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Methoctramine and gallamine inhibit PI hydrolysis in guinea-pig gallbladder

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Abstract

The present study aimed to determine the effect of two M_2/M_4-selective muscarinic receptor antagonists on blocking the hydrolysis of carbachol (CCh) stimulated phospho-inositide (PI) breakdown in order to address the possibility that a muscarinic receptor other than the M_3 receptor is involved in PI hydrolysis in this tissue. Gallbladder tissue slices labeled with myo-[2-^{3}H] inositol were incubated with increasing concentrations of antagonists and agonist. After the reactions were terminated by the addition of chloroform/methanol, labeled inositol phosphates were separated using anion exchange chromatography. Muscarinic M_2 antagonists methoctramine and gallamine both inhibited carbachol-induced PI breakdown at high concentrations, with log IC_{50} values of -5.145 and -6.049, respectively. Gallamine at 10^{-5}M concentration failed to displace the dose-response curve for carbachol-induced accumulation of inositol triphosphate (IP_3). Our data suggest that M_3 receptors play a major role in stimulation of PI hydrolysis in the guinea-pig gallbladder.

Keywords: Muscarinic receptors; Gallbladder; Phosphoinositide hydrolysis; Gallamine; Methoctramine

1. Introduction

Muscarinic receptors are known to mediate many diverse physiological processes including glandular secretion, modulation of cardiac rate and force and smooth muscle contraction. Muscarinic receptors are also involved in many important events in the central nervous system such as learning, memory, temperature regulation and motor control (Caulfield and Birdsall, 1998). Five distinct muscarinic acethylcholine receptor (mAChR) subtypes (M_1-M_5) have been identified using molecular and pharmacological techniques (Buckley et al., 1989; Hulme et al., 1990; Dörje et al., 1991). Muscarinic receptors belong to the seven-transmembrane receptors superfamily, which regulates different effector systems by activating heterotrimeric G proteins. The M_1, M_3 and M_5 receptor subtypes are coupled efficiently to the pertussis toxin-insensitive Gq family of G proteins, leading to activation of Phospholipase C-β (PLC-β) and subsequent hydrolysis of inositol 4,5-biphosphate, while M_2 and M_4 receptors inhibit adenylyl cyclase via pertussis toxin-sensitive Gi/Go family of G proteins (reviewed in Caulfield, 1993; Felder, 1995).

Most smooth muscle cells contain M_2 and M_3 mACh receptors that mediate muscle contraction. In the majority of these tissues, M_3 receptor has been demonstrated to initiate contraction, although it represents a minor fraction of total muscarinic receptor population (Eglen et al., 1994). Studies in uterine smooth muscle (Doods et al., 1993; Eglen et al., 1989; Eglen et al., 1991) and porcine basilar artery (Van Chardorp and Van Zweiten, 1989) indicate that the M_2 receptor may also mediate contraction. M_1 receptors have also been suggested to mediate the contractile response in canine saphenous and femoral veins (Eglen and Whiting, 1990). The muscarinic receptor subtype(s) involved in cholinergic contractions of the guinea-pig gallbladder is

Abbreviations: CCh, carbachol; PI, phospho-inositide; IP_1, inositol monophosphate; IP_3, inositol triphosphate; mAChR, muscarinic acethylcholine receptor; PLC, Phospholipase C.

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unclear. Different groups have suggested the involvement of M_1, M_2, M_3 and M_4 subtypes. Based on affinities of a number of selective drugs, Von Schrenck et al. (1993, 1994), Takahashi et al. (1994) and Eltze et al. (1997) have reported the presence of functional M_3 receptors in guinea-pig gallbladder smooth muscle. On the other hand, Özkutlu et al. (1993) have claimed that the contractile response in the guinea-pig gallbladder is more likely to be mediated by the M_4 receptor. Oktay et al. (1998) demonstrated the coexistence of M_2 and M_4 receptor subtypes coupled to PI hydrolysis and inhibition of adenylyl cyclase by using Western blot analysis, ligand binding and biochemical assays. Subsequent studies using reverse transcription-polymerase chain reaction showed the presence of m_2, m_3 (Cabadak et al., 2000) and m_4 (unpublished data) transcripts in this tissue. Parkman et al. (1999) have suggested that M_1, M_2 and M_3 receptor subtypes modulate cholinergic contractions in the guinea-pig gallbladder. A recent report by Akici et al. presented evidence that supports the coexistence of M_2, M_3 and M_4 receptors in this tissue (Akici et al., 2000).

Phospholipase C-mediated hydrolysis of PI is a major transducing mechanism of cholinergic stimulation in many contractile tissues. Generation of IP_3 is considered to lead to a rapid phase of muscle contraction (Eglen et al., 1996). Stimulation of phosphoinositide hydrolysis is generally attributed to the muscarinic M_3 receptor in the majority of smooth muscle tissue. In certain cell types, M_2 and M_4 receptors mediate weak stimulation of PI hydrolysis by coupling to Gi/Go proteins (Ashkenazi et al., 1987). We have previously demonstrated that carbachol inhibited cAMP formation and also evoked a concentration-dependent increase in inositol phosphates in guinea-pig gallbladder slices (Oktay et al., 1998). Several muscarinic receptor antagonists have previously been utilized to determine the subtypes of muscarinic receptor that mediate PI hydrolysis in smooth muscle tissues (Varol et al., 1989; Moroi-Fetters et al., 1988; Harriss et al., 1995).

The aim of the present study was to address the possibility that a receptor subtype other than M_3 is linked to phosphoinositide hydrolysis in the guinea-pig gallbladder. We therefore studied the effect of gallamine and methoctramine, two M_2/M_4-selective receptor antagonists (Pedder et al., 1991; Melchioerre et al., 1987), to inhibit carbachol-induced PI accumulation in guinea-pig gallbladder slices.

2. Materials and methods

2.1. Materials

Carbamylcholine chloride (carbachol) and gallamine were purchased from Sigma Chemical. Methoctramine was a gift from Dr. Melchiorre, Italy. AGI-X8 resin (200–400 mesh formate form) was purchased from Amersham. Other chemicals used were of reagent grade. All drug solutions were prepared on the day of the experiment. Approval for the study was obtained from the University Ethical Committee for Laboratory Animals.

2.2. PI hydrolysis assay

Receptor mediated modulation of PI turnover was monitored by the accumulation of radiolabeled inositol phosphates as described Berridge (1983) and Takahashi et al. (1994). Gallbladders of guinea-pigs (300–350 g) of both sexes were used in the study. After guinea-pigs were killed by decapitation, gallbladders were removed and minced in 0.5–1 mm pieces. Slices were preincubated in Krebs-Ringer bicarbonate buffer (pH 7.6) containing (mmol/l): NaCl, 120; KCl, 5.5; $CaCl_2$, 2.5; NaH_2PO_4, 1.2; $MgCl_2$, 1,2; $NaHCO_3$, 20; glucose, 11; and equilibrated with 95% O_2–5% CO_2. After preincubation, slices were transferred to fresh Krebs Ringer buffer 300 μl, containing 5 μCi/ml of myo-[2-^{3}H] inositol (Amersham, Buckinghamshire, UK) and incubated for 2 h at 37 °C while being gassed continuously with 95% O_2–5% CO_2. Subsequently, 10 mM LiCl and carbachol at 10^{-8}–10^{-4} M were added for 10 min. The muscarinic receptor antagonists when used were added 20 min prior to adding the agonist. Reactions were terminated after 10 min by the addition of chloroform/methanol (1:2, v/v); samples were thereafter extracted and separated according to the method described by Berridge (1993). The radioactivity of the phosphorylated inositol fractions was measured in a liquid scintillation counter. Tissue protein was measured by the method of Lowry et al. (1951) after solublization with 1 ml, 0.1 N NaOH. Bovine serum albumin was used as the standard.

2.3. Data analysis

Log IC_{50} (inhibitory concentration of antagonist required to elicit 50% of the maximal response) values were calculated from the data with GraphPAD InPlot Software. Statistical significance was determined by Student's unpaired t test ($p<0.05$). All data are presented as mean ± S.E.M of 3 experiments performed in duplicate.

3. Results

3.1. Time-dependent stimulation of PI hydrolysis in guinea-pig gallbladder slices

Gallbladder slices labeled with myo-[2-^{3}H] inositol were exposed to 10^{-5} M carbachol at different time points. This concentration of carbachol caused an increase over basal values in IP_1 (Fig. 1), IP_2 and IP_3 (data not shown) fractions at all time points examined. The basal level increased from 13.36 pmol/mg protein to 27.70 pmol/mg protein at 30 min, when the maximal response was attained.