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Short communication

Pertussis toxin increases isoproterenol induced relaxation in field-stimulated ileum

Gregory Sawyer, Frederick Ehlert *

Department of Pharmacology, College of Medicine, University of California, Irvine CA 92697, USA

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Abstract

We investigated the effects of pertussis toxin on contraction in field-stimulated guinea pig ileum in the absence and presence of isoproterenol. Field-stimulation elicited pertussis toxin-insensitive contractions. Cumulative addition of isoproterenol produced a maximal 52% reduction in the contractile response. Following pertussis toxin-treatment, the maximal inhibitory effect of isoproterenol increased to 83%. Pertussis toxin had no effect on the ability isoproterenol to inhibit contractions elicited by histamine agonists. Our results suggest that the increased effectiveness of isoproterenol in pertussis toxin-treated ileum is due to an uncoupling of the muscarinic M_2 receptor contractile mechanism.

Keywords: Acetylcholine release; Pertussis toxin; Muscarinic receptor; Ileum, field-stimulated; Muscarinic M_2 receptor

1. Introduction

One of the highest densities of muscarinic receptors occurs in the smooth muscle of the guinea pig ileum (Yamamura and Snyder, 1974), where both muscarinic M_2 and M_3 receptors are expressed in a ratio of approximately four to one (see review, Ehlert et al., 1997a). It is the less abundant muscarinic M_3 receptor that mediates contraction when muscarinic receptor agonists are applied to isolated strips of guinea pig ileum. These contractions are pertussis toxin-insensitive (Eglen et al., 1987; Thomas and Ehlert, 1994) and are selectively inhibited by muscarinic receptor antagonists in a manner consistent with an M_3 mechanism. However, following covalent inactivation of muscarinic M_3 receptors with the aziridinium ion of *N*-(2-chloroethyl)-4-piperidyldiphenylacetic acid (4-DAMP mustard), muscarinic receptor agonists can elicit a highly potent contractile response in the guinea pig ileum, provided that histamine and isoproterenol are present (Thomas et al., 1993; Thomas and Ehlert, 1994). Together, histamine and isoproterenol are without effect because the relaxant mechanism of isoproterenol opposes the contraction elicited to histamine. Under these conditions, muscarinic receptor agonists elicit a pertussis toxin-sensitive contractile response that displays an M_2 antagonistic profile. Presumably, the contractile mechanism involves an muscarinic M_2 receptor-mediated inhibition of the relaxant effect of isoproterenol on histamine-induced contraction.

Muscarinic M_2 receptors also participate in contraction when muscarinic M_3 receptors are intact. When measured in the presence of isoproterenol, the contractile response to muscarinic receptor agonists is moderately pertussis toxin-sensitive, demonstrating that muscarinic M_2 receptors mediate an inhibition of the relaxant effect of isoproterenol on muscarinic M_3 receptor-mediated contractions (Thomas and Ehlert, 1994). Thus, the contractile role of the muscarinic M_2 receptor depends on the presence of a cAMP stimulating agent, like isoproterenol or forskolin, and this role can be detected with pertussis toxin, which selectively uncouples M_2-mediated responses but not those of the muscarinic M_3 receptor (see reviews, Ehlert et al., 1997a,b).

In the present report, we have used pertussis toxin to investigate the role of the muscarinic M_2 receptor in the contractile response of the guinea pig ileum to field-stimulation. Field-stimulation causes a pertussis toxin-insensitive twitch response that is mediated by the action of acetylcholine on muscarinic M_3 receptors (Kilbinger et al.,

* Corresponding author. Tel.: +1-949-824-6208; Fax: +1-949-824-4855; E-mail: fjehlert@uci.edu

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1984; Tucker, 1984). We found that, in the presence of isoproterenol, the field-stimulated twitch response is moderately pertussis toxin-sensitive, suggesting that neuronally released acetylcholine acts on both muscarinic M_2 and M_3 receptors to mediate contraction in the presence of isoproterenol.

2. Materials and methods

2.1. Isolated ileum

Ileal segments were harvested from male Hartely guinea pigs asphyxiated with CO_2 followed immediately by exsanguination (300–400 g) and mounted in an organ bath for contractile measurements as described previously (see Thomas et al., 1993). In some experiments, ilea were mounted between platinum ring electrodes spaced 2.54 cm apart in a manner similar to that described by Puig et al. (1990). Ilea were subjected to alternating periods (5 min) of rest and field-stimulation (Grass SD9 stimulator set at 100 mV, 8 ms duration, 0.2 Hz) until steady, reproducible contractions were observed for three successive stimulation periods (five to seven stimulation periods were needed to achieve consistent contractions). Ileal segments were allowed to equilibrate 40 min, and the relaxant responses to isoproterenol and forskolin were measured subsequently during a 10 min period of field-stimulation. A cumulative concentration–response curve was measured for each relaxant agent, and the EC_{50} value and maximal inhibition were estimated from the data as described previously (Candell et al., 1990). Ileal segments were washed three times and allowed to equilibrate for 30 min before additional measurements were made. In some experiments, ilea were harvested from guinea pigs that had been injected i.p. with pertussis toxin (70 μg/kg body weight) 3 days prior to being euthanized.

2.2. Materials

Drugs and chemicals were obtained from the following sources: pertussis toxin, LIST Biological Laboratories, Campbell, CA; [[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido [2,3b][1,4] benzodiazepine-6-one (AF-DX 116), Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT; isoproterenol, Sigma, St. Louis, MO; forskolin, Calbiochem, La Jolla, CA; 4-DAMP mustard was synthesized as described previously (Thomas et al., 1992).

3. Results

3.1. Isolated guinea pig ileum

Isoproterenol caused a concentration-dependent inhibition of the contractions elicited to histamine (0.32 μM), the histame H_1 receptor selective agonist 2-(2-methylaminoethyl)-pyridine (2-MAP, 6.4 μM), and the muscarinic receptor agonist, oxotremorine-M (80 nM) (Fig. 1a,b,c, respectively). The EC_{50} values and maximal effects of isoproterenol against the three contractile agonists were 7.5, 8.5 and 23 nM and 100, 100 and 56% inhibition, respectively. Pertussis toxin-treatment had no effect on isoproterenol-induced relaxation of histamine- and 2-MAP-induced contractions, but enhanced the relaxant effects of isoproterenol against oxotremorine-M-induced contraction. Both the potency (2.1-fold decrease in EC_{50}) and maximal effect (91% inhibition) of isoproterenol were increased with pertussis toxin-treatment.

3.2. Field-stimulated guinea pig ileum

Field-stimulation of the ileum elicited contractions (tension equivalent to a load of 3 to 4 g) that were inhibited 88% by *N*-methylscopolamine (0.1 μM) and 90% by prior treatment (1 h) with 4-DAMP mustard (40 nM) in combination with AF-DX 116 (1.0 μM) followed by extensive washing. This treatment has been shown to cause a selective inactivation of M_3 over M_2 muscarinic receptors (Thomas et al., 1992). These results show that the contractile response to field-stimulation in the present study is due to the release of acetylcholine. Isoproterenol and forskolin caused a concentration-dependent inhibition of the contractile response to field-stimulation (Fig. 2). The maximal inhibitory effects of isoproterenol and forskolin were 58 and 31%, respectively, and their corresponding EC_{50} values were 18 and 44 nM. Pertussis toxin-treatment had no effect on the contractile response to field-stimulation, but enhanced the relaxant effects of isoproterenol and forskolin. Both the maximal relaxant effect (83% inhibition) and potency (2.2-fold decrease in EC_{50}) of isoproterenol were

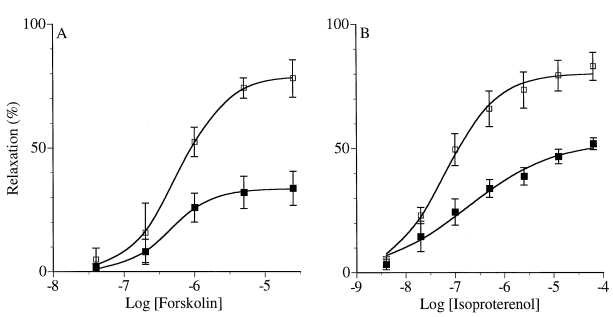

Fig. 1. Effects of pertussis toxin on isoproterenol-induced relaxation of contractions elicited by histamine (0.32 μM) (A), 2-(2-methyaminoethyl)-pyridine (6.4 μM) (B) and oxotremorine-M (80 nM) (C) in isolated guinea pig ileum. Control, (■) and pertussis toxin-treated ileum (□). Each data point represents the mean contractile response ± S.E.M. of three to four experiments.

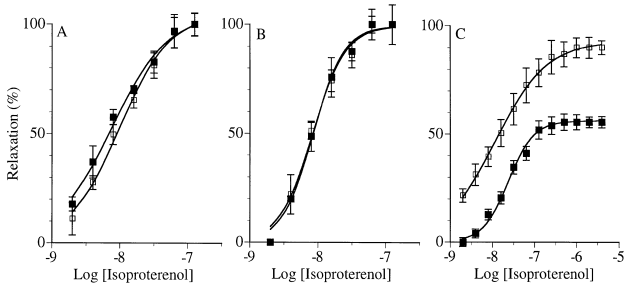

Fig. 2. Effects of pertussis toxin on forskolin (A) and isoproterenol (B) induced relaxation of contraction elicited by field-stimulation of guinea pig ileum in untreated (■) and pertussis toxin-treated ilea (□). Each data point represents the mean contractile response ± S.E.M. of four to five experiments.

increased with pertussis toxin-treatment, whereas only the maximal relaxant effect (78%) of forskolin was increased.

4. Discussion

Pertussis toxin catalyzes the ADP-ribosylation of G_i and G_o, and thereby uncouples muscarinic M_2 and M_4 receptor-mediated responses, without affecting muscarinic M_1, M_3 and M_5 receptor-mediated responses (Kurose and Ui, 1983; Peralta et al., 1988). In the smooth muscle of the guinea pig ileum, most known actions of muscarinic receptor agonists can be classified as either pertussis toxin-sensitive M_2 responses or pertussis toxin-insensitive M_3 responses. For example, in the absence of relaxant agents, muscarinic receptor agonists elicit contraction and phosphoinositide hydrolysis, and both responses are mediated by the muscarinic M_3 receptor and are pertussis toxin-insensitive (Eglen et al., 1987; Candell et al., 1990; Thomas and Ehlert, 1994). Muscarinic receptor agonists also elicit a pertussis toxin-sensitive inhibition of isoproterenol- and forskolin-stimulated cAMP accumulation, and this response is mediated by the muscarinic M_2 receptor (Candell et al., 1990; Thomas and Ehlert, 1994). As described under Section 1, muscarinic M_2 receptors have been shown to elicit a pertussis toxin-sensitive contraction by inhibiting the relaxant effects of cAMP-stimulating agents (e.g., isoproterenol and forskolin) on histamine-induced contraction. Collectively, these results indicate that pertussis toxin is a useful tool for discriminating between muscarinic M_2 and M_3 receptor-mediated contractile responses in the field-stimulated guinea pig ileum.

The field-stimulated guinea pig ileum is a model cholinergic neuroeffector junction used for studying acetylcholine release. Field-stimulation causes a tetrodotoxin-sensitive release of acetylcholine (Cowie et al., 1978; Kilbinger, 1982; Kilbinger et al., 1984), resulting in a pertussis toxin-insensitive contractile response (Tucker, 1984; Lux and Schulz, 1986) mediated by the muscarinic M_3 receptor (Kilbinger et al., 1984; Kilbinger and Stein, 1988). In this investigation, the twitch response elicited to field-stimulation was insensitive to pertussis toxin-treatment and inhibited by 4-DAMP mustard-treatment, consistent with previous observations that the muscarinic M_3 receptor mediates contraction to endogenous acetylcholine.

The addition of either isoproterenol or forskolin renders the twitch response elicited to field-stimulation sensitive to pertussis toxin (Fig. 2). These results strongly suggest that endogenous acetylcholine acts on both muscarinic M_2 and M_3 receptors. The muscarinic M_3 receptor mediates a direct contractile response through calcium mobilization, whereas the muscarinic M_2 receptor mediates an inhibition of the relaxant effects of cAMP-stimulating agents, like isoproterenol and forskolin (see reviews, Ehlert et al., 1997a,b).

In the longitudinal muscle–myenteric plexus of rat ileum, pertussis toxin-treatment uncouples muscarinic autoreceptor-mediated inhibition of acetylcholine release (Dolezal et al., 1989). Such an effect would tend to facilitate contraction in a field-stimulated ileum. Therefore, this mechanism cannot account for our observations because we observed a pertussis toxin-mediated inhibition of field-stimulated contraction in the presence of isoproterenol and forskolin.

It seems unlikely that a change in the field-stimulated release of norepinephrine could account for the enhanced effectiveness of isoproterenol in pertussis toxin-treated ileum, because the twitch response was unaffected by pertussis toxin-treatment. Also, pertussis toxin-treatment enhanced the relaxant effects of isoproterenol against both field-stimulated and oxotremorine-M-mediated contractions. Presumably, under the latter condition, release of norepinephrine is insignificant.

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