexyphenidyl (Artane) displays a 10-fold higher affinity for the muscarinic receptors of the brain than for those of the ileum [8].

The aim of this investigation was to verify whether trihexyphenidyl shows selectivity for muscarinic receptor subtypes of the ileum and brain and to determine whether this compound, like pirenzepine, indicates that receptors of intestinal smooth muscle are different from those of intestinal epithelial cells.

Materials and methods

Chemicals. [^{3}H]Quinuclidinyl benzilate (QNB, 33.2 Ci/mmole) was purchased from the New England Nuclear Corp., Boston, MA. (-)-Scopolamine methyl bromide, trihexyphenidyl hydrochloride and trizma hydrochloride and base were obtained from the Sigma Chemical Co., St. Louis, MO.

Methods. Trihexyphenidyl and methyl scopolamine displacement of [^{3}H]QNB binding were carried out on cerebral cortex, heart and colonic tissues obtained from male Sprague–Dawley rats (200–300 g). Colonic epithelial cells were vibrated off from the colon, and the remaining colonic tissue (which contains nerve plexuses in addition to the muscle fibers) was used as colonic smooth muscle. All the tissues were homogenized in Tris buffer (pH 7.4) and were centrifuged. Pellets were collected and used for binding assays. Binding experiments were conducted according to the procedure of Yamamura and Snyder [9]. The binding incubation mixture contained 1 nM [^{3}H]QNB, sufficient membrane protein to give approximately 1000 cpm bound in the absence of unlabeled competitors, and various concentrations of either methyl scopolamine or trihexyphenidyl in a final volume of 1.5 ml. Incubation was at 37° for 30–40 min. Bound and free [^{3}H]QNB were separated by filtration. The results from each experiment were plotted, and the IC_{50} values (concentration of unlabeled competitor that reduced [^{3}H]QNB binding to half of that observed with no competitor) were determined directly from the graphs. Means were calculated using log IC_{50} from three separate experiments. Statistical significance was evaluated using Duncan's multiple comparison test.

Results and discussion

Methyl scopolamine and trihexyphenidyl were studied for the ability to displace [^{3}H]QNB binding. A representative curve for each antagonist in the four tissues is shown in Fig. 1. The methyl scopolamine displacement curves were very close to each other in the four tissues. In contrast, the trihexyphenidyl displacement curves fell into two groups such that displacement occurred at a lower concentration of trihexyphenidyl in cerebral cortex and colonic epithelial cells than in heart and colonic smooth muscle. Finally, in all tissues, methyl scopolamine was somewhat more potent than trihexyphenidyl at displacing [^{3}H]QNB binding.

Means from replicate data are summarized in Table 1. For methyl scopolamine displacement of [^{3}H]QNB binding,

* X. Y. Tien, R. Wahawisan, L. J. Wallace and T. S. Gaginella, manuscript submitted for publication.

Table 1. IC_{50} values (nM) and Hill coefficients for methyl scopolamine and trihexyphenidyl displacement of [^{3}H]QNB binding*

	Methyl scopolamine		Trihexyphenidyl		
	IC_{50}corr	Hill	IC_{50}corr	Hill	Ratio (T/M)
Cerebral cortex	0.158 (0.147–0.176)	0.78	1.32 (1.11–1.76)	1.00	8.4 (7.2–9.8)
Colon cells	0.299 (0.220–0.439)	0.82	1.75 (1.46–2.49)	1.14	5.8 (4.5–7.6)
Heart	0.534 (0.464–0.590)	0.89	16.6 (15.2–17.7)	0.98	31.0 (17.8–29.0)
Colon muscle	0.827 (0.641–1.07)	1.02	18.8 (12.8–24.2)	0.75	22.7 (24.4–33.8)

* Membrane preparations were incubated with 1 nM [^{3}H]QNB and various concentrations of either methyl scopolamine (M) or trihexyphenidyl (T) for 30–40 min. Bound and free QNB were separated by filtration. The IC_{50} values were corrected for the radioligand occupancy shift using the equation $IC_{50}corr = IC_{50}/(1 + [L]/K_d)$, where $[L]$ and K_d represent concentration and dissociation constant of the radioligand. K_d values for QNB binding were 0.0623, 0.0790, 0.0921, and 0.166 nM for cerebral cortex, colon cells, heart, and colon muscle respectively. IC_{50}corr are means of three separate determinations, and values in parentheses are the extremes of the three determinations. Hill coefficients are means of three determinations using linear regression to calculate the slope of the equation plotting log $((100\text{-}Bd)/Bd)$ vs log $([C])$, where Bd represents QNB bound expressed as a percentage of specific binding in the absence of unlabeled competitor and $[C]$ represents concentration of unlabeled competitor. Ratio (T/M) was calculated as the antilog of the difference in mean IC_{50}corr values for T and M using log transformed data, and values in parentheses are antilogs of difference of mean ± S.E.M., where S.E.M. = square root of (S.E.M.T + S.E.M.M).

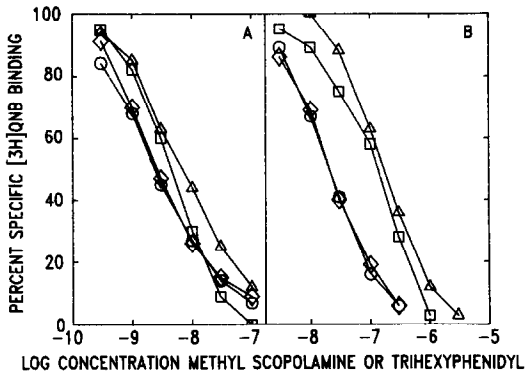

Fig. 1. Displacement of [^{3}H]QNB binding by methyl scopolamine (A) and trihexyphenidyl (B). Membrane preparations were incubated with 1 nM [^{3}H]QNB and various concentrations of either methyl scopolamine or trihexyphenidyl for 30–40 min. Bound and free QNB were separated by filtration. Symbols: (◇) cerebral cortex, (○) colonic epithelial cells, (□) colonic smooth muscle, and (△) heart.

the range of IC_{50} values was relatively narrow (about 5-fold). In spite of this, the IC_{50} for cerebral cortex was significantly different ($P < 0.01$) from those determined for colonic smooth muscle and heart. In other studies, methyl scopolamine binding affinity is nearly the same in all tissues tested [2]. Thus, the small range of IC_{50} values (less than 5-fold) observed in the present investigation is similar to that reported by other investigators [2].

In contrast to the data for methyl scopolamine displacement of [^{3}H]QNB binding, IC_{50} values for trihexyphenidyl displacement of [^{3}H]QNB binding showed a greater amount of variation from one tissue to the next. There was approximately a 13-fold difference between cerebral cortex and heart, and about an 11-fold difference between colonic epithelial cells and colonic smooth muscle. The IC_{50} values from both cerebral cortex and colonic epithelial cells were significantly different ($P < 0.01$) from those of both colonic muscle and heart. These data are consistent with the concept that the muscarinic receptors of the cerebral cortex and of the colonic epithelial cells are different from those of the heart and of colonic smooth muscle.

The conclusion that trihexyphenidyl, but not methyl scopolamine, shows tissue-dependent variations in potency of displacement of [^{3}H]QNB binding is supported by the values of the ratio of IC_{50} values for trihexyphenidyl to methyl scopalamine in each tissue (Table 1). Thus, cerebral cortex and colonic epithelial cells, reported to contain mostly M-1 receptors, show ratios of 8.4 and 5.8. In contrast, heart and colonic smooth muscle, which contain mostly M-2 receptors, show ratios of 31 and 23.

Our data showing a 14-fold difference between intestinal smooth muscle and cerebral cortex in IC_{50} values of trihexyphenidyl displacement for [^{3}H]QNB binding compare favorably with the 10-fold difference reported by Snyder and Yamamura [8]. Thus, data from both reports suggest a tissue-dependent variation in the affinity of trihexyphenidyl for muscarinic receptors. A comparison of our results using trihexyphenidyl with those using pirenzepine [10],* shows that tissues with high affinity trihexyphenidyl binding also have high affinity pirenzepine binding, and that those with a low affinity trihexyphenidyl binding also have a low affinity pirenzepine binding. Based on this, both ligands appear to show selectivity for the postulated muscarinic receptor subtypes. The affinity of these ligands appears to be greater for M-1 receptors than for M-2 receptors.

Trihexyphenidyl is used clinically in the treatment of Parkinson's disease [11]. Although not lacking side effects, it does produce fewer side effects than the natural anti-muscarinic alkaloids such as atropine and scopolamine [11]. This may be partly explained by lower affinity of trihexyphenidyl for the muscarinic receptors in heart and smooth muscle as compared to the affinity in the cerebral structures where the drug acts to produce its therapeutic effect.

* X. Y. Tien, R. Wahawisan, L. J. Wallace and T. S. Gaginella, manuscript submitted for publication.

In summary, our results further support the hypothesis that muscarinic receptors can be divided into subclasses with cerebral cortex and intestinal epithelial cells containing one class and heart and intestinal smooth muscle containing the other. In addition, our results support the hypothesis that trihexyphenidyl (Artane), like pirenzepine [1–6], is a drug that shows selectivity for the M-1 subclass of the muscarinic receptors.

Acknowledgement—This work was supported by USPHS Grant AM 28299.

Division of Pharmacology
College of Pharmacy
The Ohio State University
Columbus, OH 43210, U.S.A.

XIAO-YING TIEN
LANE J. WALLACE*

REFERENCES

1. M. Watson, H. I. Yamamura and W. R. Roeske, *Life Sci.* **32**, 3001 (1983).
2. R. Hammer, C. P. Berrie, N. J. M. Birdsall, A. S. V. Burgen and E. C. Hulme, *Nature, Lond.* **283**, 90 (1980).
3. R. Hammer, *Scand. J. Gastroent.* **17** (Suppl. 72), 59 (1982).
4. R. Hammer and A. Giachetti, *Life Sci.* **31**, 2991 (1982).
5. N. J. M. Birdsall and E. C. Hulme, *Trends pharmac. Sci.* **4**, 459 (1983).
6. F. J. Ehlert, W. R. Roeske and H. I. Yamamura, *Trends Neurosci.* **5**, 336 (1982).
7. R. E. Gibson, W. J. Rzeszotarski, W. C. Eckelman, E. M. Jagoda, D. J. Weckstein and R. C. Reba, *Biochem. Pharmac.* **32**, 1851 (1983).
8. S. H. Snyder and H. I. Yamamura, *Archs gen. Psychiat.* **34**, 236 (1977).
9. H. I. Yamamura and S. H. Snyder, *Molec. Pharmac.* **10**, 861 (1974).
10. N. J. M. Birdsall, A. S. V. Burgen, R. Hammer, E. C. Hulme and J. Stockton, *Scand. J. Gastroent.* **15**, (Suppl. 66), 1 (1980).
11. J. R. Bianchine, in *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Eds. A. G. Gilman, L. S. Goodman and A. Gilman), 6th Edn, p. 485. Macmillan, New York (1980).

* Author to whom all correspondence should be addressed.