Investigation of the Mechanism of Nicotine-Induced Relaxation in Guinea Pig Gallbladder

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Background. Muscular contraction of the gallbladder is the primary determinant of bile delivery into duodenum. Gallbladder filling and emptying are influenced by both inhibitory and excitatory stimuli, and NO plays a key role in normal relaxation. In this study, to determine whether nicotine acts on the gallbladder muscle, the mechanism of its effect on strips of guinea pig gallbladder was studied in vitro.

Materials and methods. Guinea pig gallbladder muscle strips were mounted in organ bath with modified Krebs-Henseleit solution and aerated with Carbogen. Tension was measured with isometric force transducers, and muscle relaxation was expressed as percent decrease of precontraction induced by carbachol.

Results. Nicotine produced concentration dependent relaxation when preparations were precontracted by carbachol (10⁻⁶ M). Nicotine-induced relaxation was 51.6 ± 3.2% of phenylephrine contraction and was not affected by guanethidine (10⁻⁵ M), propranolol (10⁻⁶ M), hexamethonium (10⁻⁴ M), indomethacin (10⁻⁶ M), N⁰-nitro L-arginine methyl ester (L-NAME) (3 × 10⁻⁶ M), methylene blue (10⁻⁶ M), glibenclamide (10⁻⁵ M), clotrimazole (10⁻⁶ M), tetraethylammonium (3 × 10⁻⁴ M), or 4-aminopyridine (10⁻³ M). Nicotine did not exhibit a calcium antagonizing effect.

Conclusions. From these results, we concluded that nicotine-induced relaxation of the guinea pig gallbladder is not mediated by the release of noradrenaline, nitric oxide (NO), prostaglandins, or a related substance, or by the activation of potassium channels, or by the stimulation of nicotinic cholinoreceptors. Further work is needed to determine the cellular mechanism(s) of action by which nicotine acts on gallbladder smooth muscle. © 2003 Elsevier Science (USA)

INTRODUCTION

Active and significant relaxation of the human gallbladder must be one of the facets of its motility during both the filling and emptying cycles. The gallbladder has an inhibitory nonadrenergic noncholinergic (NANC) innervation in several species, including human and dog [1, 2]. NO is released from NANC nerves in many smooth muscles such as anococcygeus, esophagus, uterus, gallbladder, gastrointestinal system, and vascular tissues [3–9]. There is evidence to support the idea that nitric oxide or related substances of the L-arginine/NO pathway functions as an NANC neurotransmitter in the gallbladder of the human and guinea pig [1, 2, 10, 11]. The studies on the diminished gallbladder neuronal NO synthase in gallbladder stasis and impaired contraction and relaxation in muscle strips from stone-diseased gallbladders have been reported in the literature [10, 12, 13]. Therefore, it is speculated that NO plays a key role in normal gallbladder relaxation. Recently, nicotine has been reported to cause relaxation of lower esophageal sphincter, anococcygeus, and gastrointestinal smooth muscle through stimulation of nicotinic receptors located on NANC inhibitory nerves [14–16] and this relaxation is mediated largely by the release of nitric oxide or a related substance. The aim of this study was to investigate the mechanism of nicotine-induced relaxation in guinea pig gallbladder muscle using isolated strips from guinea pig gallbladder.

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MATERIALS AND METHODS

After an overnight fast, guinea pigs were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and killed by exsanguination. The gallbladder was rapidly excised, put into Kreb’s solution (composition see below), and cut into longitudinal strips 2-mm wide and 10-mm long. The strips were transferred to 20-ml organ baths containing Kreb’s solution maintained at 37°C by a thermostated water circuit and continuously aerated with 95% O2 and 5% CO2. The pH of the solution was 7.4. Tension was measured with an isometric force transducer (Grass FT03) and recorded on a polygraph (model 79E, Grass Instruments, Quincy, MA). All tissues were allowed to equilibrate for 60 min prior to beginning of the experiments. During this period, the bath fluid was routinely changed every 15 min. Resting tension was set at 1 g by repeat adjustments and remained unchanged throughout the experiment.

After the equilibration period, the muscle strip was contracted with a submaximal concentration of carbachol (10⁻⁷ M) and after the tonic force had reached a stable plateau, concentration-response relationships for nicotine (10⁻¹⁰⁻¹⁰⁻⁴ M) were obtained cumulatively. After the addition of each dose, a strip was waited until a plateau response was obtained before adding the next one. At the end of the experiment, papaverine (10⁻⁴ M) was added to the organ bath to obtain the maximal relaxation. Thirty minutes after guanethidine (10⁻⁵ M), hexamethonium (10⁻⁴ M), propranolol (10⁻⁵ M), methylene blue (10⁻⁵ M), clotrimazole (10⁻⁵ M), glibenclamide (10⁻⁵ M), tetroethylammonium (3 × 10⁻⁴ M), 4-aminopyridine (10⁻⁵ M), L-NAME (3 × 10⁻⁴ M), or indomethacin (10⁻⁵ M) was added to the tissue bath, the same protocol was repeated. These doses were chosen based on previous studies, indicating their ability to block nicotine-induced relaxation in precontracted tissues [14–16]. In most studies, to look at the effects of antagonist drugs, tissue was exposed to the drug for 25 to 30 min. Therefore, the incubation times for the antagonists were evaluated by comparing the response before and after the addition of antagonist or inhibitors in the same preparation. To this end, the bath fluid was routinely changed every 15 min. Resting tension was set at 1 g by repeat adjustments and remained unchanged throughout the experiment.

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RESULTS AND DISCUSSION

In basal tonus, no effect with nicotine was observed. Nicotine (10⁻⁴–3 × 10⁻³ M) produced concentration-dependent relaxation with a mean pEC₅₀ value of 3.26 ± 0.07 and a mean maximum effect (E₅₀) at 3 × 10⁻³ M, amounting to 51.6 ± 3.2% (n = 7) of the phenylephrine-induced submaximal contraction. At the end of the experiment, papaverine (10⁻⁴ M) caused relaxation that was 100% of submaximal phenylephrine contraction. None of the antagonists investigated had a significant influence on basal tonus.

Nicotine produces a concentration-dependent relaxation of precontracted gallbladder strips from the guinea pigs (Fig. 1 and Table 1). The response is not mediated by the stimulation of nicotinic cholinoreceptors located in inhibitory neurons because it was not inhibited by hexamethonium (Table 1). Although the exact nature of the relaxations remains unknown, nicotine is known to elicit a TTX-resistant response in several peripheral organs [17–19]. There are two types of inhibitory neurons that are known to affect the smooth muscle motility of gastrointestinal tract, noradrenergic neurons and NANC neurons. In the present study, one of the inhibitory neurotransmitters responsible for the nicotine-induced relaxation was unlikely to be noradrenaline, because the relaxation was not reduced by propranolol, a beta-adrenoceptor antagonist (Table 1). Guanethidine that works via the inhibition of the release of noradrenaline, was also ineffective in blocking nicotine-induced relaxation in gallbladder strips (Table 1). Furthermore, the role of any substance released from adrenergic nerve endings could be ruled out.

There is now good evidence that NO-like substance(s) might be inhibitory NANC neurotransmitters in the mammalian gallbladder [2, 10, 20]. In the present study, l-NAME, an inhibitor of NO synthase, did not influence the nicotine-induced relaxation (Table 1). These findings indicate that the relaxant action of nicotine on the guinea pig gallbladder is not mediated by the release of NO or related substance from the L-arginine/NO pathway. However, it was recently reported that in bovine retractive muscle, the relaxation induced by nicotine is inhibited by methylene blue [21]. In our study, methylene blue did not inhibit the nicotine-induced relaxation (Table 1). Therefore any role for guanylate cyclase/cGMP pathway could be excluded in nicotine-induced relaxation of guinea pig gallbladder strips. To further explore the possibility of
prostaglandins in gallbladder relaxation, we examined the effect of indomethacin on nicotine-induced gallbladder muscle relaxation. Indomethacin had no significant effect on nicotine-induced gallbladder relaxation, suggesting nicotine-induced relaxation is not mediated by the release of prostaglandins (Table 1).

In this study, nicotine did not exhibit any Ca\(^{2+}\) antagonizing effect as it did not induce any relaxation in the gallbladder depolarized and contracted by high K\(^+\) and Ca\(^{2+}\) respectively, in a Ca\(^{2+}\)-free medium. However, a very well-known Ca\(^{2+}\) antagonist, verapamil, completely relaxed the gallbladder contracted by Ca\(^{2+}\) (data not shown).

Recently it has been shown that dilatation of various smooth muscles is mediated by the activation of different types of potassium channels [22]. Moreover, the presence of ATP-sensitive potassium channels in human gallbladder has been demonstrated [23]. The possibility that potassium channels play an important role in the relaxation due to nicotine is also unlikely, because glibenclamide, an inhibitor of ATP-sensitive potassium channels, tetraethylammonium and clotrimazole, inhibitors of calcium-activated potassium channels, and 4-aminopyridine, a nonselective potassium channel blocker, did not significantly influence the response (Table 1).

Previous studies have shown that VIP and ATP are the two leading candidates as inhibitory neurotransmitters of the noncholinergic nonadrenergic inhibitory innervation of the gastrointestinal tract [24, 25]. As a VIP- or ATP-antagonist was not used in the present study, we cannot draw any conclusion regarding the involvement of VIP or ATP at the present time.

In conclusion, nicotine produced relaxation of gallbladder strips from the guinea pigs and this relaxation is not mediated by the release of noradrenaline, nitric oxide, prostaglandins, or a related substance, or by the activation of potassium channels, or by the stimulation of nicotinic cholin receptors. Further experiments are required to establish whether VIP or ATP contributes to nicotine-induced relaxation.

### REFERENCES


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<th>E(<em>{\text{max}}) (% of 10(^{-6}) M Carbachol) and pD(</em>{2}) Values (−log EC(_{50})) for Nicotine in the Absence (Control) or Presence of Antagonists or Inhibitors in Guinea Pig Gallbladder Strips</th>
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*Note. The data are arithmetic means ± SEM from 5–8 of the experiments.*


