Imidazole-induced contractions in bovine tracheal smooth muscle are not dependent on the cAMP pathway

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ABSTRACT. The mechanism of imidazole-induced contraction on the bovine tracheal smooth muscle was investigated. Imidazole induced muscle contraction in a concentration-dependent manner on bovine, porcine and guinea-pig tracheas, but not in rat or mouse. In bovine tracheas, imidazole was cumulatively applied and induced muscle tension and increased intracellular Ca2+ level in a concentration-dependent manner. Imidazole, even at 300 µM, the concentration at which maximum contractile response occurs, did not significantly increase in cAMP content relative to control. Atropine inhibited imidazole-induced contraction in a concentration-dependent manner and pretreatment of hemicholinium-3 almost abolished imidazole-induced contraction. Conversely, pretreatment of tripeprennamine, indomethacin or tetrodotoxin did not affect imidazole-induced contraction. Acetylcholine or eserine induced contraction in bovine, porcine, guinea pig, rat and mice trachea in a concentration-dependent manner. However, there was little difference in the rank order of maximum contraction of these agents. Imidazole-induced contraction was greater in bovine trachea compared to the other species tested. Further, cAMP did not appear to play a role in imidazole-induced contraction, suggesting other mechanisms, such as the release of endogenous acetylcholine.

KEY WORDS: cAMP, imidazole, smooth muscle, trachea

Cyclic adenosine monophosphate (cAMP), as well as cyclic guanosine monophosphate (cGMP), is important second messenger and closely involved in development of diseases. Many drugs targeting cAMP have been launched to treat cardiovascular and respiratory diseases. cAMP is synthesized by adenylyl cyclase (AC) stimulating with β adrenergic agonist, and degraded by phosphodiesterase (PDE). Therefore, β adrenergic agonists, AC activators, PDE inhibitors and membrane permeable cAMP are used as therapeutic agents for heart failure.

Currently, PDEs are classified into 11 families [1] and numerous drug discovery studies have been done because of specific distribution on various organ [12]. On the other hand, imidazole is known as a PDE activator, but it will be not a therapeutic agent because it is predicted to accelerate the hydrolysis of cAMP, but it is important research tool for promoting AC/cAMP/PDE signaling [2].

Tracheal smooth muscle is an important research tool in the study of respiratory diseases, and bronchial smooth muscle is directly linked to disease pathogenesis [16]. However, since bronchus of rodents such as mice, is small to use research and from the concept of 3R of laboratory animal studies, porcine and bovine tracheas, which are waste organs of food animals, are useful for research. Especially, it is extremely important to study of AC/cAMP/PDE signaling in tracheal smooth muscle. It is also well known that the trachea is one of the organ, which has animal species difference for drug reaction [11, 19]. We have continued research on the agents related to signal transduction of cAMP and cGMP in bovine and porcine smooth muscle [6–9].

In this study, we compared the effect of imidazole in tracheal smooth muscle contraction of various animals and investigated the mechanism of imidazole induced muscle tension in bovine tracheal smooth muscle.

MATERIALS AND METHODS

Muscle preparations and tension measurement

Tracheas from adult bovine and porcine of either sex were obtained from a local abattoir. Male Hartley guinea-pig (300–450 g),
Cumulative addition of KCl also induced a graded increase on \([\text{Ca}^{2+}]_i\) level. Imidazole induced muscle tension and increases on \([\text{Ca}^{2+}]_i\) level at a concentration-dependent manner in bovine trachea (Fig. 2A). Moreover, imidazole-induced muscle tension and increases on \([\text{Ca}^{2+}]_i\) were completely inhibited by 1 \(\mu\)M atropine (Fig. 2A).

**Effects of Imidazole on Muscle Tension and \([\text{Ca}^{2+}]_i\) Level in Bovine Trachea**

It has been suggested that imidazole induced muscle tension by increases \(\text{Ca}^{2+}\) influx in rat uterus [15]. Therefore, the effects of imidazole on \([\text{Ca}^{2+}]_i\) level in bovine trachea.

After confirming KCl (65 mM) induced muscle tension and increases on \([\text{Ca}^{2+}]_i\), level, imidazole was cumulatively applied. Imidazole induced contraction in bovine, porcine and guinea pig trachea in a concentration-dependent manner, but did not affect in rat and mice (Fig. 1). Based on KCl-induced contraction, the rank order of maximum contraction was bovine>porcine>guinea-pig=rat=mice.

**Effects of Imidazole on cAMP Contents in Bovine Trachea**

Imidazole, even at 300 \(\mu\)M, the concentration at which maximum contractile response occurs, did not increase significant increase in cAMP content relative to control (control [n=4]; 63.4 ± 5.0 pmol/g wet weight; imidazole [n=4]; 70.2 ± 7.2 pmol/g wet weight).
Effects of various inhibitors on imidazole-induced contraction in bovine trachea

Pretreatment of bovine tracheas with atropine (1–100 nM) inhibited imidazole-induced contraction in a concentration-dependent manner (Fig. 3A).

On the other hand, pretreatment of tripelemamine (1 μM), a H₁ receptor antagonist, indomethacin (10 μM), a cyclooxygenase inhibitor, or tetrodotoxin (0.3 μg/ml), Na⁺ channel blocker did not affect imidazole-induced contraction. However, pretreatment of hemicholinium-3 (HC-3, 1 mM), a choline reuptake blocker, almost abolished imidazole-induced contraction (Fig. 3B).

Acetylcholine- and eserine-induced contraction in trachea of various animals

After confirming KCl (65 mM) induced sustained contraction, acetylcholine (ACH) or eserine, a cholinesterase inhibitor, was cumulatively applied. ACH induced contraction in bovine, porcine, guinea pig, rat and mice trachea in a concentration-dependent manner (Fig. 4A). Based on KCl-induced contraction, the rank order of maximum contraction was bovine=porcine=rat=mouse>guinea-pig.

Eserine, also induced contraction in bovine, porcine, guinea pig, rat and mice trachea in a concentration-dependent manner (Fig. 4B). Based on KCl-induced contraction, the rank order of maximum contraction was the same with the order of ACH-induced contraction.

DISCUSSION

The present study results show that imidazole-induced contraction of tracheal smooth muscle was greater in bovine tissue than in...
the other animal species tested. 1) Specifically, when we compared imidazole-induced maximum contraction based on KCl-induced contraction between species, the rank order was bovine>porcine>guinea-pig>rat=mouse. 2) In our investigation of the underlying imidazole-induced muscle tension in bovine tracheal smooth muscle, we found that (1) imidazole did not affect cAMP content, (2) atropine inhibited imidazole-induced muscle tension and increases of [Ca$^{2+}$]i level and (3) HC-3 almost abolished imidazole-induced muscle tension.

PDE4 inhibitors induce more effective relaxation compare to the other selective PDEs inhibitors in tracheal smooth muscle [5, 17]. Moreover, it is known that PDE4 selectively hydrolyzes cAMP [1]. Imidazole, a PDE activator [2], can enhance rhythmic contraction of the rabbit uterus by stimulating PDE [4], suggesting that cAMP may be involved in imidazole-induced muscle tension. However, in our study, imidazole did not affect cAMP contents in bovine trachea, even at 300 µM, the concentration at which maximum contractile response occurs. This result indicates that imidazole-induced contraction in bovine trachea is not due to the decreases in cAMP content by activating PDE.

It has been reported that imidazole induced muscle contraction in rat uterus by increases of Ca$^{2+}$ influx [18]. In the current study, we also found that imidazole induced muscle contraction and increases on [Ca$^{2+}$], level in a concentration-dependent manner in bovine trachea. However, cumulative addition of imidazole induced greater contraction than high K+ at a given [Ca$^{2+}$], level. Cumulative addition of carbachol induced greater contraction than high K+ at a given [Ca$^{2+}$], level in tracheal smooth muscle [15]. Additionally, in our study, atropine completely inhibited imidazole-induced muscle tension and increases on [Ca$^{2+}$], level in bovine trachea. These data suggest that the imidazole-induced increases on [Ca$^{2+}$], level by endogenous ACh release. Imidazole can induce contraction by augmenting the release of ACh in guinea pig ileum [10, 13]. In the present study, pretreatment with atropine or
HC-3, but not with indomethacin or TTX, inhibited imidazole-induced contraction in bovine trachea. It is well known that HC-3 is choline uptake inhibitor. Recently, it has been reported that HC-3, at low concentrations, act as inhibitors of high affinity choline transporter-1 (CHT1), at high concentrations, act as inhibitors of low affinity choline transporters [14]. In this study, we used HC-3 (1 mM) at high concentration. Differences in the efficacy of imidazole-induced contractions in various animals may be due to differences in expression of such transporters. Further research will clarify this difference.

Creese and Denborough [3] suggest that imidazole inhibited histaminase, since imidazole was able to contract guinea-pig isolated tracheal smooth muscle and also enhanced muscle tension induced by histamine, and H1 antagonist inhibited imidazole-induced muscle tension. However, in our study, triphenlenamine, a H1 antagonist, did not affect imidazole-induced contraction in bovine trachea. These data suggest that imidazole-induced contraction in bovine trachea may be due to stimulate release of endogenous ACh.

In this study, exogenous ACh and eserine, a cholinesterase inhibitor, concentration-dependently induced contractions in bovine, porcine, guinea pig, rats and mice trachea with similar efficacy of maximum contractions. Therefore, it is suggested that the difference of imidazole-induced contraction in various animals do not involve in difference of affinity of muscarinic receptor or activity of cholinesterase.

The parasympathetic nerve regulates the symptoms and inflammation of allergic responses primarily by signaling via the peripheral muscarinic receptor.

Tracheal smooth muscle is as a classical effector of parasympathetic signaling and has been a target of research in conditions such as airway narrowing.

Isolated trachea from rodents such as mouse and rat are usually used for ex vivo experiments. However, we showed that imidazole-induced contraction in the bovine trachea was greater than that in mouse and rat, and that imidazole-induced contraction was due to stimulate release of endogenous ACh. Thus, our study demonstrates that bovine trachea may be a useful tool to investigate the asthma regulating parasympathetic nerve.

In conclusion, we showed that imidazole-induced muscle tension in bovine trachea was greater than the muscle tension in other species tested. Further, CAMP did not appear to play a role in imidazole-induced muscle tension, suggesting another mechanism is involved. It is possible that imidazole-induced muscle tension in bovine trachea may have been due to stimulate release of endogenous ACh.

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