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European Journal of Pharmacology 425 (2001) 129–133

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Lidocaine potentiates atrial natriuretic peptide-induced relaxation of bovine tracheal smooth muscle

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Received 17 January 2001; received in revised form 12 June 2001; accepted 19 June 2001

Abstract

The effect of lidocaine on the changes in tension and guanosine 3′,5′-cyclic monophosphate (cGMP) content induced by atrial natriuretic peptide (ANP) and nitric oxide (NO) was examined in bovine tracheal smooth muscle preparations contracted with methacholine (0.3 μM). Lidocaine (10 μM) did not affect the methacholine-induced tensions, whereas 100 μM lidocaine significantly ($P < 0.01$) attenuated methacholine-induced ones. Treatment of the tracheal preparations with lidocaine (10 and 100 μM) significantly ($P < 0.05$) augmented the relaxant responses to ANP, whereas the same procedure did not alter the responses to sodium nitroprusside, (±)-(*E*)-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexeneamide (NOR 3) or 8-bromo-cGMP. Lidocaine (100 μM) enhanced cGMP accumulation induced by ANP (0.1 μM) but not by sodium nitroprusside (0.3 μM). In contrast, mexiletine (100 μM), another class Ib antiarrhythmic, did not affect ANP- and sodium nitroprusside-induced relaxations. These results suggest that lidocaine augments ANP-induced relaxation and cGMP accumulation, probably by modulating activation mechanism of particulate guanylyl cyclase. © 2001 Published by Elsevier Science B.V.

Keywords: ANP (Atrial natriuretic peptide); Antiarrhythmic drug, class Ib; cGMP (Guanosine 3′,5′-cyclic monophosphate); Smooth muscle, tracheal

1. Introduction

Agents that cause elevations of intracellular guanosine 3′,5′-cyclic monophosphate (cGMP), as well as those that increase adenosine 3′,5′-cyclic monophosphate (cAMP), have been demonstrated to exert relaxant effects in airway smooth muscles (Katsuki and Murad, 1977; Fiscus et al., 1984; Ishii and Murad, 1989; Ijioma et al., 1995). Atrial natriuretic peptide (ANP) activates the particulate (membrane-spanning) isoenzyme form of guanylyl cyclase. On the other hand, nitric oxide (NO)-donating compounds activate soluble isoenzyme form of guanylyl cyclase via the release of NO. Activation of either form of guanylyl cyclase increases the formation of cGMP from GTP and thereby causes relaxation of smooth muscles by activating cGMP-dependent protein kinase (Torphy et al., 1982; Fiscus et al., 1984). Our recent study showed that lidocaine, a class Ib antiarrhythmic drug, augmented relaxant responses to cAMP-elevating agents, such as salbutamol and forskolin (Nakahara et al., 2000). However, effect of lidocaine on responses to drugs that augment cGMP production remains unknown.

The purpose of this study, therefore, was to examine how lidocaine affected the effects of agents that are known to increase the level of intracellular cGMP through activation of different isoforms of guanylyl cyclases in airway smooth muscle. For this purpose, the effect of lidocaine on changes in tension and cGMP content of bovine tracheal smooth muscle induced by ANP and NO donors (sodium nitroprusside and (±)-(*E*)-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexeneamide (NOR 3)) was evaluated. The effect of mexiletine, another class Ib antiarrhythmic drug, on the relaxant responses to ANP and sodium nitroprusside was also examined.

2. Materials and methods

2.1. Preparation of bovine tracheal smooth muscle segments

Fresh bovine tracheas were obtained from a local abattoir and transported to the laboratory in ice-cold Krebs–

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0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)01154-2

Ringer bicarbonate buffer (KRB, composition in mM: NaCl, 118.5; KCl, 4.47; $MgSO_4$, 1.18; KH_2PO_4, 1.18; $CaCl_2$, 2.54; $NaHCO_3$, 24.9; glucose, 10.0 and pyruvic acid, 1.0) (pH = 7.4). The smooth muscle layers were dissected from the cartilage, mucosa, and connective tissues while immersed in ice-cold KRB gassed with 95% O_2–5% CO_2 as described previously (Nakahara et al., 2000). Segments ($1 \times 2 \times 10$ mm) of smooth muscle were used for measurement of mechanical responses and of cGMP content.

2.2. Measurement of mechanical activity

Muscle tension changes were recorded isometrically. One end of each muscle was attached by cotton thread to a force displacement transducer (model TB-611T, Nihon Kohden, Tokyo, Japan), and the other end was tied to a stainless-steel holder. The muscle segments were mounted in 20-ml jacketed organ baths containing Krebs–Ringer bicarbonate buffer gassed with 95% O_2–5% CO_2 at 37 °C, and subsequently allowed to equilibrate for 1 h under an initial tension of 0.75 g. The bath solution was changed every 15 min during the incubation period. The resting tension was adjusted to 0.5 g 10 min before starting each experiment.

When plateau tone was reached 15 min after the addition of methacholine (0.3 μM), tissues were exposed to vehicle (KRB), lidocaine (10 or 100 μM) or mexiletine (100 μM). After an additional 15-min incubation period, the tissues were relaxed by the administration of ANP (0.0003–0.3 μM), sodium nitroprusside (0.001–10 μM), NOR 3 (0.001–10 μM) or 8-bromo-cGMP (1–300 μM). Only one concentration–response curve was constructed with each preparation.

2.3. Measurement of cGMP content

The effect of lidocaine on changes in tissue content of cGMP induced by ANP (0.1 μM) and sodium nitroprusside (0.3 μM) under treatment with methacholine (0.3 μM) and 3-isobutyl-1-methylxanthine (IBMX, 300 μM) was examined. IBMX was used in this study to prevent the degradation of cGMP by phosphodiesterases. Each tissue was equilibrated for 1 h in an organ chamber that was warmed to 37 °C and filled with 5 ml of KRB gassed with 95% O_2–5% CO_2. After the equilibration period, tissues were exposed to both methacholine and IBMX. Then, the tissues were treated with vehicle (KRB) or lidocaine (10 or 100 μM) 15 min after the additions of methacholine and IBMX. After an additional 15-min incubation period, the tissues were incubated for 10 min with vehicle (KRB), ANP (0.1 μM) or sodium nitroprusside (0.3 μM). At the end of the incubation, the tissues were rapidly frozen in liquid nitrogen and at stored −80 °C until homogenization in 2 ml of ice-cold 6% trichloroacetic acid, using a glass homogenizer. The homogenate was centrifuged at $1500 \times g$ for 10 min at 4 °C. The supernatant was extracted three times with 5 ml of diethyl ether. We determined the cGMP content using a method of radioimmunoassay (cGMP assay kit, Yamasa Shoyu, Choshi, Japan). The tissue residue was dissolved in 2 N NaOH and the protein content was determined using a protein assay kit (Bio-Rad protein Assay, Bio-Rad Laboratories, Hercules, CA, USA) with bovine serum albumin as the standard. The tissue content of cGMP is presented as pmol/mg protein.

2.4. Data analysis and statistics

Data were expressed as the mean ± S.E.M. Relaxant responses were expressed as percentages of methacholine-induced tension obtained just before the cumulative addition of drugs. IC_{50} values (the concentration required to decrease methacholine-induced tension by 50%) were calculated by linear regression analysis by using the two data points that bracketed the 50% relaxant concentration. Data were analyzed using either Student's *t*-test or Scheffé's multiple comparison test after one-way analysis of variance (one-way ANOVA). A P value smaller than 0.05 was considered significant.

2.5. Drugs

The following drugs were used: acetyl-β-methylcholine chloride (methacholine), 3-isobutyl-1-methylxanthine (IBMX), lidocaine hydrochloride, mexiletine hydrochloride, sodium nitroprusside (Sigma, St. Louis, MO, USA), and (±)-(*E*)-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexeneamide (NOR 3) (Dojindo Lab., Kumamoto, Japan). Recombinant α-human atrial natriuretic peptide (ANP) was a generous gift from Suntory (Osaka, Japan). Stock solutions of IBMX and NOR 3 were prepared in dimethyl sulfoxide (DMSO) and those of other agents were prepared in distilled water. Stock solutions were diluted appropriately using KRB.

3. Results

3.1. Effects of lidocaine and mexiletine on tension developed with methacholine

The results presented in Fig. 1 show the effects of lidocaine and mexiletine on tension developed with methacholine. The mean tensions developed with methacholine (0.3 μM) were 7.4 ± 0.3 g ($n = 30$). This concentration produced approximately 50% of the maximum methacholine-induced tension in bovine tracheal smooth muscle preparations (data not shown). Lidocaine (100 μM) attenu-

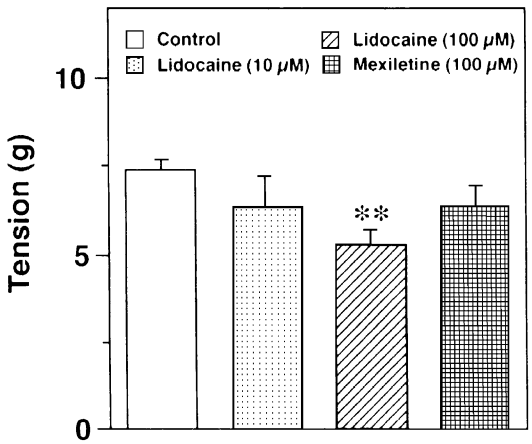

Fig. 1. Tensions developed with methacholine (0.3 μM) and effects of lidocaine (10 and 100 μM) and mexiletine (100 μM) on the developed tensions in bovine tracheal smooth muscle. Each column represents the mean + S.E.M. from 5 to 30 separate preparations. $^{**}P < 0.01$ vs. corresponding values obtained with 0.3 μM methacholine.

ated methacholine-induced tensions, whereas neither 10 μM lidocaine nor 100 μM mexiletine significantly affected methacholine-induced tensions.

3.2. Effects of lidocaine on ANP-, sodium nitroprusside-, NOR 3- and 8-bromo-cGMP-induced relaxations

The results presented in Fig. 2 show that lidocaine (10 and 100 μM) significantly ($P < 0.05$) augments relaxant responses to ANP in bovine tracheal smooth muscle contracted with methacholine (0.3 μM). In contrast, lidocaine (100 μM) did not significantly affect the concentration–response curves for sodium nitroprusside- and 8-bromo-cGMP-induced relaxations (Fig. 3). Similarly, the concentration of lidocaine did not change the relaxant responses to NOR 3 (IC_{50}s: control, 92 ± 16 vs. lidocaine, 90 ± 31 nM)($n = 4$).

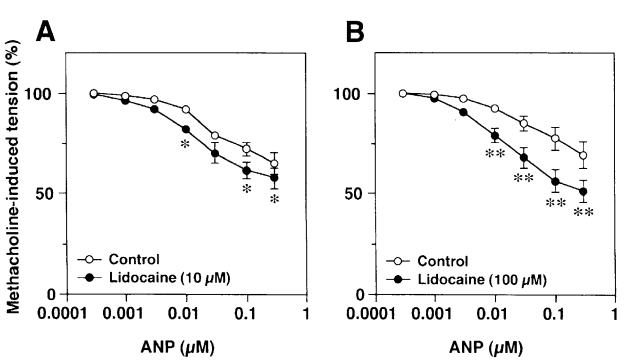

Fig. 2. Effects of lidocaine (A, 10 μM; B, 100 μM) on concentration–response curves for the relaxant responses to atrial natriuretic peptide (ANP). The bovine tracheal smooth muscle preparations were precontracted with methacholine (0.3 μM). Each point with a vertical bar represents the mean ± S.E.M. from five separate preparations. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. corresponding control values.

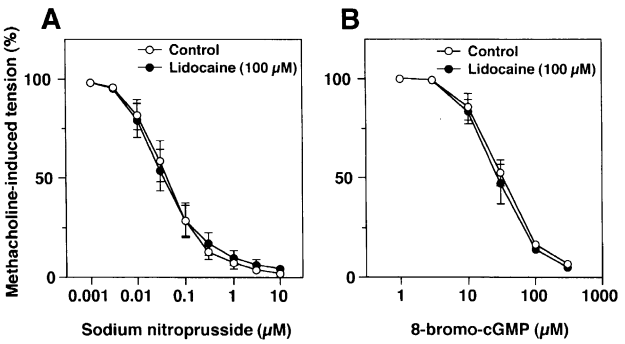

Fig. 3. Effects of lidocaine (100 μM) on concentration–response curves for the relaxant responses to sodium nitroprusside (A) and 8-bromo-cGMP (B). The bovine tracheal smooth muscle preparations were precontracted with methacholine (0.3 μM). Each point with a vertical bar represents the mean ± S.E.M. from four separate preparations.

3.3. Effects of mexiletine on ANP- and sodium nitroprusside-induced relaxations

The results presented in Fig. 4 show effects of mexiletine (100 μM) on ANP- and sodium nitroprusside-induced relaxations. Unlike lidocaine, mexiletine did not significantly affect relaxant responses to either ANP or sodium nitroprusside.

3.4. Effects of lidocaine on basal and ANP- and sodium nitroprusside-induced cGMP accumulation

The results presented in Fig. 5 show that lidocaine (100 μM) significantly ($P < 0.05$) increased ANP (0.1 μM)-induced cGMP accumulation without affecting basal cGMP levels. On the contrary, 100 μM lidocaine did not change sodium nitroprusside (0.3 μM)-induced accumulation (control, 4.0 ± 0.3 vs. lidocaine, 3.7 ± 0.4 pmol/mg protein; $n = 4$). Lidocaine (10 μM) slightly increased ANP-

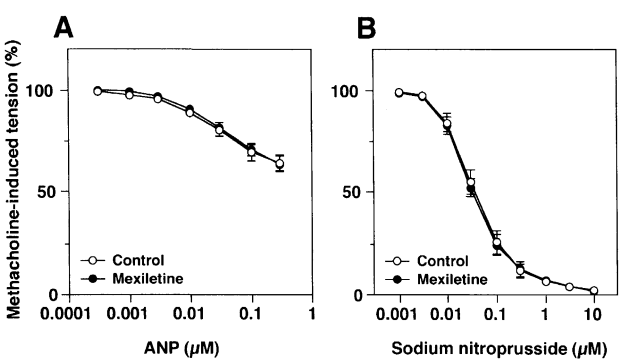

Fig. 4. Effects of mexiletine (100 μM) on concentration–response curves for the relaxant responses to atrial natriuretic peptide (ANP) (A) and sodium nitroprusside (B). The bovine tracheal smooth muscle preparations were precontracted with methacholine (0.3 μM). Each point with a vertical bar represents the mean ± S.E.M. from four separate preparations.

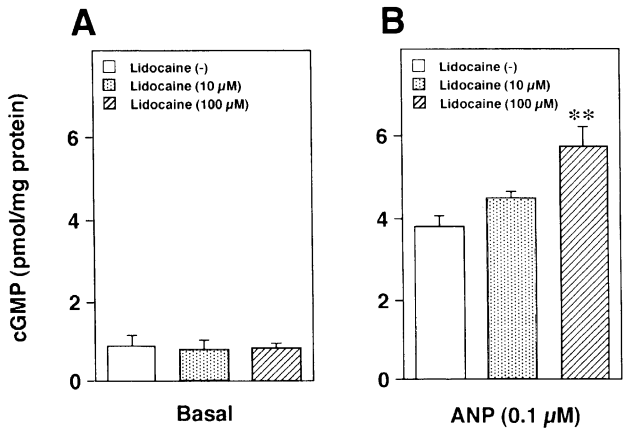

Fig. 5. Basal (A) and atrial natriuretic peptide (ANP, 0.1 μM) (B)-induced cGMP accumulations in the absence and presence of lidocaine (10 and 100 μM). The bovine tracheal smooth muscle preparations were treated with methacholine (0.3 μM) and 3-isobutyl-1-methylxanthine (300 μM). Each column with a vertical bar represents the mean + S.E.M. from four to nine separate preparations. $^{**}P < 0.01$ vs. corresponding lidocaine (−) values.

induced cGMP accumulation; however, the change was not significant.

4. Discussion

The present data demonstrate that lidocaine augments ANP-induced relaxations without affecting NO-induced relaxations in bovine tracheal smooth muscle contracted with methacholine. On the other hand, mexiletine had no significant effect on either ANP- or NO-induced relaxations.

Lidocaine is used to prevent the bronchospasm associated with airway instrumentation for general anaesthesia or bronchoscopy. Not only attenuation of vagal reflex arcs (Downes et al., 1980; Brown et al., 1995; Groeben et al., 1996) and inhibition of chemical mediator release (Weiss et al., 1978), but also the relaxing effect on airway smooth muscle (Weiss et al., 1975; Downes and Loehning, 1977; Okumura and Denborough, 1980; Kai et al., 1993) might be important for the protective action of lidocaine. The present study also showed that lidocaine exerted relaxant effect on bovine tracheal smooth muscle preparations contracted with methacholine (Fig. 1). Since lidocaine per se attenuates contraction with methacholine, the decreased levels of precontraction might affect the relaxant responses to bronchodilators. However, 100 μM lidocaine, which decreased precontraction levels induced by methacholine (~ 30%), did not significantly change the relaxation induced by 8-bromo-cGMP, a membrane-permeable cGMP analogue. It is unlikely, therefore, that the attenuation of precontraction by lidocaine affects cGMP-mediated relaxation of bovine tracheal smooth muscle.

ANP activates particulate guanylyl cyclase and NO activates soluble guanylyl cyclase. Activation of either form of guanylyl cyclase causes elevation of intracellular cGMP in tracheal smooth muscles (Katsuki and Murad, 1977; Ishii and Murad, 1989; Ijioma et al., 1995). The increased cGMP activates cGMP-dependent protein kinase and elicits several intracellular responses, such as reduction in intracellular Ca^{2+} concentration (Jones et al., 1994), activation of Ca^{2+}-dependent K^+ channels (Yamakage et al., 1996), and decrease in the Ca^{2+} sensitivity of contractile apparatus (Jones et al., 1999). Thus, both ANP and NO elicit relaxation of airway smooth muscle via cGMP-dependent mechanisms. The present study, however, showed that lidocaine exhibited distinct effects on ANP- and sodium nitroprusside-induced relaxations. The difference might be attributable to the different isoforms that are activated by these agents, because lidocaine has no effect on responses evoked by cGMP per se. This idea also was supported by the results obtained from the biochemical studies investigating the cGMP levels. In this study, the elevations of cGMP induced by ANP and sodium nitroprusside, unlike the relaxations, were examined in the presence of a phosphodiesterase inhibitor. Nevertheless, similar to the finding obtained with relaxation, lidocaine enhanced ANP-induced cGMP responses without affecting sodium nitroprusside-induced ones.

Like lidocaine, mexiletine is classified as a class Ib antiarrhythmic drug and reduces Na^+ currents in cardiac myocytes. Despite the similarities of electrophysiological properties, mexiletine had no significant effect on either relaxation evoked by ANP or by sodium nitroprusside. These results suggest that the inhibitory effect on Na^+ channel does not account for the augmentation by lidocaine of ANP-induced relaxant responses. As discussed above, lidocaine appears to act on the step of cGMP production in the signaling pathway stimulated by ANP (i.e., activation of particulate guanylyl cyclase). Due to the amphipathic properties, lidocaine might affect not only Na^+ channel conductance but also activity of membrane-bound enzymes. For example, benzyl alcohol, a neutral anaesthetic drug, has been shown to cause stimulation or inhibition of several membrane-bound enzymes, such as 5′-nucleotidase (Gordon et al., 1980), Na^+, K^+-ATPase (Gordon et al., 1980), sarcoplasmic reticulum Ca^{2+}-ATPase (Almeida et al., 1986), and adenylyl cyclase (Gordon et al., 1980; Friedlander et al., 1987), due to increase in plasma membrane fluidity. However, at present, whether increase in membrane fluidity modulates particulate guanylyl cyclase is unknown. Moreover, there is no evidence that lidocaine changes the activity of membrane-bound enzymes via a modification of membrane fluidity in airway smooth muscle. Thus, although the most likely site of action of lidocaine is plasma membrane, the precise mechanisms by which lidocaine potentiates ANP-mediated responses in tracheal smooth muscle remain to be clarified. Nevertheless, we would like to emphasize that this is the first evidence which indicates that lidocaine modifies the activity of particulate guanylyl cyclase.

ANP is of interest as a bronchodilator because it relaxes airway smooth muscle by a mechanism different from that of β_2-adrenoceptor agonist. Although ANP has a potent bronchodilating action in both normal and asthmatic subjects when given intravenously (Chanez et al., 1990; Hulks et al., 1990), inhaled ANP has only a modest effect (Hulks and Thomson, 1994). The prevention of ANP degradation by neutral endopeptidase inhibitor enhanced the protective effect of inhaled ANP against histamine-induced bronchoconstriction (Angus et al., 1995). The present data suggest that not only inhibition of neutral endopeptidase but also treatment with lidocaine may become an alternative approach to increase the therapeutic potential of inhaled ANP on the airway. In this study, even 10 μM lidocaine showed a tendency to augment ANP-induced responses in tracheal smooth muscle. Therefore, it is speculated that lidocaine in a therapeutic concentration range used for the treatment of arrhythmias (5.6–19 μM) could exert a synergistic effect on the prevention by ANP of muscarinic receptor-mediated bronchospasm. Moreover, although ANP at physiological concentrations appears unlikely to have any influence on airway diameter (Hulks et al., 1990), circulating ANP may play a role in regulating airway smooth muscle tone in the presence of lidocaine.

In summary, lidocaine augments ANP-induced relaxation and cGMP accumulation in bovine tracheal smooth muscle without affecting NO-mediated responses. Thus, lidocaine modulates activation mechanism of particulate guanylyl cyclase and thereby affects ANP-induced responses.

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