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The Effect of Lidocaine on Tracheal Smooth Muscle Tension in Guinea-pigs

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= 국문초록 =

기니픽 기관평활근장력에 대한 Lidocaine효과

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고신옥 · 김원옥 · 길혜금 · 김종래

서 론: Lidocaine은 기관내관 삽관시나 흡인시에 기도반사작용을 감소시키기 위하여 투여된다. 본 연구는 기니픽의 기관평활근에 대한 lidocaine의 작용과 그 기전을 평가하고자 시행하였다.

방 법: 근육표본은 30 ml의 Krebs용액으로 채워진 organ bath에 수직으로 고정하며, 근육장력은 등척성 변환기를 이용하여 측정하였다. 제 1 군은 carbachol 10^{-6} M에 의하여 일정한 상태의 긴장도를 유지한 표본에 lidocaine을 10^{-5} M~3× 10^{-3} M 까지 농도를 누적하여 적용하였고, 제 2 군은 carbachol투여 전에 10^{-5} M의 verapamil 로 전처치했다.

결 과: Lidocaine은 두 군에서 모두 용량에 비례하여 근육 장력을 감소시켰고, 제 1 군보다 제 2 군에서 많이 감소시켰다. ED_{50} 와 ED_{95} 의 값은 제 2 군에서 보다 제 1 군에서 컸다.

결 론: Verapamil은 carbachol에 의한 기도근육 장력을 감소시키며 lidocaine에 의한 이완효과를 높였다. 그러나 근육 장력과 같이 세포내 칼슘농도를 동시에 측정하지 않았기 때문에 기니픽에서 기도 이완효과의 세포내 기전을 설명할 수 없었다. **(Korean J Anesthesiol 1996; 30: 414~418)**

핵심용어: 기도: 기관, 기관평활근 장력. 국소미취제: 리도카인. 동물: 기니픽. 약리: 베라파밀.

INTRODUCTION

Lidocaine is often administered intravenously to suppress those airway reflexes associated with tracheal intubation or tracheal suction in patients. In addition, intravenous lidocaine has spasmolytic effects against bronchospasm[1~2].

Effects of an airway relaxant and the mechanism of relaxation may include followings: interruption of reflex arcs[3~4], inhibition of the chemical mediator release[5] and direct relaxation of airway smooth muscles[6~8] as well as on vascular smooth muscles[9]. The underlying mechanisms which are responsible for the direct effect of lidocaine on smooth muscles relaxation may be related to Ca^{2+} mobilization[10~12]. However, there is little direct evidence to support this hypothesis with regard to airway or other smooth muscles.

The present study was designed to examine the effect of lidocaine on tracheal smooth muscles tension induced by carbachol, potent muscarinic receptor agonist and the mechanism involved in the inhibitory effect of lidocaine

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on tracheal smooth muscles pretreated with verapamil, an L-typed voltage-operated calcium channel blocker, in guinea-pigs.

MATERIALS AND METHODS

1) Animal preparation

The study protocol was approved by the animal investigation committee at university. Male guinea-pigs weighing 350~450 grams each were anesthetized with ether inhalation. Explo-thoracotomy and neck dissection using a midline approach were done. The trachea was excised, cleaned of adhering adipose and connective tissue, and the trimmed trachea was opened by cutting longitudinally through the cartilage rings diametrically opposite the trachealis muscle. The opened trachea was cut into small segments each containing 3-4 cartilage rings.

2) Perfusion

Every preparation was mounted vertically in an organ bath which was filled with 30 ml of Krebs solution maintained at 37°C and aerated with 5 % carbon dioxide in 95% oxygen. The modified Krebs solution contained the followings (mmol / liter): sodium chloride 120.7, potassium chloride 5.9, calcium chloride 2.5, magnesium chloride 1.2, sodium bicarbonate 15.5, sodium dihydrogen phosphate 1.2, and glucose 11.5. The upper end of the strip was connected by a small clip with cotton thread to a strain gauge transducer(Grass, Quincy), while the lower end of the strip was held vertically by a cotton thread and mounted in an organ bath.

Each preparation was set at 1.5 grams of resting tension and allowed to equilibrate for ninety minutes. Isometric tension was induced to increase muscle length to the maximum level. This was done with 10^{-6}M carbamylcholine chloride(carbachol), (Aldrich Chemical Co., Milwaukee, WI), a stable and potent muscarinic receptor agonist. After a steady state was maintained (considered as 100% in each preparation), lidocaine hydrochloride, (Bulk Medicine and Pharmaceuticals, Hamburg, Germany) was cumulatively applied at a dose of 10^{-5} to 3×10^{-3} M while the specimens were kept at a steady state of tension (group 1). In group 2, after pretreatment with 10^{-5} M verapamil hydrochloride(Isoptin), (Sigma Chemicals, St. Louis, MO), an L-type voltage-operated calcium channel blocker, muscle tension was induced by 10^{-6} M carbachol and lidocaine was applied cumulatively at a dosage of 10^{-5} to 3×10^{-3} M. The changes in isometric tension of each sample was recorded at each concentration of lidocaine in group 1 and 2 on two channnels of Grass model 7 polygraph recorder (Grass medical instrument polygraph, Quincy, USA) simultaneously via force transducers (FT-03) and calculated as % relaxation of initial tension induced carbachol.

Before end of the experiment, all tissue was washed repeatedly with fresh Krebs solution for 15 minutes. After washing out lidocaine with normal Krebs solution for 15 minutes, carbachol caused the same extent of response, reversing the effect of the lidocaine and the next experiment was proceeded.

3) Data analysis

The measured values were expressed as mean±SE (n=number of observation). For each observation of group 1 and group 2, a strip from a different part of trachea tissue was used. One-way analysis of variance for repeated measurement was used to determine concentration- dependent effects. An unpaired t-test was done to compare the percent relaxation of tracheal muscle contraction and ED_{50} and ED_{95} between group 1 and group 2. A P value of less than 0.05 was considered to be significant in each case. The ED_{50} and ED_{95} values, the lidocaine concentration needed to decrease tension to 50 % and 95% of the maximal response induced by 10^{-6} M carbachol, were calculated, using a four-parameter logistic equation[13].

RESULTS

1) Effect of lidocaine on muscle tension induced by carbachol

In group 1, 4.9±0.5, 17.5±1.4, 37.5±2.7 and 60.6±2.5, 78.3±2.0% of maximal muscle contracture induced

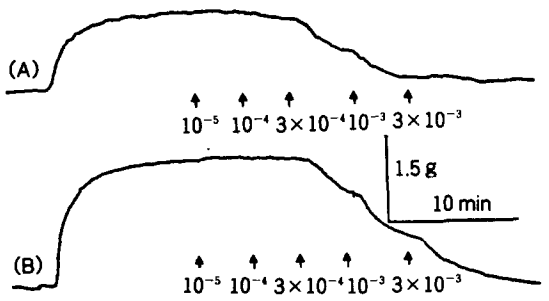

Fig. 1. Effect of lidocaine on tension induced by carbachol. This figure represents the time course of the effect of cumulatively applied lidocaine($10^{-5}\sim3\times10^{-3}$ M) on tension induced by 10^{-6} M carbachol. Upper trace(A): Muscle contraction was induced by 10^{-6} M carbachol after pretreating the muscle strip with 10^{-5} M verapamil(Group 2). Lower trace(B): Muscle contraction was induced by 10^{-6} M carbachol(Group 1) .

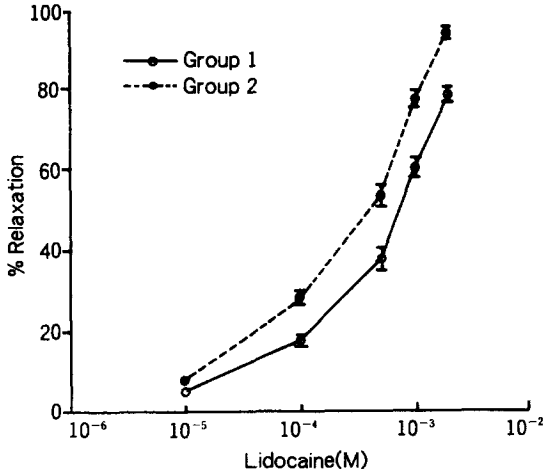

Fig. 2. This figure represents the effect of lidocaine on tension induced by carbachol. Note the representative time course of the effect of cumulatively applied lidocaine($10^{-5}\sim3\times10^{-3}$ M) on tension induced by 10^{-6} M carbachol. Group 1: The concentration dependent effect of lidocaine on elevated tension as induced by 10^{-6} M carbachol. Group 2: The concentration dependent effect of lidocaine on elevated tension as induced by 10^{-6} M carbachol after pretreating the muscle strip with 10^{-5} M verapamil.

Table 1. Relaxation Effect of Lidocaine on Airway Tension Induced by Carbachol

	Group 1	Group 2
Lidocaine concentration(M)	% Relaxation of Muscle Contraction	
10^{-5}	4.9 ± 0.5^a	7.3 ± 0.8
10^{-4}	17.5 ± 1.4^a	27.9 ± 1.9
3×10^{-4}	37.5 ± 2.7^a	53.2 ± 2.7
10^{-3}	60.6 ± 2.5^a	77.2 ± 2.2
3×10^{-3}	78.3 ± 2.0^a	93.9 ± 1.3

Values are mean±SE (%) (n=18, means number of observations from 18 animals). Group 1 refers to that group of guinea-pigs in which the muscle tension was induced by 10^{-6} M carbachol. Group 2 refers to that group of guinea-pigs in which muscle tension was induced by 10^{-6} M carbachol after pretreatment with 10^{-5} M verapamil. $^a p<0.05$ versus Group 2

Table 2. Values of ED_{50} and ED_{95} of Group 1 and Group 2

	Group 1	Group 2
ED_{50}	1.21 ± 0.10^a	0.74 ± 0.05
ED_{95}	2.45 ± 0.08^a	1.85 ± 0.06

Values are (mean±SE)$\times10^{-3}$ M. Group 1 refers to those guinea-pigs in which contraction was induced by 10^{-6} M carbachol. Group 2 refers to those guinea-pigs in which contraction was induced by 10^{-6} M carbachol after pretreatment with 10^{-5} M verapamil. ED_{50} and ED_{95} refer to the lidocaine concentration that decreased tension to 50 and 95 percent of the maximal response induced by 10^{-6} M carbachol. $^a p < 0.05$, versus Group 2

by 10^{-6} M carbachol was decreased at a concentration of 10^{-5}, 10^{-4}, 3×10^{-4}, and 10^{-3}, 3×10^{-3} M lidocaine in a concentration - dependent manner ($p<0.05$) (Table 1, Figure 1 B). In group 2 , 7.3±0.8, 27.9±1.9, 53.2±2.7 and 77.2±2.2, 93.9±1.3 % of maximal muscle contracture induced by 10^{-6} M carbachol was decreased at a concentration of 10^{-5}, 10^{-4}, 3×10^{-4} and 10^{-3}, 3×10^{-3} M lidocaine in a concentration dependent manner ($p<0.05$) (Table 1, Figure 1 A). At the same concentrations of lidocaine, $10^{-5}\sim3\times10^{-3}$ M, the percent of depression

was greater in group 2 than that of group 1 ($p<0.05$) (Table 1, Fig. 2).

2) Values of ED_{50} and ED_{95} of group 1 and group 2

The ED_{50} values in group 1 and 2 were $(1.21\pm0.10)\times10^{-3}$ M, $(0.74\pm0.05)\times10^{-3}$ M. The ED_{95} values in group 1 and 2 were $(2.45\pm0.08)\times10^{-3}$ M and $(1.85\pm0.06)\times10^{-3}$ M and. The values of ED_{50} and ED_{95} were greater in group 1 than those of group 2 ($p<0.05$) (Table 2).

DISCUSSION

Increase in intracellular Ca^{2+} can be triggered by two mechanisms: ① via the release of Ca^{2+} from intracellular stores, especially the sarcoplasmic reticulum and ② the entry of extracellular Ca^{2+} through voltage-operated or receptor-operated channels. It is well known that smooth muscle contraction is maintained by a Ca^{2+} influx through voltage-operated and receptor-operated channels[14].

Although it is generally accepted that smooth muscle contraction is primarily regulated by Ca^{2+}, smooth muscle contractile forces do not simply depend on Ca^{2+}. Morgan and Morgan[15] first showed that agonists can increase the effectiveness of intracellular Ca^{2+} on the contractile apparatus.

Our results indicated that lidocaine decreased the muscle tension induced by carbachol when administered in a range of concentration of 10^{-5} to 3×10^{-3} M in a concentration - dependent manner. Verapamil, an L-type voltage-operated calcium channel - blocker, decreased muscle contraction induced by 10^{-6} M carbachol almost to the resting level and appears to enhance the the inhibitory effect of lidocaine. This implied that the increase of Ca^{2+} through voltage operated calcium channels may play an important and perhaps even a controlling role in the maintenance of smooth muscle contraction. Therefore lidocaine may decrease Ca^{2+} by regulating at least voltage - operated calcium channels[16].

Hay and Wadsworth[17] state that the inhibitory action of lidocaine on KCl responses in rat vas deferens was reversed by raising the extracellular Ca^{2+} concentration. Spedding and Berg[18] report that drugs such as lidocaine can also interact with calcium channels as deduced from similar observations in guinea-pig taenia. Our study also indicated this.

Carbachol activated Ca^{2+}- nondependent muscle contraction; Carbachol, a muscarinic receptor agonist, activated phospholipase C via the G-protein linked to it. The phospholipid component of the cell membranes was broken down into second messengers, inositol 1, 4, 3-triphosphate and diacylglycerol(DAG). Carbachol induced a sustained increase in the DAG content of intact tracheal smooth muscles[19]. The only well characterized effect of DAG was the activation of phospholipid / Ca^{2+}-dependent PKC.

Under basal conditions, PKC is thought to be located mainly in cytosol and to be deactivated, but after carbachol stimulation, PKC is rapidly translocated to the membrane, where it begins to associate with membrane phospholipid DAG[20]. It has been shown that PKC plays an important role in tracheal smooth muscle contraction, which is independent of Ca^{2+} [16].

The ED_{50} and ED_{95} values, the lidocaine concentration that decreased tension to 50 % and 95 % of the maximal response were $(1.21\pm0.10)\times10^{-3}$ M and $(2.45\pm0.08)\times10^{-3}$ M, higher in group 1 (this contraction was induced by 10^{-6} M carbachol) than those of group 2 (this contraction was induced by 10^{-6} M carbachol after pretreating the muscle strip with 10^{-5} M verapamil). This was supported by the data of Table 1 and Figure 2, also, showing that the relaxation percent of muscle contraction was greater in group 2 at the same concentrations of lidocaine. ED_{50} values for Ca^{2+} and tension induced by 40 mM K^+ in adult pigs were approximately 3.4×10^{-4} M and 1.2×10^{-4} M [11]. If tension was induced by 10^{-6} M acetylcholine, the ED_{50} values for Ca^{2+} and tension were approximately 2.8×10^{-4} M and 1.5×10^{-4} M. Differences relating to the ED_{50} values between our data and that of Kai's group may have been due to differences in species, or to the fact that the muscle contraction was induced by such different agents from carbachol as

potassium or acetylcholine. Another handicap to our study was that we did not measure the intracellular calcium concentration simultaneously with muscle tension as in the Kai et al[12] group study.

In conclusion, lidocaine decreased the tracheal smooth muscle contraction induced by carbachol in a dose dependent manner. Verapamil, an L-type voltage-operated calcium channel blocker, antagonized tracheal muscle contraction induced by carbachol and thus appears to enhance the inhibitory effect of lidocaine. However, we did not measure intracellular calcium simultaneously with muscle tension and can not explain the exact intracellular mechanism of the inhibitory effect of lidocaine on airway tension of guinea-pigs.

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REFERENCES

1. Brandus V, Joffe S, Benoit CV, Wolf WI: Bronchial spasm during general anaesthesia. Can Anaesth Soc J 1970; 17: 269-74.
2. 최윤, 문선학, 임승운: 기관내 삽관시 lidociane정주가 혈역학적 변화에 미치는 영향. 대한마취과학회지 1991; 24: 373-8.
3. Downes H, Gerber N, Hirshman CA: IV Lignocaine in reflex and allergic bronchoconstriction. Br J Anaesth 1908; 52: 873-8.
4. Downes H, Hirshman CA: Lidocaine aerosols do not prevent allergic bronchoconstriction. Anesth Analg 1981; 60: 28-32.
5. Weiss EB, Hargraves WA, Viswanath SG: The inhibitory action of lidocaine on anaphylaxis guinea pig trachealis muscle. Am Rev Respir Dis 1978; 117: 859-69.
6. Weiss EB, Anderson WH, O'brien KP: The effect of local anesthetic, lidocaine, on guinea pig trachealis muscle in vitro. Am Rev Respir Dis 1975; 112: 393-400.
7. Downes H, Loehing RW: Local anesthetic contracture and relaxation of airway smooth muscle. Anesthesiology 1977; 47: 430-6.
8. Okumura F, Denborough MA: Effects of anaesthetics on guinea pig tracheal smooth muscle. Br J Anaesth 1980; 52: 199-204.
9. Altura BM, Altura BT: Effects of local anesthetics, antihistamines and glucocorticoids on peripheral blood flow and vascular smooth muscle. Anesthesiology 1980; 41: 197-214.
10. Feinstein MB, Paimre M: Pharmacological action of local anesthetics on excitation- contraction coupling in striated and smooth muscle. Fed Proc 1969; 28: 1643-8.
11. Fleisch JH, Titus E: Effect of local anesthetics on pharmacologic receptor system of smooth muscle. J Pharmacol Exp Ther 1973; 186: 44-51.
12. Kai T, Nishimura J, Kobayashi S, Takahashi S, Yoshitake JI, Kanaide H: Effect of lidocaine on intracellular Ca^{2+} and tension in airway smooth muscle. Anesthesiology 1993; 78: 954-65.
13. De Lean A, Munson PJ, Rodbard D: Simultaneous analysis of families of sigmoidal curves: Application to bioassay, radioligand assay and physiological dose-response curves. Am J Physiol 1978; 235: E97-E102.
14. Karaki H, Weiss GB: Calcium release in smooth muscle. 1988; Life Sci 42: 111-22.
15. Morgan JP, Morgan KG: Stimulus-specific patterns of intracellular calcium levels in smooth muscle of ferret portal vein. J Physiol 1984; 351: 155-67.
16. Yamakage M: Direct inhibitory mechanisms of halothane on canine tracheal smooth muscle contraction. Anesthesiology 1992; 77: 546-53.
17. Hay DWP, Wadsworth RM: Local anesthetic activity of organ calcium antagonist: Relevance to their actions on smooth muscle. Eur J Pharmacol 1982; 77: 221-8.
18. Spedding M, Berg C: Antagonism of Ca^{2+} induced contractions of K^{+}- depolarized smooth muscle by local anesthetics. Eur J Pharmacol 1985; 108: 143-50.
19. Takuwa Y, Takuwa N, Rasmussen H: Carbachol induces a rapid and sustained hydrolysis of polyphosphoinositide in bovine tracheal smooth muscle measurements of the mass of polyphosphoinositides, 1,2-diacylglycerol, and phosphatidic acid. J Biol Chem 1986; 261: 14670-5.
20. Yamakage M, Kohro S, Kawamata T and Namiki A: Inhibitory effect of four inhaled anesthetics on canine tracheal smooth muscle contraction and intracellular Ca^{2+} concentration. Anesth Analg 1993; 77: 67-72.