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Role of K^+ channels in *N*-acetylprocainamide-induced relaxation of bovine tracheal smooth muscle

Tsutomu Nakahara [a,*], Hiroshi Moriuchi [a], Yoshio Tanaka [b], Motonari Yunoki [a], Yuko Kubota [a], Kenji Sakamato [a], Koki Shigenobu [b], Kunio Ishii [a]

[a] *Department of Molecular Pharmacology, Kitasato University School of Pharmaceutical Sciences, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan*
[b] *Department of Pharmacology, Toho University School of Pharmaceutical Sciences, 2-2-1 Miyama, Funabashi City, Chiba 274-8510, Japan*

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Abstract

We examined the relaxant effects of *N*-acetylprocainamide, the major hepatic metabolite of procainamide, on bovine tracheal smooth muscle, focusing on the possible involvement of K^+ channels. *N*-acetylprocainamide produced a concentration-dependent and full inhibition of the tension development elicited by methacholine (0.3 or 1 μM). The potency of *N*-acetylprocainamide in diminishing methacholine-elicited tension development was one-half of that of procainamide. By comparison, *N*-acetylprocainamide inhibited high-K^+ (40 mM)-induced contraction more potently than procainamide though both inhibitions were largely reduced when compared to those against methacholine-induced contraction. Iberiotoxin (30 nM), Ba^{2+} (1 mM) or a combination of both agents significantly attenuated the relaxant effect of *N*-acetylprocainamide on methacholine-induced contraction, whereas apamin (100 nM), 4-aminopyridine (300 μM), and glibenclamide (10 μM) did not affect it. These results suggest that *N*-acetylprocainamide, similar to procainamide, elicits tracheal smooth muscle relaxation mainly through the activation of plasma membrane K^+ channels.

Keywords: Ba^{2+}; (Bovine); Iberiotoxin; K^+ channel, inward rectifier; Maxi-K^+ channel; *N*-Acetylprocainamide; Procainamide; Smooth muscle, tracheal

1. Introduction

Procainamide is an antiarrhythmic drug which is classified into class I (class Ia) category (Vaughan Williams, 1984). In addition to the effect on cardiac muscles, we have recently shown that procainamide exhibits relaxation of the isolated bovine tracheal smooth muscle contracted with methacholine (Nakahara et al., 2000). Therefore, procainamide could be also expected to ameliorate bronchoconstriction in a similar way as the class Ib antiarrhythmics, lidocaine and mexiletine (Brown et al., 1995; Groeben et al., 1996).

When administered systemically, procainamide is eliminated by renal excretion as unchanged drug and by hepatic metabolism. The major hepatic metabolite of procainamide is *N*-acetylprocainamide (Dreyfuss et al., 1972), which is produced as a consequence of conjugation by *N*-acetyl transferase (Roden, 1996). *N*-acetylprocainamide also exerts antiarrhythmic action and its mechanism is similar to that of class III antiarrhythmics (i.e., blockade of cardiac K^+ channels)(Elson et al., 1975; Minchin et al., 1978; Dangman and Hoffman, 1981; Komeichi et al., 1990). During chronic therapy with procainamide, the plasma concentration of *N*-acetylprocainamide often exceeds that of procainamide (Bagwell et al., 1976; Drayer et al., 1974; Reidenberg et al., 1975). Therefore, *N*-acetylprocainamide may play some important roles in determining pharmacological responses to systemic administration of procainamide. However, the actions of *N*-acetylprocainamide on the smooth muscles including tracheal smooth muscle have not been elucidated so far.

The present study was thus carried out to investigate the effect of *N*-acetylprocainamide on tracheal smooth muscle. Since the relaxant effects of procainamide on bovine tracheal smooth muscle have been shown to be mainly attributable to the activation of some K^+ channels

* Corresponding author. Tel.: +81-3-3444-6205; fax: +81-3-3444-6205.
E-mail address: nakaharat@pharm.kitasato-u.ac.jp (T. Nakahara).

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(Nakahara et al., 2000), special care was taken to focus on the possible contribution of K^+ channel activation to the action of *N*-acetylprocainamide. Our findings indicate that, as in the case of procainamide-induced relaxation, activation of K^+ channels accounts for a substantial component of the relaxant action of *N*-acetylprocainamide in bovine tracheal smooth muscle.

2. Materials and methods

2.1. Tissue preparation

We obtained freshly-excised bovine tracheas from the local abattoir and transported them to the laboratory immersed in cold Krebs–Ringer bicarbonate buffer of the following composition (in mM): 118.5 NaCl, 4.47 KCl, 1.18 $MgSO_4$, 1.18 KH_2PO_4, 2.54 $CaCl_2$, 24.9 $NaHCO_3$, 10.0 glucose, and 1.0 pyruvic acid (pH = 7.4). We carefully separated smooth muscle from cartilage, mucosa, and connective tissues, immersing the muscle in ice-cold Krebs–Ringer bicarbonate buffer gassed with 95% O_2–5% CO_2 as described previously (Nakahara et al., 2000).

2.2. Measurement of mechanical activity

We used smooth muscle segments (1–2 mm in width × 10 mm in length) for measurement of mechanical responses. One end of each muscle was attached with a 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. Muscle tension changes were recorded isometrically. 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.

2.3. Experimental procedure

In the first series of experiments, we examined the effects of *N*-acetylprocainamide on the contractions produced by methacholine (0.3 or 1 μM) and high-K^+ (40 mM). When the contractile responses had reached a stable plateau level, *N*-acetylprocainamide (0.1–10 mM) was added cumulatively to the bath solution. In separate experiments, we examined the effect of procainamide (0.1–10 mM) on 0.3 μM methacholine- and 40 mM K^+-induced contractions. In experiments with high-K^+ solution, Na^+ in the bathing medium was replaced with an equimolar amount of K^+.

In the second series of experiments, we examined the effects of K^+ channel blockers on the *N*-acetylprocainamide-induced relaxations. When the tension development due to methacholine (0.3 μM) reached a steady-state plateau level (usually attained at 20–30 min after its administration), the tissues were exposed to vehicle or iberiotoxin (30 nM), Ba^{2+} (1 mM), iberiotoxin (30 nM) plus Ba^{2+} (1 mM), apamin (100 nM), 4-aminopyridine (300 μM), and glibenclamide (10 μM). After an additional 15-min incubation period, we added *N*-acetylprocainamide cumulatively to the bath medium to construct the concentration–response relationships for its relaxant action. Only one concentration–response relationship was made for each preparation.

2.4. Data analysis and statistics

The data were expressed as the mean ± S.E.M. Relaxant responses were presented as percentages of the methacholine- or high-K^+-induced tension increase obtained just

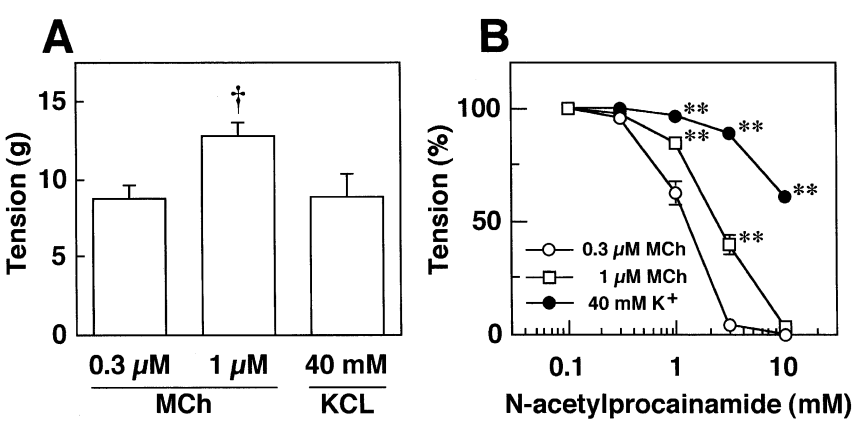

Fig. 1. Tensions developed with methacholine (MCh) (0.3 and 1 μM) and KCl (40 mM) and effect of *N*-acetylprocainamide on the developed tensions in bovine tracheal smooth muscle. Each column and point represents the mean ± S.E.M. from five to six separate preparations. S.E.M. values are within the symbols for some points. †$P < 0.05$ vs. 0.3 μM MCh or 40 mM KCl; **$P < 0.01$ vs. corresponding values obtained with 0.3 μM methacholine (Tukey's multiple comparison test).

before the cumulative drug application. IC_{50} values (the concentrations required to decrease the methacholine- or high-K^+-induced tension by 50%) were calculated by linear regression analysis using the data points that bracketed the 50% relaxant concentration, and were expressed as their negative logarithm (pIC_{50}) for statistical analysis. Significance of differences between means was evaluated by either unpaired Student's *t*-test (if required with Welch's correction), or Tukey's multiple comparison test after one-way analysis of variance (one-way ANOVA) using GraphPad Prism™ (version 2.01) (GraphPad Software, San Diego, CA, USA). A P value smaller than 0.05 was considered statistically significant.

2.5. Drugs

The following drugs were used: acetyl-β-methylcholine chloride (methacholine), 4-aminopyridine, barium chloride, *N*-acetylprocainamide hydrochloride, procainamide hydrochloride (Sigma, St. Louis, MO, USA); glibenclamide (Nacalai Tesque, Kyoto, Japan); apamin, iberiotoxin (Peptide Institute, Osaka, Japan). Glibenclamide was dissolved in dimethyl sulfoxide (DMSO). Other drugs were dissolved in distilled water and diluted further with Krebs–Ringer bicarbonate buffer.

3. Results

3.1. Effect of N-acetylprocainamide on tracheal preparations contracted with methacholine or high-K^+

Fig. 1A shows the tension developments due to methacholine (0.3 and 1 μM) and high-K^+ (40 mM). Although the tension developed with 1 μM methacholine was larger than those of 0.3 μM methacholine and 40 mM K^+ ($P < 0.05$), the tensions due to 0.3 μM methacholine and 40 mM K^+ were not significantly different.

Fig. 1B shows the relaxant effects of *N*-acetylprocainamide on preparations contracted with methacholine (0.3 and 1 μM) and 40 mM K^+. *N*-acetylprocainamide decreased tensions in a concentration-dependent manner, and almost full relaxation was obtained at 10 mM when the preparations were contracted with either concentration of methacholine. The pIC_{50} values for *N*-acetylprocainamide against the contractions induced by 0.3 and 1 μM methacholine were 2.80 ± 0.03 and 2.65 ± 0.02, respectively ($P < 0.05$, $n = 5–6$). Thus, the relaxant effect of *N*-acetylprocainamide was found to decrease when the pre-applied tension level was elevated with higher concentration of methacholine. By contrast, in preparations contracted with 40 mM K^+, *N*-acetylprocainamide-induced relaxation was more strongly diminished, and the relaxation was less than 40% ($38.9 \pm 3.0\%$, $n = 6$) even with 10 mM *N*-acetylprocainamide.

3.2. Effects of K^+ channel blockers on relaxant responses to N-acetylprocainamide

Iberiotoxin (30 nM), Ba^{2+} (1 mM) and a combination of both agents increased methacholine-induced tension (control, 9.3 ± 1.0 vs. iberiotoxin, 10.9 ± 1.3 g; control, 9.7 ± 0.2 vs. Ba^{2+}, 10.7 ± 0.7 g; control 10.7 ± 0.5 vs. iberiotoxin and Ba^{2+}, 13.6 ± 1.6 g)($n = 4–5$). However, methacholine-induced tension changes by the treatments with these K^+ channel blockers were not significantly different from their corresponding controls ($P > 0.05$).

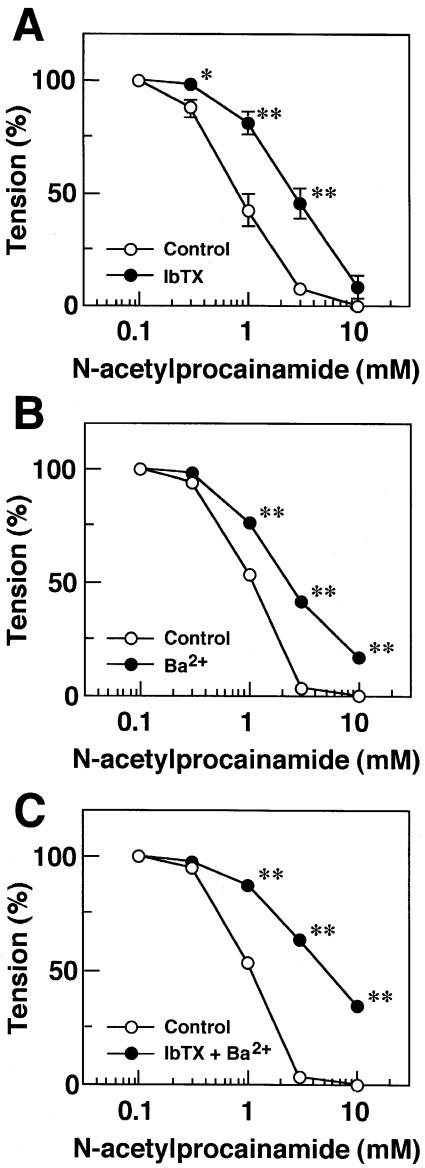

Fig. 2. Effects of iberiotoxin (IbTX, 30 nM)(A), Ba^{2+} (1 mM)(B) and a combination of iberiotoxin and Ba^{2+} (IbTX + Ba^{2+})(C) on the *N*-acetylprocainamide-induced relaxation in bovine tracheal smooth muscle contracted with 0.3 μM methacholine. Each point with a vertical bar represents the mean ± S.E.M. from four to five separate preparations. S.E.M. values are within the symbols for some points. $^{**}P < 0.01$ vs. corresponding control values (unpaired Student's *t*-test).

Fig. 2 and Table 1 show the effects of iberiotoxin (30 nM), Ba^{2+} (1 mM) and a combination treatment with both agents on the relaxant responses to *N*-acetylprocainamide in preparations contracted with 0.3 μM methacholine. Iberiotoxin, Ba^{2+} and the combination of both agents shifted rightwards the concentration–response relationships for *N*-acetylprocainamide-induced relaxation (Fig. 2), and this was evidently reflected as the significant decreases in pIC_{50} values for *N*-acetylprocainamide (Table 1). These treatments also significantly decreased the maximal relaxant response to *N*-acetylprocainamide. The combination treatment with iberiotoxin and Ba^{2+} significantly and more potently inhibited *N*-acetylprocainamide-induced relaxation than the single treatment with iberiotoxin or Ba^{2+} per se (Table 1).

Neither apamin (100 nM), 4-aminopyridine (300 μM) nor glibenclamide (10 μM) caused significant changes in pIC_{50} values for the *N*-acetylprocainamide-induced relaxations (control, 2.94 ± 0.04 vs. apamin, 3.00 ± 0.04; control, 2.89 ± 0.04 vs. 4-aminopyridine, 2.96 ± 0.07; control, 3.02 ± 0.02 vs. glibenclamide, 3.00 ± 0.09)($n = 4$–5). Furthermore, the maximal relaxant response to *N*-acetylprocainamide was not affected by these K^+ channel blockers (data not shown).

3.3. Effect of procainamide on tracheal preparations contracted with methacholine or high-K^+

Fig. 3 shows the effect of procainamide on preparations contracted with 0.3 μM methacholine and 40 mM K^+. Procainamide (0.1–10 mM) produced concentration-dependent relaxations in methacholine-contracted bovine tracheal smooth muscle with a pIC_{50} value of 3.17 ± 0.05 mM ($n = 4$). Procainamide almost abolished the methacholine-induced tones at the highest concentration examined (10 mM), whereas it exhibited only a small relaxant effect on the contraction induced by high-K^+ ($6.6 \pm 0.1\%$ relaxation, $n = 4$). These observations were almost identical with our previous findings (Nakahara et al., 2000).

Table 1
Effects of iberiotoxin (30 nM), Ba^{2+} (1 mM) and a combination of both agents on relaxant responses to *N*-acetylprocainamide in methacholine (0.3 μM)-contracted bovine tracheal smooth muscle

Treatment	pIC_{50}	Maximal response (%)
Control	2.98 ± 0.02	100.0 ± 0.0
Iberiotoxin	2.53 ± 0.08[a]	91.8 ± 4.8[b]
Ba^{2+}	2.61 ± 0.01[a]	83.1 ± 2.7[a]
Iberiotoxin + Ba^{2+}	2.20 ± 0.01[a,c]	65.4 ± 1.9[a,c]

The values represent the mean ± S.E.M. from 4 to 14 separate preparations.
[a] $P < 0.01$ vs. control.
[b] $P < 0.05$.
[c] $P < 0.01$ vs. iberiotoxin or Ba^{2+} (Tukey's multiple comparison test).

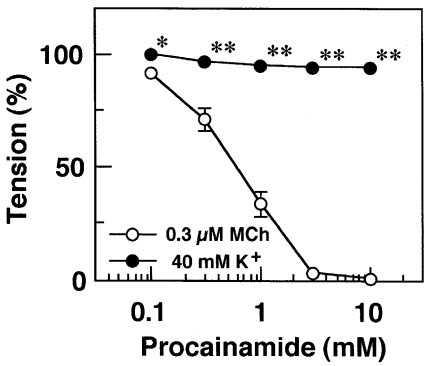

Fig. 3. Effect of procainamide on the tension of bovine tracheal smooth muscle contracted with 0.3 μM methacholine (MCh) or 40 mM K^+. Each point with a vertical bar represents the mean ± S.E.M. from four separate preparations. S.E.M. values are within the symbols for some points. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. corresponding values obtained with 0.3 μM methacholine (unpaired Student's *t*-test).

Based on the calculation with the pIC_{50} values obtained in the muscle contracted with 0.3 μM methacholine, *N*-acetylprocainamide was found to be 0.43 times as potent as procainamide to relax tracheal smooth muscle.

4. Discussion

N-acetylprocainamide, a major hepatic metabolite of procainamide, blocks cardiac K^+ channels, and thus exerts class III-like antiarrhythmic actions (Komeichi et al., 1990). In this study, we have shown that *N*-acetylprocainamide relaxes bovine tracheal smooth muscle contracted with methacholine and a high-K^+. Furthermore, we indicated that the relaxant effect of *N*-acetylprocainamide, like that of procainamide, might involve activation of K^+ channels.

Both *N*-acetylprocainamide and procainamide were more effective in attenuating methacholine-induced contraction than 40 mM K^+-induced one. This is consistent with the property of the relaxants with K^+ channel-opening actions since the driving force for K^+ efflux and the subsequent membrane hyperpolarization would be considerably reduced in the medium containing high-K^+. Indeed, we have shown that the relaxant effects of cromakalim, an opener of ATP-sensitive K^+ channel, and salbutamol, the action of which is partly mediated via an activation of maxi-K^+ (large conductance Ca^{2+}-activated K^+) channel, on 40 mM K^+-induced contraction were markedly weaker than those on methacholine-induced tension development in bovine tracheal smooth muscle (Nakahara et al., 2000). Thus, it is likely that activation of K^+ channels is significantly responsible for the tracheal smooth muscle relaxation induced by *N*-acetylprocainamide as well as procainamide.

In the previous study, we showed that procainamide-induced relaxation of bovine tracheal smooth muscle was susceptible to the inhibitions by both iberiotoxin, a selec-

tive blocker of maxi-K^+ channels (Galvez et al., 1990), and Ba^{2+}, which is able to block inward rectifier K^+ channels (Nelson and Quayle, 1995; Partiseti et al., 1998), and proposed the contribution of two types of K^+ (maxi-K^+ and inward rectifier K^+) channels to the relaxant response. In this study, we found that the relaxation induced by *N*-acetylprocainamide was also significantly sensitive to the inhibitions by iberiotoxin and by Ba^{2+}. On the other hand, other K^+ channel blockers tested (apamin, 4-aminopyridine and glibenclamide) did not affect the relaxant responses to *N*-acetylprocainamide, which rules out the possibility of involvement of the K^+ channels sensitive to these blockers (small-conductance Ca^{2+}-activated K^+ channel, voltage-dependent K^+ channel, and ATP-sensitive K^+ channel). Thus, as in the case of procainamide-induced relaxation, maxi-K^+ and inward rectifier K^+ channels seem to contribute significantly to the relaxant response to *N*-acetylprocainamide in bovine tracheal smooth muscle.

Iberiotoxin or Ba^{2+} caused an additional tension development in the preparations pre-contracted with methacholine (0.3 μM)(see Section 3). Therefore, the attenuation of *N*-acetylprocainamide-induced relaxation in the presence of iberiotoxin or Ba^{2+} could also be explained by the alteration of the pre-applied tension level. Indeed, the concentration–response relationship for *N*-acetylprocainamide-induced relaxation was shifted to the right by about 2-fold when the pre-applied tension level was significantly and strongly increased from 8.8 ± 0.8 g to 12.9 ± 0.8 g (Fig. 1A) by increasing methacholine concentration (from 0.3 to 1 μM). However, the attenuation of relaxant responses to *N*-acetylprocainamide by iberiotoxin or Ba^{2+} cannot be ascribed merely to the additional tension developments due to these K^+ channel blockers because of the following reasons: (1) First, as already addressed above, *N*-acetylprocainamide-induced relaxation was dramatically diminished when tracheal smooth muscle was precontracted with high-K^+ instead of methacholine (Fig. 1B). This observation strongly indicates that K^+ channel activation substantially contributes to the tracheal smooth muscle relaxation induced by *N*-acetylprocainamide. (2) We showed in the previous report (Nakahara et al., 2000) that the relaxant effect of diltiazem, a Ca^{2+} channel blocker, was not altered by iberiotoxin or Ba^{2+} in the muscle precontracted with methacholine. This finding indicates that tracheal smooth muscle relaxations mediated via the mechanisms other than the K^+ channel opening cannot be susceptible to the inhibitions by iberiotoxin or Ba^{2+}, and thus the pharmacological selectivity of these K^+ channel blockers was shown. (3) Iberiotoxin (30 nM) increased the precontraction level from 9.3 ± 1.0 to 10.9 ± 1.3 g ($n = 5$), Ba^{2+} (1 mM) from 9.7 ± 0.2 to 10.7 ± 0.7 g ($n = 5$) and iberiotoxin plus Ba^{2+} from 10.7 ± 0.5 to 13.6 ± 1.6 g ($n = 4$). However, these tension increases are not statistically significant ($P > 0.05$, unpaired *t*-test or unpaired *t*-test with Welch's correction). Therefore, the alteration of pre-applied tension level by iberiotoxin, Ba^{2+} or iberiotoxin plus Ba^{2+} per se can be ruled out as the primarily determinant factor which is able to affect the *N*-acetylprocainamide-induced relaxation. (4) When pre-applied tension level was elevated by about 50% (47%) (8.8 ± 0.8 g vs. 12.9 ± 0.8 g) with increasing methacholine concentration from 0.3 to 1 μM, the potency of *N*-acetylprocainamide to relax tracheal smooth muscle was reduced to 70% (100% = the relaxant potency for 0.3 μM methacholine-induced tension)(calculated based on the changes in pIC_{50} values). By contrast, the potency of *N*-acetylprocainamide in relaxing the muscle was reduced to 30% even when the precontraction level was elevated only by 20% (17%) in the presence of iberiotoxin (30 nM). This strongly indicates that the inhibition by iberiotoxin of *N*-acetylprocainamide is largely attributed to its blockade of K^+ (maxi-K^+) channel than the elevation of precontraction level by this toxin blocker. (5) Substantial inhibitory effects of iberiotoxin and Ba^{2+} are also evident from the significant additional changes in both pIC_{50} value and maximal response when the tracheal muscle was simultaneously treated with both iberiotoxin and Ba^{2+} as compared to the inhibitory effect of their single treatment (Table 1). In this case, *N*-acetylprocainamide-induced relaxation was dramatically reduced as compared to the response in 1 μM methacholine-contracted muscle even when both iberiotoxin and Ba^{2+} increased 0.3 μM methacholine-elicited tension by about 30% (27%). To confirm the idea of the significant role of these K^+ channels in *N*-acetylprocainamide-induced relaxation of tracheal smooth muscle, electrophysiological evidence using isolated cells would be necessary. Since unlike procainamide, *N*-acetylprocainamide lacks the Na^+ channel blocking action (Dangman and Hoffman, 1981), blockade of this type of cation channel can be ruled out as the mechanism responsible for the K^+ channel-mediated relaxation induced by *N*-acetylprocainamide.

N-acetylprocainamide was less potent than procainamide in attenuating methacholine-induced contraction at the concentration range below 3 mM. This suggests that *N*-acetylprocainamide is less potent than procainamide as a K^+ channel activator. On the other hand, at 10 mM, both *N*-acetylprocainamide and procainamide exhibited almost equal inhibitions against methacholine-induced contraction. Since 10 mM *N*-acetylprocainamide also inhibits 40 mM K^+-induced contraction by about 40%, some mechanisms other than K^+ channel opening seem to be operating over this concentration. High-K^+ (40 mM)-induced contraction of bovine tracheal smooth muscle was completely abolished by a Ca^{2+} channel blocker, nifedipine (Nakahara et al., 2000). Therefore, *N*-acetylprocainamide but not procainamide, may block Ca^{2+} influx through voltage-gated L-type Ca^{2+} channel in tracheal smooth muscle cells, and this action could explain the different relaxant potencies between procainamide and its metabolite against high-K^+-induced contraction.

The plasma concentration of *N*-acetylprocainamide obtained when procainamide is clinically used is 10–20 μg/ml (that is, 32–64 μM). In this study, concentrations of *N*-acetylprocainamide producing relaxation of bovine tracheal smooth muscle are extremely high. Therefore, *N*-acetylprocainamide per se may not exert airway smooth muscle dilatation in in vivo while procainamide is employed as the antiarrhythmic in a therapeutic concentration range. However, since *N*-acetylprocainamide is able to produce relaxation of tracheal smooth muscle, procainamide and its major metabolite (*N*-acetylprocainamide) may exert their actions so that both of them ameliorate bronchoconstriction associated with various stimuli including enhanced parasympathetic nerve activity.

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