*Br. J. Pharmac.* (1987), **91**, 6–8

# Differential effects of pertussis toxin and lithium on muscarinic responses in the atria and ileum: evidence for receptor heterogeneity

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Pretreatment with pertussin toxin was shown to inhibit selectively muscarinic responses in the atria, but have no effect on responses in the ileum. The converse was true when the animals were pretreated with lithium. These data are consistent with the hypothesis that muscarinic receptors in the atria are coupled to an inhibition of adenylate cyclase, whilst those in the ileum are coupled to inositol phospholipid hydrolysis and indicate differences in the muscarinic receptors in these two tissues.

**Introduction** Muscarinic receptors are designated as $M_1$ or $M_2$. Ileal and atrial muscarinic receptors exhibit a low affinity towards pirenzepine, and are classified as $M_2$. Muscarinic receptors in muscle differ from those in atria, because smooth muscle receptors exhibit a high affinity for the antagonists 4-diphenylacetoxy-N-methyl-piperidine methiodide (4-DAMP: Barlow & Shepherd, 1985) and hexahydro siladiphenidol (Fuder *et al.*, 1985) whilst atrial receptors exhibit a high affinity towards AF-DX 116 (Giachetti *et al.*, 1986) and himbacine (Anwar-ul *et al.*, 1986). However, antagonists with selectivity greater than 50 fold are unavailable. To characterize further muscarinic subtypes we have investigated second messenger responses in atria and ileum.

Muscarinic receptors may be classified according to the effector system (Harden *et al.*, 1986): it has been proposed that the $M_1$ receptor is coupled, by a regulatory N protein ($N_p$), to inositol phospholipid hydrolysis and the $M_2$ receptor type by a different regulatory protein ($N_i$) to inhibition of adenylate cyclase activity. However, this has been complicated by reports that $M_1$ and $M_2$ receptors may be coupled to both effectors (Kelly *et al.*, 1985; Brown *et al.*, 1985). The relationship of the $M_1/M_2$ classification to effector coupling, therefore, remains ambiguous.

**Methods** All tissues were removed from Dunkin-Hartley guinea-pigs (male, 250 g b.w.) and placed in physiological salt solution (PSS) at 30°C, pH 7.4, under 1 g tension for isometric tension recording. Left atrial tissue was incubated in Krebs PSS: (mmol $l^{-1}$: NaCl 118.41, KCl 4.69, $MgSO_4.7H_2O$ 1.18, $KH_2PO_4$ 1.18, glucose 11.1, $NaHCO_3$ 24.99 and $CaCl_2.6H_2O$ 2.52) and electrically paced (2 Hz, threshold voltage). Ileal tissues were placed in Tyrode PSS: (mmol $l^{-1}$: NaCl 136.89, KCl 2.68, $MgCl_2.6H_2O$ 1.05, $NaH_2PO_4.2H_2O$ 0.42, glucose 5.55, $NaHCO_3$ 11.90 and $CaCl_2.6H_2O$ 1.80). After 60 min equilibration, agonists were added either cumulatively (atria), or non-cumulatively (ileum; 30 s exposure on a 5 min dose-cycle). Antagonist affinities ($pA_2$) were estimated by the method of Arunlakshana & Schild (1959).

*Animal pretreatment* (a) *Pertussis toxin*: Pertussis toxin (10 $\mu g\,kg^{-1}$) or equivalent volume of vehicle was injected into the jugular vein under ether anaesthesia. Three days later, the animals were killed and the tissues were isolated.

(b) *Lithium:* Lithium chloride (6.8 mmol $kg^{-1}$) or equivalent volume of vehicle was injected i.p., on a daily basis for 1 to 3 days. Eighteen hours after the final injection the animals were killed and tissues were isolated.

All drugs were obtained from Sigma Chemical Co. Ltd, with the exception of pertussis toxin, which was obtained from List Biological Labs, U.S.A.

**Results** Muscarinic receptors in the atria were found to be selectively inhibited by pretreatment with pertussis toxin (0.3–10 $\mu g\,kg^{-1}$, i.v.). This agent ADP-ribosylates the regulatory $N_i$ protein coupling the muscarinic receptor to the enzyme, adenylate cyclase, thus functionally inactivating the receptor. Pertussis toxin (10 $\mu g\,kg^{-1}$, i.v.) abolished the responses in the atria to the muscarinic agonists carbachol, bethanechol and pilocarpine. In contrast, pretreatment of animals with this dose of pertussis was without effect on responses to muscarinic agonists in the ileum.

Muscarinic receptor function in the ileum was selectively inhibited by pretreatment with lithium. Lithium has been shown to inhibit myoinositol-1-phosphate activity (Allison & Steward, 1971), and

thus decrease receptor-mediated inositol phospholipid hydrolysis. Following three days' pretreatment, the responses to the partial agonist pilocarpine (Table 1b) were significantly attenuated both in terms of potency and maximum response. The treatment also produced a similar effect on the action of bethanechol but somewhat surprisingly, the responses to the agonist of highest efficacy, carbachol, were unaffected. The responses to muscarinic agonists in the atria were completely unaffected by lithium pretreatment.

Pretreatment of the animals with equimolar doses (6.8 mmol $kg^{-1}$, i.p.) of myoinositol and lithium, in order to circumvent the lithium blockade, reversed the effects of lithium pretreatment and the potencies and maximum responses of the agonists were restored to control values (data not shown). There was no effect of the myoinositol alone in either ileal or atrial tissues. The effects of lithium pretreatment did not affect the $pA_2$ values for pirenzepine. (Control animals, atrial $pA_2 = 6.8 \pm 0.05$: ileal $pA_2 = 6.9 \pm 0.08$; lithium-treated animals, atrial $pA_2 = 6.8 \pm 0.07$: ileal $pA_2 = 6.8 \pm 0.05$. All slopes not significantly different from unity; values are mean ± s.e.mean, $n = 4-6$).

**Discussion** We have observed that pretreatment of animals with pertussis toxin selectively inhibits muscarinic receptor function in the atria, but is without effect on responses in the ileum. In contrast, pretreatment of animals with lithium inhibits muscarinic receptor function in ileum without affecting atrial muscarinic responses. These data are consistent with there being different effector systems coupled to atrial and ileal muscarinic receptors.

The data are, therefore, in agreement with the proposal that muscarinic receptors may be defined according to their effector systems (Harden *et al.*, 1986). The hypothesis that muscarinic receptors differ in the atria and smooth muscle has been postulated on the basis of differential antagonist affinities and the

**Table 1** Effect of pertussis toxin or lithium on the potency ($-\log EC_{50}$) and maximum response relative to carbachol (α) of muscarinic agonists

a) *Pertussis toxin*

| | *Control* | | | |
|---|---|---|---|---|
| | *Atria* | | *Ileum* | |
| | $-\log EC_{50}$ | α | $-\log EC_{50}$ | α |
| Carbachol | 6.8 ± 0.03 | 1.0 | 6.8 ± 0.06 | 1.0 |
| Bethanechol | 5.8 ± 0.05 | 1.0 | 5.6 ± 0.06 | 1.0 |
| Pilocarpine | 5.6 ± 0.08 | 0.9 | 5.8 ± 0.07 | 0.6 |

| | *Pertussis toxin* (10 μg $kg^{-1}$, pretreated) | | | |
|---|---|---|---|---|
| | *Atria* | | *Ileum* | |
| | $-\log EC_{50}$ | α | $+\log EC_{50}$ | α |
| Carbachol | No response* | | 6.7 ± 0.05 | 1.0 |
| Bethanechol | No response* | | 5.5 ± 0.03 | 1.0 |
| Pilocarpine | No response* | | 5.9 ± 0.08 | 0.6 |

b) *Lithium*

| | *Control* | | | |
|---|---|---|---|---|
| | *Atria* | | *Ileum* | |
| | $-\log EC_{50}$ | α | $-\log EC_{50}$ | α |
| Carbachol | 6.7 ± 0.15 | 1.0 | 6.5 ± 0.15 | 1.0 |
| Bethanechol | 5.7 ± 0.14 | 1.0 | 5.2 ± 0.13 | 1.0 |
| Pilocarpine | 5.5 ± 0.16 | 0.8 | 5.9 ± 0.12 | 0.6 |

| | *Lithium* (6.8 mmol $kg^{-1}$, pretreated) | | | |
|---|---|---|---|---|
| | *Atria* | | *Ileum* | |
| | $-\log EC_{50}$ | α | $-\log EC_{50}$ | α |
| Carbachol | 7.0 ± 0.13 | 1.0 | 6.5 ± 0.15 | 1.0 |
| Bethanechol | 5.8 ± 0.09 | 1.0 | 4.3 ± 0.03* | 0.8* |
| Pilocarpine | 5.3 ± 0.05 | 0.8 | 4.8 ± 0.08* | 0.4* |

Values are mean ± s.e.mean, $n = 5$. *Significantly different from control ileal value ($P < 0.05$).

finding that these receptors are differently coupled is in agreement with this hypothesis. However, both these receptors exhibit low affinities towards pirenzepine and could thus be designated as $M_2$. These data, combined with previously reported studies which show that $M_1$ receptors are coupled to both inhibition of adenylate cyclase activity and inositol phospholipid hydrolysis (Harden *et al.*, 1986), further highlight the problems associated with the classification based solely upon the affinity of pirenzepine.

We conclude that investigation of effector-coupling systems provides evidence of differences in coupling between muscarinic receptors and indicates that those receptors in the ileum differ from those in the atria.

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*(Received January 19, 1987.*
*Accepted February 26, 1987.)*