JPP
Journal of
Pharmacy and Pharmacology

JPP 2009, 61: 949–952

Received October 20, 2008
Accepted April 06, 2009
DOI 10.1211/jpp/61.07.0015
ISSN 0022-3573

Short Communication

Characterization of the effect of penehyclidine hydrochloride on muscarinic receptor subtypes mediating the contraction of guinea-pig isolated gastrointestinal smooth muscle

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Abstract

Objectives The aim was to characterize the effect of penehyclidine hydrochloride, which mediates the relaxation of guinea-pig isolated gastrointestinal smooth muscle, on muscarinic receptor subtypes.

Methods Radioimmune assay was used to determine cAMP levels in isolated guinea-pig gastrointestinal smooth muscle to compare the selective effects of penehyclidine hydrochloride on muscarinic receptor subtypes.

Key findings The results indicated that the relaxing effect of penehyclidine hydrochloride on isolated gastrointestinal smooth muscle contraction induced by acetylcholine was stronger than that of atropine (based on PA_2 values). In the radioimmune assay, penehyclidine hydrochloride increased the cAMP content in isolated guinea-pig stomach smooth muscle and decreased the cAMP content in isolated guinea-pig intestinal smooth muscle, but the difference was not statistically significant at a dose of 10 μmol/l.

Conclusions The results suggest that penehyclidine hydrochloride has little or no effect on M_2 receptor subtypes in guinea-pig gastrointestinal smooth muscle.

Keywords cAMP; guinea-pig gastrointestinal smooth muscle; muscarinic receptor; penehyclidine hydrochloride; selective antagonist

Introduction

Penehyclidine hydrochloride (PHC; (2-hydroxyl-2-cyclopentyl-2-phenyl-ethoxy)quinuclidine) is a new anticholinergic drug with both antimuscarinic and antinicotinic activity while retaining potent central and peripheral anticholinergic activity.[1] In China, PHC is widely used in the clinic as an antagonist of organic phosphorus poisoning and soman.[2,3] Receptor binding assays showed that PHC had far greater selectivity for the central M_3 muscarinic receptor subtype over the M_1 subtype,[4] and some molecular mechanisms of PHC have also been investigated. Li *et al.*[5] investigated the protective effects of PHC against lung injury after cardiopulmonary bypass in paediatric patients with congenital heart disease and found that the lung protective effect was related to inhibiting the release of the cytokines tumour necrosis factor-α, interleukins 6 and 8 and matrix metalloproteinase-9. Zhang *et al.*[6] investigated the protective effects of PHC in septic mice and found that treatment with 0.45 mg/kg PHC markedly decreased tumour necrosis factor-α, malondialdehyde content and inducible nitric oxide synthase mRNA expression, and enhanced superoxide dismutase activity ($P < 0.05$ and $P < 0.01$). Thus, PHC may have a protective effect against sepsis. However, little is known about the effects of PHC on guinea-pig gastrointestinal smooth muscle contraction or its underlying mechanism.

Muscarinic receptors are classified by pharmacological and signal transduction criteria into five major subtypes: M_1, M_3 and M_5 (coupled to the stimulation of phosphoinositide hydrolysis), and M_2 and M_4 (linked to the inhibition of adenylate cyclase). The functional properties and binding profiles of the M_1, M_2, M_3, M_4 and M_5 receptor subtypes closely correspond to those of the m_1, m_2, m_3, m_4 and m_5 receptor subtypes that have been

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identified in recent receptor cloning studies. M_2 and M_3 receptor subtypes have been identified in guinea-pig gastrointestinal smooth muscle.[7] It has been shown that the muscarinic contractile response is greatly inhibited in the ileum and urinary bladder of M_3 knockout mice, whereas a much smaller decrease in contractile function was noted in M_2 knockout mice. In mice lacking both M_2 and M_3 muscarinic receptors (M_2/M_3 knockout mice), the muscarinic contractile response was nearly completely eliminated in the ileum and urinary bladder.[7]

We previously investigated the effects of PHC on guinea-pig gastrointestinal smooth muscle contraction induced by acetylcholine and found that, according to PA_2 values, the antagonizing effect of PHC on isolated guinea-pig gastrointestinal smooth muscle contraction induced by acetylcholine was stronger than that of atropine.[8,9] This indicates that PHC can relax guinea-pig gastrointestinal smooth muscle. In the present study, we characterized the underlying mechanism of PHC relaxation of guinea-pig gastrointestinal smooth muscle, in particular the muscarinic receptor subtypes it acts upon, using a radioimmune assay method to determine tissue cAMP production in the guinea-pig gastrointestinal smooth muscle.

Materials and Methods

All protocols were approved by the institutional ethics committee. The guinea-pigs were obtained from the Animal Center of Sichuan University, Chengdu, China. All regents were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA) unless otherwise specified. The PHC powder (purity 99.99%) was donated by Chengdu List Pharmaceutical Co., Ltd, Chengdu, China. All compounds were dissolved in normal saline (0.9% NaCl solution) before use.

Radioimmune assay

The protocol was used as previously reported[10] with minor modifications. Briefly, guinea-pigs (200–250 g) of either sex were fasted for 24 h and then killed by stunning and cervical dislocation. The isolated intestines and stomach were dissected from the peritoneal cavity and washed with cold Krebs-bicarbonate buffer. The segments were then individually opened along the mesenteric border along the circular and longitudinal axis and cut into strips (approx. 40–50 mg).

To measure cAMP accumulation, all experiments were conducted in the presence of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (300 μM). The isolated intestinal and stomach strips were incubated in Krebs solution with the following composition (mM): NaCl 119; KCl 4.5; $MgSO_4$ 2.5; $NaHCO_3$ 25; KH_2PO_4 1.2; $CaCl_2$ 2.5; glucose 11.1, at 37°C, gassed with a mixture of 95% O_2 and 5% CO_2 for 30 min and then exposed to 10 μmol/l acetylcholine, bethanechol, PHC, gallamine or atropine for 10 min. Strips without application of drugs were used as untreated controls. Control and treated strips were studied in parallel. After incubation for the appropriate time, the muscle strips were quickly frozen in liquid nitrogen and homogenized in alcohol solution using a Polytron homogenizer. After centrifugation at 2200*g* for 15 min, twice, the alcohol in the supernatant was removed and the cAMP in the supernatant was measured using an [^{125}I]cAMP kit (Shanghai University of Traditional Chinese Medicine, Shanghai, China). Sample data were divided by the weight of the wet tissue and the results were expressed in pmol/g for the guinea-pig gastrointestinal tissue wet weight.

Statistical analysis

Values are expressed as mean ± SD. One-way analysis of variance was used to determine the differences among groups. The significance level was set at $P < 0.05$.

Results

Table 1 shows the effects of PHC on cAMP levels in isolated guinea-pig intestinal and stomach smooth muscle. A dose of 10 μmol/l PHC increased the cAMP content in isolated guinea-pig stomach smooth muscle, and decreased the cAMP content in isolated guinea-pig intestinal smooth muscle, but the difference was not statistically significant. At the same dose, both acetylcholine and bethanechol decreased the cAMP content in isolated guinea-pig intestinal and stomach smooth muscle and the difference was statistically significant ($P < 0.05$). Both atropine and gallamine increased the cAMP content in isolated guinea-pig intestinal and stomach smooth muscle and the difference was also statistically significant ($P < 0.001$).

Discussion

It is well established that M_3 muscarinic receptor-mediated activation of phospholipase C, resulting in the formation of inositol triphosphate and diacylglycerol, is a key event in the signalling cascade leading to gastrointestinal smooth muscle contraction. Muscarinic M_2 receptors are linked to the inhibition of adenylate cyclase activity, and activation of these receptor subtypes lowers tissue cAMP levels.[11] In our assays, acetylcholine and bethanechol are muscarinic M_2 receptor agonists, while atropine and gallamine are muscarinic M_2 receptor antagonists.[12] Table 1 shows that at a dose of 10 μmol/l, both acetylcholine and bethanechol were able to significantly decrease the cAMP content in intestinal and stomach strips, and both atropine and gallamine could significantly increase the cAMP content in isolated intestinal and stomach strips. Although PHC could increase the cAMP content in isolated stomach strips and decrease the cAMP

Table 1 Effects of penehyclidine hydrochloride on cAMP levels in isolated guinea-pig intestinal and stomach smooth muscle

Drugs (10 μmol/l)	cAMP pmol/g (intestinal)	cAMP pmol/g (stomach)
Control	119.01 ± 4.63	171.64 ± 30.35
Acetylcholine	59.19 ± 32.87*	134.95 ± 45.50*
Bethanechol	74.30 ± 32.01*	51.09 ± 16.71***
Atropine	188.58 ± 11.19***	231.35 ± 41.47*
Gallamine	172.65 ± 14.76***	298.46 ± 50.12***
Penehyclidine hydrochloride	109.86 ± 26.74	185.17 ± 55.35

Data are mean ± SD, $n = 6$. *$P < 0.05$, ***$P < 0.001$, significantly different compared with the control group.

content in isolated intestinal strips, the difference was not statistically significant. Therefore, according to our results, we can conclude that PHC has little or no effect on M_2 receptor subtypes in guinea-pig isolated intestinal and stomach strips.

In gastrointestinal smooth muscles, the M_2 and M_3 muscarinic receptor subtypes are preferentially expressed, with a preponderance of the former subtype.[13] However, a recent reverse transcriptase–polymerase chain reaction study has reported the possible expression of all five subtypes (M_1, M_2, M_3, M_4 and M_5) in gastric smooth muscles. To elucidate the functional roles of each muscarinic receptor subtype, the contractile responses to muscarinic agonists, including carbachol, have been extensively studied using various muscarinic receptor antagonists. Most, but not all, of the studies indicate that the contractile responses are mediated exclusively by M_3 receptors, and that M_2 receptors appear non-functional or may act only indirectly. Nonetheless, the recent use of mutant mice lacking certain muscarinic receptor subtypes has revealed that not only the M_3, but also M_2 receptors, may have a direct role in inducing contraction in gastric and intestinal smooth muscles. It is therefore possible that both M_2 and M_3 receptors take part in mediating contractions induced by stimulation of cholinergic nerves. However, this has not been addressed experimentally so far.

PHC can effectively relax the contraction of guinea-pig intestinal and stomach strips induced by acetylcholine *in vitro*.[8,9] As PHC is an anticholinergic drug, if the antagonizing effect of PHC on isolated guinea-pig intestinal and stomach strips contraction induced by acetylcholine is stronger than that of atropine, at a receptor level PHC should act on the muscarinic receptor according to our knowledge. Moreover, we measured the effect of PHC on the Ca^{2+} changes in guinea-pig intestinal and stomach strips and found that PHC could inhibit Ca^{2+} release (unpublished data). This suggests that PHC could act on the M_3 receptor-mediated activation process through an effect on the Ca^{2+} changes.

According to our results, we conclude that PHC has little or no effect on M_2 receptor subtypes in guinea-pig isolated intestinal and stomach strips, and PHC has a more selective effect on muscarinic M_3 receptor subtypes than M_2 receptor subtypes. Hyoscyamine, developed from Chinese medicinal plants, acts mainly by blocking muscarinic acetylcholine receptors, and shows a wide range of biological activities, including antioxidant and cytoprotective actions. PHC is a new type of hyoscyamus drug and, compared with other hyoscyamines, the notable advantage of PHC is that it has few M_2 receptor-associated cardiovascular side-effects.[14] Recently, clinical results demonstrated that PHC had curative effects for soman poisoning and pulmonary dysfunction in chronic obstructive pulmonary disease.[15] Other than improving microcirculation, PHC can inhibit lipid peroxidation, attenuate the release of lysozyme, and depress microvascular permeability.[16] Moreover, it can significantly decrease brain nuclear factor-κB expression in cerebral ischaemia/reperfusion injury.[17] Qiao *et al.*[18] studied the pharmacokinetics of PHC in mice after administration of 0.05, 0.15 and 0.45 mg/kg (i.m.) and found that PHC was well absorbed, rapidly distributed and excreted via urine, mainly as inactive metabolites. Thus, PHC has good pharmacokinetic characteristics and greater muscarinic receptor selectivity in some organs. Our results suggest that, compared with atropine, PHC might be a more selective drug for muscarinic receptors in gastrointestinal smooth muscle for use in relieving abdominal pain. Because atropine is a non-selective muscarinic antagonist, side-effects include blurred vision, constipation, decreased sweating, difficulty sleeping, dizziness, drowsiness, dry mouth, nose or skin, headache, loss of appetite, loss of taste, nausea and nervousness. Further research on the effects of PHC on gastrointestinal smooth muscle and abdominal pain should be undertaken.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Acknowledgement

Hong-tao Xiao and Zhi Liao both contributed equally to this paper.

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