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Nitric oxide-mediated relaxation induced by bradykinin in the isolated mouse trachea

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Abstract

We examined the nature of the relaxant effect of bradykinin on mouse isolated tracheal rings. Bradykinin produced a concentration-dependent relaxation in mouse tracheal rings contracted by carbachol. Potentiation of the contractile effect of carbachol and inhibition of the relaxant effect of bradykinin by pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME), L-glutamine (L-Gln) and methylene blue (MeB) suggested that the peptide activated the L-arginine nitric oxide (NO) pathway. Part of the relaxant effect of bradykinin was also mediated through the release of cyclooxygenase metabolites of arachidonic acid, as evidenced by the inhibition of this response by lysine acetylsalicylic acid (ASA) pretreatment. Bradykinin also caused a relaxant response in precontracted tracheal rings in the presence of lower but not higher concentrations of K^+ (>60 mM). N^G-nitro-L-arginine methyl ester and L-Gln did not alter the contractile effect of K^+. K^+ channel blockers partially inhibited the relaxant effect of bradykinin in carbachol-induced precontracted tracheal rings. Tetraethylammonium, a non-selective blocker of K^+ channels, completely abolished the relaxant response to the peptide. Among the other channel blockers, the inhibitory effect of glibenclamide was slightly greater than that of apamine and iberiotoxin, indicating the involvement of K_{ATP} channels in the relaxant response to the peptide. These results suggest that the mechanisms of the relaxation induced by bradykinin in carbachol-induced precontracted mouse tracheal muscle primarily involve activation of L-arginine NO and arachidonic acid cyclooxygenase pathways and secondly K^+ channels.

Keywords: Bradykinin; Trachea; (Mouse); N^G-L-arginine methyl ester; K^+ channel; Carbachol

1. Introduction

Earlier studies from this laboratory have indicated that bradykinin produces a relaxant response in cat isolated tracheal muscle which can not be blocked by β-adrenoceptor antagonists (Türker and Kiran, 1965). Subsequent studies have also indicated that bradykinin, angiotensin I and angiotensin II also produce a relaxation through the release of prostaglandins in cat (Türker and Ercan, 1976) and dog (Ercan and Türker, 1977; Ercan et al., 1978; Türker and Khairallah, 1969) isolated tracheal muscles contracted by 5-hydroxytryptamine. Recently, Van Heuven-Nolsen et al. (1997), working with mouse isolated tracheal muscles, have shown that bradykinin exerts a prostaglandin-mediated relaxant response via stimulation of bradykinin B_2 receptors.

Another group of peptides, endothelin-1 and endothelin-3, has been shown to produce concentration-dependent relaxation in carbachol-contracted guinea-pig isolated trachea (Hadj-Kaddour et al., 1996). There is evidence that the relaxant effect of endothelin-1 is mediated through the activation of the nitric oxide (NO) pathway while that of endothelin-3 is mediated through the activation of charybdotoxin sensitive K^+ channels (Hadj-Kaddour et al., 1996).

In addition to classical adrenergic bronchodilator neural mechanisms, several other endogenous substances may also cause an inhibitory response in airway smooth muscles. The release of vasoactive intestinal polypeptide and related peptides, such as histidine–isoleucine–methionine, may also produce relaxation of respiratory smooth muscles (for review, see Jorens et al., 1993; Raenburn and Giebycz, 1991). Ellis and Farmer (1989) have shown that α-chymotrypsin, a degrading enzyme of peptides, partly reduces the relaxant effects of the peptides, indicating that part of the response is mediated by a molecule other than a protein. Thus all these investigations clearly indicate that

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endogenously bioactive polypeptides participate to the respiratory smooth muscle tone.

As described for anococcygeus muscle (Gillespie et al., 1989), it is assumed that L-arginine-NO also has a role in the inhibitory response of respiratory smooth muscle (for review see Jorens et al., 1993; Raenburn and Giebycz, 1991).

In the present study, the relaxant effect of bradykinin was studied on tracheal rings isolated from mice. An attempt was also made to investigate the possible mechanisms underlying the relaxant effect of the peptide. In addition, the inhibitory effect of L-glutamine (L-Gln), which has been described as an inhibitor of intracellular L-arginine generation (Sessa et al., 1990; Hecker et al., 1990; Arnal et al., 1995), on bradykinin-induced relaxation was also assessed.

2. Materials and methods

2.1. Preparation of tracheal rings

Experiments were carried out on tracheal rings isolated from Swiss albino mice of either sex weighing 20–30 g. The mice were anesthetized with urethane (1.5 g/kg, i.p.) and after anesthesia was established the tracheas were excised and transferred to a beaker containing oxygenated Krebs solution of the following composition (mM): Na^+ 138.2, K^+ 5, Ca^{2+} 2.5, Mg^{2+} 0.5, Cl^- 123, HCO_3^- 25, $H_2PO_4^-$ 1.2, dextrose 11.5. The tracheas were cleaned of adhering fat and tissues, two rings were prepared from one animal and were suspended in 10-ml jacketed isolated organ baths containing Krebs solution warmed to 37°C and gassed with 95% O_2 + 5% CO_2. An initial tension of 1.0 g was applied and the contractility was recorded isometrically via a force–displacement transducer (Grass FT.03) on a four-channel polygraph (Grass Model 7 E). The rings were equilibrated for 45–60 min with fresh Krebs solution added every 15 min before drug application.

2.2. Experimental protocol

In a series of control experiments the concentration–response curves of carbachol were assessed and repeated in the presence of acetylsalicylic acid (ASA), N^G-nitro-L-arginine methyl ester (L-NAME) and L-Gln. ASA, L-NAME and L-Gln (10^{-5} M) were added to the organ bath and kept in contact with the preparations for 30–45 min. Similar concentration–response studies were also assessed with KCl (20–100 mmol/l) before and after incubation of the rings with L-NAME and L-Gln.

After a submaximal contraction (70–80%) was elicited with carbachol added to the organ bath in concentrations of 3×10^{-7}–10^{-6} M, cumulative concentration–response curves were made for bradykinin. Concentration–response studies were also repeated after addition of L-NAME, L-Gln and *des*-Arg^9-Leu^8-bradykinin, a bradykinin B_1 receptor antagonist (Regoli et al., 1993), in a separate series of experiments.

A series of experiments was also performed with various K^+ channel blockers in order to assess the participation of these channels in the relaxant effect of bradykinin in submaximally contracted tracheal rings. Tetraethylammonium as a nonselective, apamine and iberiotoxin as Ca^{2+}-activated and glibenclamide as ATP-dependent K^+ channel blockers (for review see Brayden, 1996 and Rusch et al., 1996) were used. Finally, methylene blue (MeB), a soluble guanylate cyclase inhibitor (Martin et al., 1985), was added to the bath in order to assess the participation of cGMP to the relaxant effect of bradykinin.

2.3. Chemical agents

The following drugs were used in this study: carbamylcholine HCl, bradykinin, N^G-nitro-L-arginine methyl ester, L-Gln, iberiotoxin, apamine, glibenclamide, tetraethylammonium, *des*-Arg^9-Leu^8-bradykinin (Sigma) and lysine ASA (Bayer, Germany). The peptides were dissolved in 0.1 M acetic acid as stock solution (10^{-3} M) and kept frozen −20°C and further dilutions were made in Krebs just before use. ASA, carbachol, L-NAME and L-Gln were freshly dissolved in saline for daily use.

2.4. Statistical analysis

Data are expressed as the means ± S.E.M. One-way analysis of variance (ANOVA) with modified Bonferroni *t*-test was used to assess the statistical significance between groups. Wilcoxon Matched-Pairs Signed-Rank test

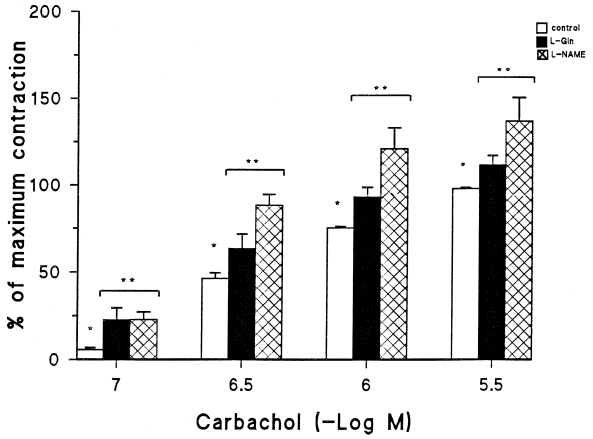

Fig. 1. Concentration–response relationship for carbachol in tracheal rings isolated from mice before (control) and after pretreatment with L-NAME or L-Gln. The concentrations of L-NAME and L-Gln were 10^{-5} M in bathing medium. When compared with the corresponding controls (*), responses were significant after L-NAME and L-Gln (**) ($P < 0.05$). Each column represents the mean value of eight experiments. Vertical bars on columns show S.E.M.

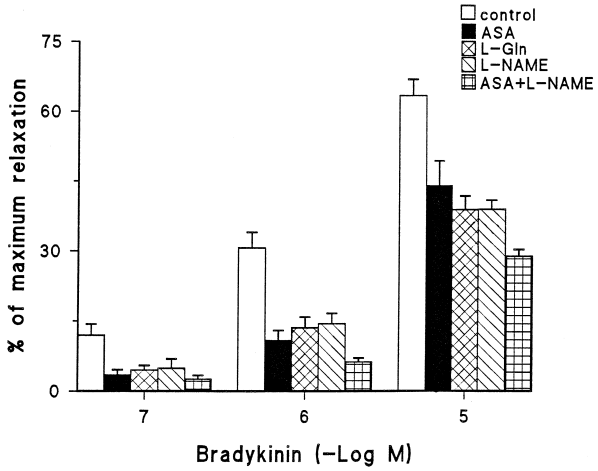

Fig. 2. Relaxation induced by bradykinin in mouse isolated tracheal rings precontracted by carbachol (3×10^{-7} M) before (control) and after pretreatment with lysine ASA ($n=12$), L-Gln ($n=8$), L-NAME ($n=8$) and ASA + L-NAME ($n=12$). The concentrations of antagonists were 10^{-5} M. All antagonists significantly ($P<0.05$) but not completely inhibited the relaxing effect of bradykinin. Columns indicate the mean value of ($n=$ number) experiments. Vertical bars on columns represent S.E.M. The comparison of inhibition by ASA and ASA + L-NAME of the relaxant effect of bradykinin for the concentrations of 10^{-6} and 10^{-5} M was statistically significant ($P<0.05$).

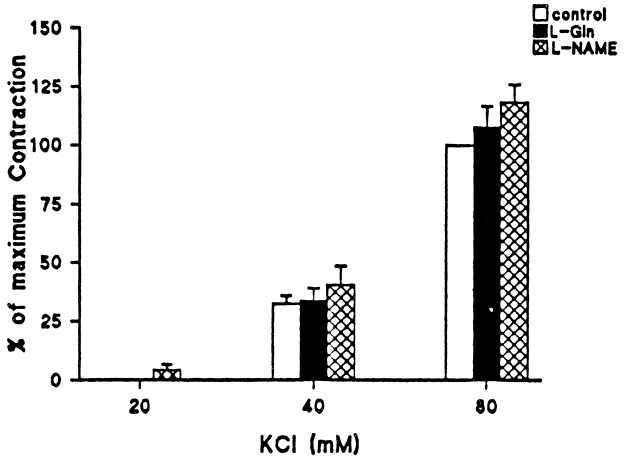

Fig. 3. Concentration–response study of KCl before (control) and after pretreatment of mouse tracheal rings with L-NAME and L-Gln. The concentrations used in this series of experiments were the same as Figs. 1 and 2. No change was observed before and after pretreatment with antagonists. Columns represent the mean value of six experiments ± S.E.M.

was used for intergroup comparisons. The level of significance was $P<0.05$.

3. Results

3.1. Contractile effect of carbachol and alteration by L-NAME and L-Gln

Carbachol produced a concentration-dependent contractile response in tracheal rings when tested at concentrations between 10^{-7} and 3×10^{-6} M, as shown in Fig. 1. Addition of L-NAME (10^{-5} M) to the organ bath for 30 min produced a significant ($P<0.05$) potentiation at all concentrations tested. An almost identical significant potentiation was obtained when the preparation was pretreated with L-Gln (10^{-5} M) (Fig. 1).

3.2. Effects of ASA, L-NAME, L-Gln and des-Arg^9-Leu^8-bradykinin on bradykinin-induced relaxation

Submaximal contractions were obtained with 3×10^{-7}–10^{-6} M carbachol in intact tracheal rings. However, when the rings were incubated with L-NAME or L-Gln (10^{-5} M), a lower concentration of carbachol elicited an identical submaximal contraction (about 80% of maximum contraction). L-NAME, L-Gln and ASA (10^{-5} M) caused a significant inhibition of the relaxant response to bradykinin (Fig. 2). L-NAME and, in another series of experiments L-Gln when incubated with tracheal rings caused an almost equal significant ($P<0.01$) inhibition of the relaxant response to bradykinin when compared with their corresponding control values. When the rings were pretreated with L-NAME plus ASA, a further inhibition of the response to bradykinin was seen. Such inhibition was significant ($P<0.05$) for the concentrations of 10^{-7}–10^{-6} M but not for 10^{-5} M of bradykinin when compared with the corresponding control values and with the responses obtained after pretreatment with L-NAME and L-Gln (Fig. 2). In another series of experiments, the bradykinin B_1 receptor antagonist with relatively high concentration partly inhibited the relaxant effect of bradykinin but addition of L-NAME to the organ bath did not cause a further inhibition of the relaxant effect of bradykinin. The calculated results are summarized in Table 1.

Table 1
Inhibition by *des*-Arg^9-Leu^8-bradykinin (1.5×10^{-5} M) of the relaxant response induced by bradykinin (% of maximum) in mouse isolated tracheal rings contracted by carbachol (10^{-7} M)

Bradykinin	Control	*des*-Arg^9-Leu^8-bradykinin	*des*-Arg^9-Leu^8-bradykinin plus L-NAME (10^{-5} M)
10^{-7} M	14.4 ± 1.7^a	2.4 ± 1.0^b	0.7 ± 0.1^b
10^{-6} M	34.9 ± 3.1^a	23.9 ± 0.9^b	21.3 ± 4.1
10^{-5} M	70.4 ± 4.3	63.3 ± 2.5	56.1 ± 6.6

Mean ± S.E.M. of six experiments.
[a] $P<0.05$.
[b] $P<0.05$.

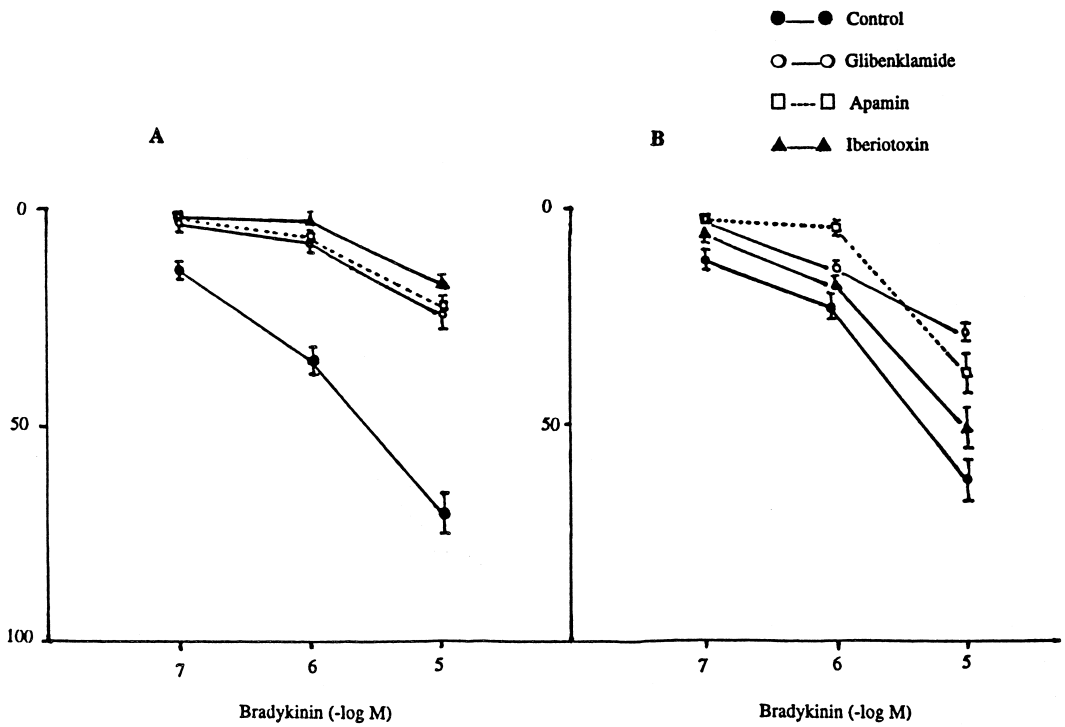

Fig. 4. Relaxing effect of bradykinin on carbachol (3×10^{-7} M) (A) and KCl (B) precontracted (80% of maximum contraction) tracheal rings from mice and the inhibition elicited by K^+ channel blockers, glibenclamide, apamine (10^{-6} M) and iberiotoxin (10^{-8} M) added to the bathing medium. The channel blockers significantly inhibited the relaxing response to bradykinin when compared with control ($P < 0.01$) in carbachol-precontracted rings. In potassium-contracted tracheal rings, inhibition was significant for glibenclamide ($P < 0.01$) and for apamine and iberiotoxin ($P < 0.05$) for the higher concentration of bradykinin (10^{-5} M). Each point represents the mean value of five experiments. Vertical bars S.E.M.

3.3. Inhibition of the relaxant effect of bradykinin by various K^+ channel inhibitors

L-NAME and L-Gln did not alter the contractile response to KCl (40–100 mmol/l), as shown in Fig. 3. The relaxant effect of bradykinin in the tracheal rings precontracted by carbachol (10^{-7} M) or KCl (60 mM) was reduced when the rings were preincubated with glibenclamide, apamine and iberiotoxin (Fig. 4A,B). No relaxant response to bradykinin was observed in the tracheal rings contracted by higher concentrations of KCl (above 80–100 mmol/l). Tetraethylammonium, however, almost completely blocked the relaxant effect of bradykinin in both carbachol- and KCl-precontracted tracheal rings. The inhibition of bradykinin-induced relaxation by glibenclamide, apamine and iberiotoxin were highly significant in tracheal rings precontracted by carbachol when compared with the inhibition observed in K^+-precontracted rings (Fig. 4A,B). MeB at the concentration of 10^{-5} M almost completely blocked the relaxant response to bradykinin in tracheal rings precontracted by carbachol. The calculated results are summarized in Table 2.

Table 2
The effect of MeB (10^{-5} M) on the relaxing response to bradykinin in isolated mouse tracheal rings contracted by carbachol (10^{-6} M)

Bradykinin (−log M)	% of maximum relaxation	
	Control	MeB pretreated
7	14.9 ± 3.6	0
6	32.3 ± 4.5[a]	4.5 ± 0.9[b]
5	68.6 ± 7.8[a]	8.6 ± 1.4[b]

Mean ± S.E.M of six experiments.
[a] $P < 0.001$.
[b] $P < 0.001$.

4. Discussion

The results of the present study indicate that bradykinin produced a relaxing response in mouse isolated tracheal rings submaximally contracted by carbachol and KCl, supporting the findings of recently published study of Van Heuven-Nolsen et al. (1997) in the same species. The present results also support our earlier observations for cat (Türker and Kiran, 1965; Türker and Ercan, 1976) and dog (Ercan and Türker, 1977; Ercan et al., 1978; Türker and Khairallah, 1969) isolated tracheal muscles. Our earlier result indicate that the relaxing effect of bradykinin on tracheal muscles from mice seems to be mediated jointly by the release of NO or NO-related substance(s) and a cyclooxygenase metabolite of arachidonic acid, probably prostaglandin E_2. This view is based upon the inhibition of the relaxing effect of bradykinin in tracheal rings after

pretreatment with ASA, a cyclooxygenase inhibitor (Vane and Botting, 1987), or L-NAME, a NO synthase inhibitor (Thiemermann and Vane, 1990). In addition, when the rings were pretreated with ASA plus L-NAME, a further inhibition of the bradykinin response was observed indicating the participation of both endogenous substances in the relaxing response. The findings obtained with bradykinin are in agreement with the observations of Schlemper and Calixto (1994) in guinea-pig isolated trachea and evidence suggests that NO acts as the nonadrenergic and noncholinergic relaxing mediators in number of tissues, including trachea (Li and Rand, 1991; Fisher et al., 1993). However, Van Heuven-Nolsen et al. (1997), working with the isolated tracheal muscles from male Balb c mice, have observed that the relaxant effect of bradykinin is probably mediated by prostaglandins but not NO, since indomethacin completely abolished the effect of the peptide. In addition, these authors have also shown that the relaxing effect of bradykinin is mediated through bradykinin B_2 receptors, as evidenced by the inhibition of the relaxant response of the peptide by HOE 140, a bradykinin B_2 receptor antagonist. The discrepancy between both studies may be due to the differences since the present study was performed with tracheal muscles isolated from Swiss albino mice. Another possibility is the involvement of bradykinin B_1 receptors to the relaxant effect of the peptide and activation of the L-arginine-NO pathway. The bradykinin B_1 receptor antagonist *des*-Arg^9-Leu^8-bradykinin (Regoli et al., 1993) partially inhibited the relaxant effect of bradykinin but no further inhibition was observed when the rings were incubated with L-NAME and the bradykinin B_1-receptor antagonist. These findings were taken as evidence of a link between bradykinin B_1 receptors and NO release. There is clear evidence that bradykinin acting through B_2 receptors causes the release of prostaglandins, which is the main mechanism of the relaxant effect of the peptide, as shown by Van Heuven-Nolsen et al. (1997). The relaxant effect of bradykinin was greatly inhibited by MeB, a soluble guanylate cyclase inhibitor (Martin et al., 1985), thus supporting the NO-mediated relaxing effect of the peptide in precontracted tracheal muscle from mice. However, the inhibition by MeB of the relaxing effect of bradykinin in precontracted tracheal rings was more pronounced and highly significant when compared with the inhibition produced by L-NAME and L-Gln indicating the possibility of a nonspecific effect of MeB.

An interesting result of the present study is the inhibition of the relaxant effect of bradykinin by L-Gln. It has been reported that L-Gln inhibits the release of endothelium-derived relaxing factor from bovine endothelial cells. This effect involves the inverse relation between intracellular levels of L-arginine and L-Gln. Removal of L-Gln from culture medium abolished the inhibitory effect of this amino acid on L-arginine generation (Sessa et al., 1990; Hecker et al., 1990). However, it has been shown that L-Gln also decreases the availability of L-arginine, the substrate of NO synthase, in bovine aortic endothelial cells (Arnal et al., 1995). In bovine venular endothelial cells L-Gln has been shown to inhibit NO synthesis from L-arginine without interfering with NO synthase activity or L-arginine transport (Meininger and Wu, 1997). Whether the inhibition by L-Gln of the relaxant effect of bradykinin in tracheal rings is due to the inverse relation between the intracellular level of L-arginine or is due to the inhibition of NO synthase remains to be studied.

The results of the present study suggest that bradykinin-induced relaxation of mouse tracheal muscle contracted by carbachol may possibly be due to the activation of K^+ channels. Several inhibitors (Brayden, 1996; Rusch et al., 1996) were used in order to assess the specificity of K^+ channels in the relaxing response to bradykinin in the contracted mouse tracheal muscle. Glibenclamide attenuated the relaxing response of bradykinin, indicating the involvement of K_{ATP} channels. However, apamine caused about 75% inhibition of the response to bradykinin, indicating that this relaxation may partly be due to the activation of small-conductance Ca^{2+} activated K^+ channels. Iberiotoxin, a large-conductance Ca^{2+} activated K^+ channel inhibitor, also caused an inhibition of about 75–80%. Tetraethylammonium, a non-selective K^+ channel blocker (Rapacon et al., 1996), at the concentration of 0.2 mmol/l completely inhibited the relaxing response to bradykinin in contracted mouse tracheal rings. High K^+ concentration (above 80–100 mmol/l) completely prevented the relaxing response to bradykinin in submaximally contracted tracheal rings, indicating that the peptide caused hyperpolarization of tracheal smooth muscle. These results are in accordance with the observation of Hadj-Kaddour et al. (1996) for isolated guinea-pig tracheal muscle, that endothelin-3 caused hyperpolarization. The only difference is that the hyperpolarization induced by endothelin-3 is mediated by the activation of charybdotoxin-sensitive K^+ channels, while that of bradykinin is mediated by the activation of K_{ATP} channels.

The results of the present study indicate that the relaxing effect of bradykinin in contracted mouse tracheal rings is mediated by the activation of the L-arginine-NO pathway and partially through the cyclooxygenase metabolites of arachidonic acid. These results also implicate K^+ channels in the relaxing effect of bradykinin, without being a great difference between various channels such as K_{ATP}, small- and large-conductance Ca^{2+}-activated K^+ channels, and support the role of bradykinin as a hyperpolarizing factor in mouse tracheal smooth muscle.

Acknowledgements

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