Two Apparently Distinct Muscarinic Cholinoceptor Mechanisms in Guinea-Pig Taenia Caecum

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Abstract—Thirty-min treatment of guinea-pig taenia caecum with 300 nM propylbenzilylcholine mustard (PrBCM) shifted the concentration-response curve for carbachol to the right with a reduction of the maximum contraction, but 90-min treatment did not result in further inhibition. Under these conditions, pilocarpine hardly contracted the preparations, and it competitively antagonized carbachol. Muscarinic agonists might interact with two types of receptor mechanisms and carbachol elicited a stimulus from both types, whereas pilocarpine did so predominantly from the PrBCM-sensitive one.

Muscarinic cholinceptors have recently been subdivided into at least two classes, M_1 - and M_2 -receptors, mainly using non-classical antagonists such as pirenzepine (1). However, in addition to the subclassification based on these antagonists, heterogeneity of muscarinic agonism has been reported (2–4). This heterogeneity is found in the preparation which predominantly contains one type of receptor as revealed by the use of the antagonists, although evidence for the origin of the heterogeneity is not yet conclusive.

M_2 -receptors exist predominantly in guinea-pig taenia caecum (our unpublished data), but heterogeneity of muscarinic agonism has not been studied. Propylbenzilylcholine mustard (PrBCM) (5), an irreversible specific muscarinic cholinergic antagonist, has been used increasingly to occlude the receptors. In this communication, we report the effect of treatment with PrBCM on the contractile responses of guinea-pig taenia caecum to carbachol and pilocarpine.

Taenia caecum was obtained from female guinea-pig (300–500 g) and suspended in an organ bath which contained 20 ml of physiological solution of the following composition (mM): NaCl, 154; KCl, 5.6; $MgCl_2$, 2.1; $CaCl_2$, 0.8; $NaHCO_3$, 6.0; and glucose, 2.8. This was kept at 30°C and bubbled with

air. Carbachol chloride or pilocarpine hydrochloride (both from Sigma Chemical Co.) was applied in the presence of 300 nM tetrodotoxin (Sankyo Co.) using a cumulative technique and the mechanical response was recorded isotonically.

For occlusion of muscarinic cholinergic receptors, after the control concentration-response curve(s) for carbachol or carbachol and pilocarpine was determined, the preparation was treated for 5 min or longer with 300 nM or 1 μ M activated PrBCM (New England Nuclear) prepared according to the method of Young et al. (5). When PrBCM was applied for more than 10 min, it was renewed every 10 min. The preparation was then allowed to re-equilibrate for 60 min, with washing every 10 min, and the concentration-response curve for carbachol or pilocarpine was determined by repeating determinations of the curves until constant ones were established. For progressive occlusion of the receptors, the same protocol was repeated.

In competition experiments, the preparation was treated with atropine sulfate (Sigma Chemical Co.) or pilocarpine, and in the presence of the drug, the concentration-response curve for carbachol was determined. Competitive antagonism was evaluated according to the method of Arunlakshana and Schild (6), and the slope of the Schild plot and pA_2 value was calculated by linear

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regression analysis (7).

Figure 1A shows the effect of progressive treatments of guinea-pig taenia caecum with 300 nM PrBCM on the carbachol-induced contraction. The concentration-response curve for carbachol was shifted in a parallel fashion to the right after 10-min treatment with activated PrBCM, and treatment for an additional 20 min produced a further displacement of the curve with a slight, but significant reduction of the maximum response. However, more prolonged treatments with PrBCM had no further significant effect on the concentration-response curve. The limiting effect of PrBCM on the concentration-

response curve for carbachol obtained after treatments using 1 μ M PrBCM or a single treatment with 300 nM PrBCM for 90 min was the same as that determined after successive treatments with 300 nM PrBCM for a combined duration of 50 min (data not shown).

It has been reported that phenoxybenzamine and dibenamine inhibits cellular responses nonspecifically to some degree (8). However, treatment with PrBCM did not inhibit KCl-induced contraction: EC_{50} 's (negative logarithm of molar concentration to produce 50% of maximum contraction) of K determined from graphical analyses were 19.3 ± 0.35 and 15.9 ± 0.49 mM in the control and the PrBCM-treated preparations (300 nM, 50 min), respectively, and the maximum response after the treatment was $107.6 \pm 4.0\%$ of the control ($n=4$). The result suggests that PrBCM would not inhibit carbachol-induced response by depression of smooth muscle contractility.

Atropine antagonized the response to carbachol of the preparation treated with PrBCM for 50 min in a competitive manner. The slope of the Schild plot and the calculated pA_2 values were 0.91 ± 0.28 and 9.06 ± 0.14 ($n=5$), respectively. The pA_2 values were not significantly different from those determined in untreated preparations: the values of slope and pA_2 were 1.20 ± 0.37 and 9.03 ± 0.13 ($n=6$), respectively. In both cases, the slope values of the Schild plot were not different from unity.

Figure 1B shows the effect of treatment with PrBCM on the concentration-response curve for pilocarpine. The intrinsic activity of pilocarpine was 0.83 ± 0.04 ($n=6$). When the preparation was treated with PrBCM for 5 min, the maximum response to pilocarpine was greatly reduced, and 20-min treatment almost abolished the response. After 50-min treatment with PrBCM, pilocarpine antagonized the residual response to carbachol in a competitive manner (Fig. 2): the values of the slope of the Schild plot and pA_2 were 0.97 ± 0.11 and 5.67 ± 0.14 ($n=6$), respectively. The slope value of the Schild plot was not different from unity.

The present study using guinea-pig taenia caecum confirmed that PrBCM irreversibly

Fig. 1. Effects of progressive treatments with propylbenzylcholine mustard (PrBCM) on the responses to carbachol (A) and pilocarpine (B) in guinea-pig taenia caecum. After establishing the control concentration-response curves for carbachol (●) and pilocarpine (○), the preparation was exposed to 300 nM PrBCM and then washed until a stable concentration-response curve could be obtained. The treatment was then repeated. Cumulative durations of the treatments with PrBCM were 5 (▲), 10 (△), 20 (■), 30 (□), 40 (▼) and 50 min (▽). Each point represents the mean $\pm$ S.E. of 7 (A) and 6 (B) determinations.

Fig. 2. Effect of pilocarpine on contraction-response curve for carbachol in preparations treated with 300 nM PrBCM for 50 min. ●: Control curve for carbachol; ○, ▲, △ and □: curves for carbachol after PrBCM-treatment in the presence of 0, 30, 100 and 300 μ M pilocarpine, respectively. Each point represents the mean $\pm$ S.E. of 6 determinations.

antagonizes the responses to muscarinic agonists (5). However, when carbachol was used as an agonist, it elicits a pharmacological stimulus through the interaction of two distinct, non-interconvertible muscarinic cholinergic mechanisms, and PrBCM but not atropine is a selective antagonist which can discriminate between the two. Since the types of muscarinic receptors subclassified so far by use of non-classical antagonists have been reported to be inactivated by PrBCM (9), the PrBCM-resistant receptors may be a novel type or a special coupling with some factor or effector system(s) may make them PrBCM-resistant.

In guinea-pig taenia caecum, pilocarpine behaved as a partial agonist. Disappearance of the response to pilocarpine by PrBCM suggests that pilocarpine produces contractions predominantly through activation of PrBCM-sensitive receptor mechanisms. Pilocarpine competitively antagonized the response of the PrBCM-treated preparations to carbachol, suggesting a possibility that pilocarpine actually binds to the PrBCM-resistant receptors but does not produce a detectable stimulus.

It has been reported that PrBCM is selective for some populations of rat heart muscarinic receptors (3) and that the drug cannot inactivate [3 H]QNB binding sites of guinea-pig heart completely (4). Therefore, our pharmacological data might provide

clues for the finding of unknown receptor subtypes or to an understanding of the mechanisms of receptor-effector coupling, though much additional experimental support such as the difference in Ca-utilization, second messenger systems or dependency on energy metabolism will be required.

References

- 1 Hammer, R., Berrie, C.P., Birdsall, N.J.M., Burgen, A.S.V. and Hulme, E.C.: Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature* **283**, 90–92 (1980)
- 2 Burgen, A.S.V. and Spero, L.: The action of acetylcholine and other drugs on the efflux of potassium and rubidium from smooth muscle of the guinea-pig intestine. *Br. J. Pharmacol.* **58**, 613–620 (1968)
- 3 Christophe, J., De Neef, P., Robberecht, P. and Waelbroeck, M.: Propylbenzilylcholine mustard is selective for rat heart muscarinic receptors having a low affinity for agonists. *Br. J. Pharmacol.* **88**, 63–70 (1986)
- 4 Mizushima, A., Uchida, S., Zhou, X.-M., Kagiya, T. and Yoshida, H.: Cardiac M_2 receptors consist of two different types, both regulated by GTP. *Eur. J. Pharmacol.* **135**, 403–409 (1987)
- 5 Young, J.M., Hiley, R. and Burgen, A.S.V.: Homologues of benzilylcholine mustard. *J. Pharm. Pharmacol.* **24**, 950–954 (1972)
- 6 Arunlakshana, O. and Schild, H.O.: Some quantitative uses of drug antagonist. *Br. J. Pharmacol.* **14**, 48–58 (1959)
- 7 Tallarida, R.J., Cowan, A. and Adler, M.W.: pA_2 and receptor differentiation: a statistical analysis

- of competitive antagonism. *Life Sci.* **25**, 637–654 (1979)
- 8 Gengo, P.J., Yousif, F., Janis, R.A. and Triggle, D.J.:** Interaction of phenoxybenzamine with muscarinic receptors and calcium channels. *Biochem. Pharmacol.* **33**, 3445–3449 (1984)
- 9 El-Fakahany, E.E., Cioffi, C.L., Abdellatif, M.M. and Miller, M.M.:** Competitive interaction of pirenzepine with rat brain muscarinic acetylcholine receptors. *Eur. J. Pharmacol.* **131**, 237–247 (1986)