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Short communication

K^+ channel blockers do not modify relaxation of guinea-pig uterine artery evoked by acetylcholine

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Abstract

The effect of K^+ channel blockers on acetylcholine-induced relaxation in guinea-pig uterine arterial rings was investigated. Acetylcholine (0.1 nM–60 μM) induced endothelium-dependent relaxation of phenylephrine-precontracted guinea-pig uterine artery. Methylene blue (30 nM–1 μM) and N^G-monomethyl-L-arginine (3–30 μM) antagonized the effect of acetylcholine, with suppression of the maximal acetylcholine-induced relaxation, in a concentration-dependent manner. The inhibition of relaxation by N^G-monomethyl-L-arginine (10 μM) was significantly overcome by L-arginine (10 μM), but not by D-arginine (100 μM). In contrast, the administration of K^+ channel blockers, tetraethylammonium (6 mM), glibenclamide (5 μM), apamin (1 μM) and 4-aminopyridine (1 mM), did not modify the relaxation of guinea-pig uterine artery induced by acetylcholine. The concomitant addition of K^+ channel blockers in the same concentrations also did not alter the inhibition of acetylcholine-induced relaxation produced by N^G-monomethyl-L-arginine (30 μM). Finally, the acetylcholine-evoked relaxations were unaltered when K^+-rich Krebs-Ringer-bicarbonate solution was used to induce tone instead of phenylephrine. Indomethacin (10 μM) and diethylcarbamazine (100 μM) had no effects on acetylcholine-induced relaxation. These findings indicate that K^+ channels are probably not involved in the endothelium-dependent guinea-pig uterine arterial relaxation elicited by acetylcholine.

Keywords: K^+ channel blocker; Acetylcholine; Uterine artery; Endothelium; Nitric oxide (NO)

1. Introduction

A few years ago, Tare et al. (1990) reported that acetylcholine applied in a high concentration (10 μM) evoked relaxation of guinea-pig uterine artery accompanied by vascular smooth muscle hyperpolarization. It was hypothesized that both the relaxation and hyperpolarization in this artery were mediated by nitric oxide (NO) (Tare et al., 1990). Additionally, it was suggested that hyperpolarization of guinea-pig uterine artery in response to acetylcholine may be due to increased K^+ conductance (Tare et al., 1990).

However, the blockade of K^+ channels did not modify the relaxation of human uterine artery in response to acetylcholine (Jovanović et al., 1994b). Taking into consideration the similarities between guinea-pig and human uterine artery (Bell, 1968, 1969), this result was unexpected in view of a previous report by Tare et al. (1990).

Accordingly, we thought that it might be interesting to study the effects of K^+ channel blockers on acetylcholine-induced relaxation in guinea-pig uterine artery and to clarify the possible contribution of K^+ channel activation to the acetylcholine action in this preparation.

2. Materials and methods

Adult female non-pregnant guinea-pigs (700–900 g) were used in this study. The animals were stunned and decapitated.

2.1. Vascular preparations

The right and left uterine arteries were carefully dissected free from surrounding fat and connective tissue and cut into 3-mm long circular segments. All

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vessel segments were immediately placed in Krebs-Ringer-bicarbonate solution (composition in mM: NaCl 118.3; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25.0; CaEDTA 0.026; glucose 11.1). The endothelium was removed from some rings by gently rubbing the intimal surface with stainless steel wire. Ring preparations were mounted between two stainless-steel triangles in an organ bath containing 10 ml Krebs-Ringer-bicarbonate solution (37° C, pH 7.4), aerated with 95% O_2 and 5% CO_2. One of the triangles was attached to a displacement unit, allowing the fine adjustment of tension, and the other was connected to a force-displacement transducer (Hugo Sachs K30). Isometric tension was recorded on a Hugo Sachs model MC 6621 recorder.

Preparations were allowed to equilibrate for 60 min in Krebs-Ringer-bicarbonate solution. Subsequently, each ring was gradually stretched to the optimal point of tension (4.2 mN, Jovanović et al., 1994a). Once at their optimal length, the segments were allowed to equilibrate for 30 min before experimentation.

2.2. Experimental procedure

The experiments were performed as has been previously described in detail (Jovanović et al., 1994a). Briefly, the following protocol was used: (1) contraction in response to EC_{80} phenylephrine (0.2–0.6 μM), addition of Ca^{2+} ionophore A23187 (1 μM), followed by three washes and rinsing at 15-min intervals for the next 60 min; (2) contraction in response to EC_{80} phenylephrine (0.2–0.6 μM) and concentration-response curves for acetylcholine (0.1 nM–60 μM), followed by three washes, addition of the antagonists and a 15-min (methylene blue, N^G-monomethyl-L-arginine, L-arginine and D-arginine), 20-min (tetraethylammonium, glibenclamide, 4-aminopyridine and apamin), 30-min (diethylcarbamazine) or 40-min (indomethacin) equilibration period; (3) contraction in response to EC_{80} phenylephrine (0.2–2.0 μM) and the concentration-response curve for acetylcholine (0.1 nM–60 μM).

In a separate series of experiments, vascular rings were precontracted with K^+-rich Krebs-Ringer-bicarbonate solution. The solution was prepared by direct replacement of 65 mM NaCl with 65 mM KCl (Plane and Garland, 1993).

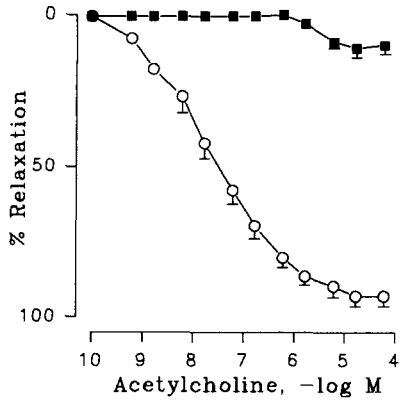


Fig. 1. Concentration-response curves for acetylcholine in guinea-pig uterine artery with intact (○) and denuded endothelium (■). Each point represents the mean ± S.E.M. (n = 11–87). Responses are expressed as a percentage of the maximal relaxation induced by papaverine (300 μM).

At the end of each experiment papaverine (300 μM) was added to the organ bath to determine the maximal relaxation of preparations.

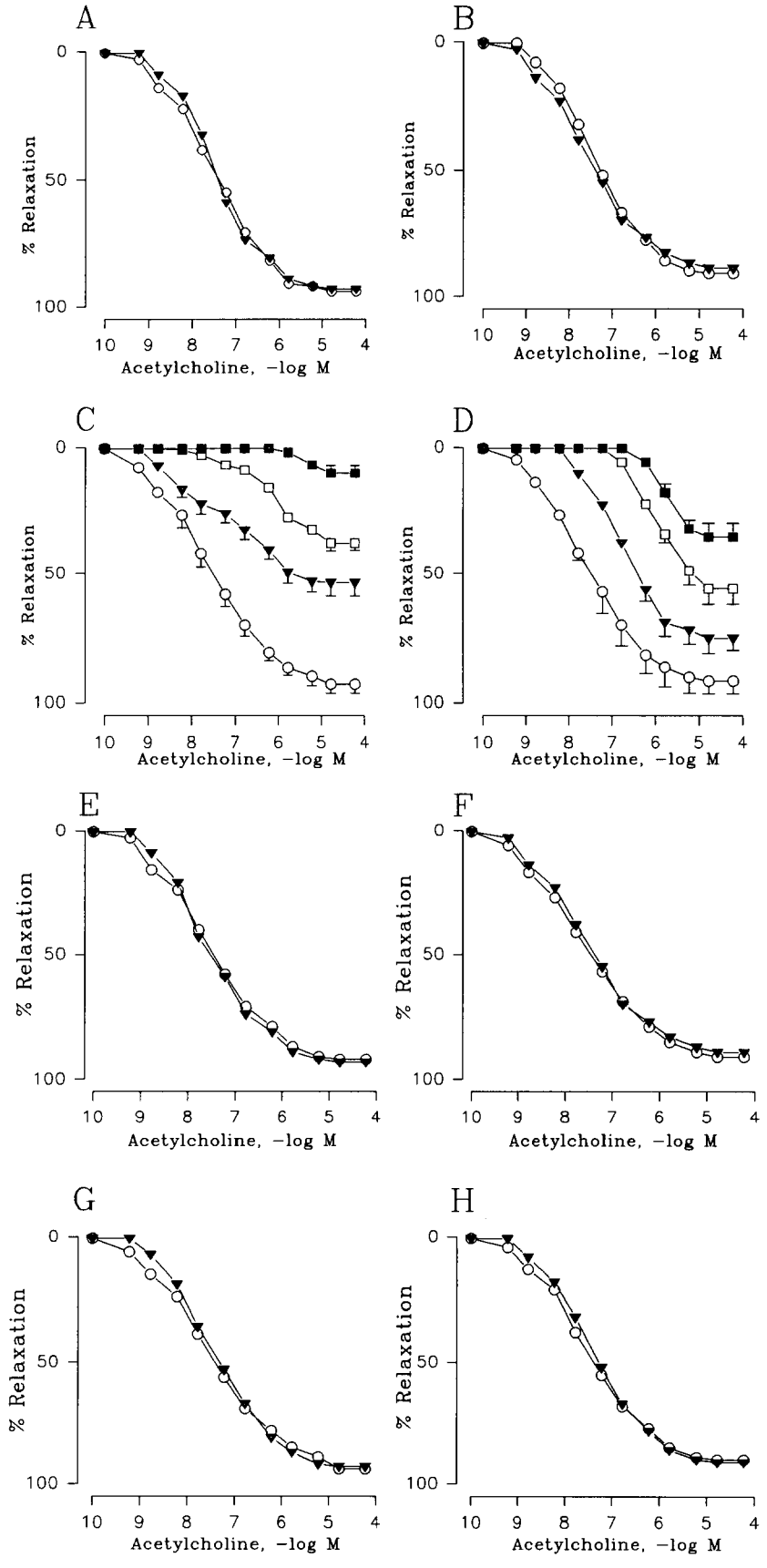
All experiments were carried out in tissues with a functionally intact endothelium precontracted with phenylephrine unless otherwise stated.

2.3. Treatment of data and statistics

The relaxation induced by each concentration of acetylcholine is expressed as a percentage of the maximum relaxation to papaverine and was used in the construction of the concentration-response curves. The concentration of acetylcholine eliciting 50% of its own maximum response (EC_{50}) was determined graphically for each curve by linear interpolation. EC_{50} values are presented as pD_2 ($pD_2 = -\log EC_{50}$).

The results are expressed as means ± S.E.M.; n refers to the number of experiments. Statistical significance of differences between two means was deter-

Fig. 2. The antagonism of the relaxant effects of acetylcholine by indomethacin, diethylcarbamazine, methylene blue, N^G-monomethyl-L-arginine and K^+ channel blockers. (A, B) Concentration-response curves for acetylcholine in guinea-pig uterine artery with intact endothelium in the absence (○) and presence (▼) of 10 μM indomethacin (A) and 100 μM diethylcarbamazine (B). Each point represents the mean of 5–6 experiments. Standard errors are excluded for clarity and do not exceed 15% of the mean value for each point. Responses are expressed as percentages of the maximal relaxation induced by papaverine (300 μM). (C, D) Concentration-response curves for acetylcholine in guinea-pig uterine artery with intact endothelium in the absence (○) and presence of 30 nM (▼), 100 nM (□) and 1 μM (■) methylene blue (C) and 3 μM (▼), 10 μM (□) and 30 μM (■) N^G-monomethyl-L-arginine (D). Each point represents the mean ± S.E.M. (n = 6–24). Responses are expressed as percentages of the maximal relaxation induced by papaverine (300 μM). (E, F, G, H) Concentration-response curves for acetylcholine in guinea-pig uterine artery with intact endothelium in the absence (○) and presence (▼) of 6 mM tetraethylammonium (E), 5 μM glibenclamide (F), 1 μM apamin (G) and 1 mM 4-aminopyridine (H). Each point represents the mean of five experiments. Standard errors are excluded for clarity and do not exceed 15% of the mean value for each point. Responses are expressed as percentages of the maximal relaxation induced by papaverine (300 μM).


A
B
C
D
E
F
G
H
% Relaxation
0
50
100
10 9 8 7 6 5 4
Acetylcholine, −log M

mined with Student's t-test for paired or unpaired observations where appropriate. One-way analysis of variance (ANOVA) followed by Dunnett's test was used when more than two groups were analyzed. A value of $P < 0.05$ was considered to be statistically significant.

2.4. Drugs used

The following drugs were used: phenylephrine hydrochloride, acetylcholine chloride, indomethacin, diethylcarbamazine, 6*S*-[6α(2*S*,3*S*),8β(*R*),9β,11α]-5-(methylamino)-2-[[3,9,11-trimethyl-8-[1-methyl-2-oxo-2-(1*H*-pyrrol-2yl)ethyl]-1,7-dioxaspiro[5.5]undec-2yl]methyl]-4-benzoxazolecarboxylic acid (Ca^{2+} ionophore A23187), apamin, tetraethylammonium bromide, L-arginine hydrochloride, D-arginine hydrochloride (Sigma, USA); 4-aminopyridine, glibenclamide (RBI, USA), N^G-monomethyl-L-arginine acetate (Wellcome, UK), methylene blue (Kemika, Croatia), papaverine hydrochloride (Merck, USA). All drug solutions were prepared immediately before the experiment and stored on ice until used. All agents were dissolved in distilled water and diluted to the desired concentration with buffer. The exceptions were indomethacin, which was dissolved in equimolar Na_2CO_3 solution, glibenclamide, which was dissolved in polyethylene glycol, and Ca^{2+} ionophore A23187, which was dissolved in dimethyl sulfoxide. Previous experiments showed that the solvents used had no effect on preparations at the concentrations applied. All drugs were added directly to the bath in a volume of 100 μl and the concentrations given are the calculated final concentration in the bath solution.

3. Results

3.1. Effect of acetylcholine on guinea-pig uterine artery

Acetylcholine (0.1 nM–60 μM) induced a concentration-dependent relaxation of the phenylephrine-precontracted guinea-pig uterine arterial rings with an intact endothelium ($pD_2 = 7.59 \pm 0.02$, maximal response = $93.1 \pm 3.4\%$, $n = 87$). After the removal of the vascular endothelium the relaxation induced by acetylcholine was almost completely abolished (maximal response was $9.0 \pm 2.0\%$, $n = 11$) (Fig. 1).

3.2. Effects of diethylcarbamazine and indomethacin on acetylcholine-induced relaxation

The administration of indomethacin (10 μM) did not modify the relaxation of guinea-pig uterine artery induced by acetylcholine (Fig. 2A).

Similar results were obtained with diethylcarbamazine (100 μM) (Fig. 2B).

3.3. Effects of methylene blue and N^G-monomethyl-L-arginine on acetylcholine-induced relaxation

In quiescent preparations, methylene blue (30 nM–1 μM) antagonized the effect of acetylcholine with suppression of the maximal acetylcholine-induced relaxation in a concentration-dependent manner ($P < 0.001$) (Fig. 2C).

Furthermore, N^G-monomethyl-L-arginine (3–30 μM) also produced a rightward shift of the concentration-response curves for acetylcholine with suppression of the maximal acetylcholine-induced relaxation in a concentration-dependent manner ($P < 0.001$) (Fig. 2D). L-Arginine (100 μM) did not affect endothelium-dependent relaxation in response to acetylcholine ($pD_2 = 7.55 \pm 0.06$, maximal response = $91.8 \pm 4.9\%$, $n = 4$, in the absence and $pD_2 = 7.51 \pm 0.07$, maximal response = $90.6 \pm 4.2\%$, $n = 4$, in the presence of L-arginine, $P > 0.05$, data not shown), but concomitant addition of L-arginine (10 μM) significantly overcame the inhibition of acetylcholine-induced relaxation produced by N^G-monomethyl-L-arginine (10 μM) (maximal response to acetylcholine in the presence of N^G-monomethyl-L-arginine was $51.8 \pm 5.9\%$ and $74.0 \pm 6.0\%$ in the presence of both N^G-monomethyl-L-arginine and L-arginine, $n = 7$, $P < 0.05$). In contrast, D-arginine (100 μM) did not alter the response to acetylcholine ($pD_2 = 7.66 \pm 0.07$, maximal response = $94.8 \pm 2.9\%$, $n = 4$, in the absence and $pD_2 = 7.55 \pm 0.07$, maximal response = $91.6 \pm 5.2\%$, $n = 4$, in the presence of D-arginine, $P > 0.05$), nor the inhibition of acetylcholine-induced relaxation produced by N^G-monomethyl-L-arginine (maximal response to acetylcholine in the presence of N^G-monomethyl-L-arginine and L-arginine was $53.7 \pm 5.7\%$ and $56.1 \pm 6.4\%$ in the presence of both N^G-monomethyl-L-arginine and D-arginine, $n = 7$, $P > 0.05$) (data not shown).

3.4. Effects of K^+ channel blockers and K^+-rich Krebs-Ringer-bicarbonate solution on acetylcholine-induced relaxation

In the guinea-pig uterine artery, the administration of the K^+ channel blockers, tetraethylammonium (6 mM, $n = 5$), glibenclamide (5 μM, $n = 5$), apamin (1 μM, $n = 5$) and 4-aminopyridine (1 mM, $n = 5$) did not modify the relaxation induced by acetylcholine (Fig. 2E–H). Furthermore, concomitant addition of the K^+ channel blockers in the same concentrations did not alter the inhibition of acetylcholine-induced relaxation produced by N^G-monomethyl-L-arginine (30 μM) (maximal response to acetylcholine in the presence of N^G-monomethyl-L-arginine was $35.6 \pm 5.5\%$, $n = 7$, and $29.2 \pm 4.7\%$, $31.1 \pm 3.9\%$, $38.9 \pm 4.4\%$ and $28.1 \pm 3.8\%$ in the presence of both N^G-monomethyl-L-arginine and tetraethylammonium, N^G-monomethyl-L-arginine

and glibenclamide, N^G-monomethyl-L-arginine and apamin and N^G-monomethyl-L-arginine and 4-aminopyridine, respectively, $n = 4$ for each, $P > 0.05$) (data not shown).

Finally, the acetylcholine-evoked relaxations were unaltered when K^+-rich Krebs-Ringer-bicarbonate solution was used to induce tone instead of phenylephrine (K^+-rich Krebs-Ringer-bicarbonate solution: $pD_2 = 7.31 \pm 0.08$, maximal response = $86.7 \pm 6.3\%$, $n = 6$; phenylephrine: $pD_2 = 7.51 \pm 0.09$, maximal response = $91.7 \pm 4.2\%$, $n = 6$, $P > 0.05$) (data not shown).

4. Discussion

In the present study we confirmed previous findings that acetylcholine induced endothelium-dependent relaxation of guinea-pig uterine artery (Tare et al., 1990; Jovanović et al., 1994a). Endothelium-dependent vasodilatation mediated by cyclooxygenase or lipoxygenase products has been observed in some blood vessels (for review see Vanhoutte, 1993). In our study, indomethacin and diethylcarbamazine did not affect the relaxant effect of acetylcholine on quiescent preparations. Accordingly, these findings do not suggest that cyclooxygenase or/and lipoxygenase cascade products of arachidonic acid are involved in the mediation of this acetylcholine effect.

Methylene blue was originally thought to inhibit the action of NO by oxidizing the haem moiety of soluble guanylate cyclase (Martin et al., 1985). Recently, it has been shown that methylene blue directly inhibits NO synthase as well (Mayer et al., 1993). Consequently, the inhibitory action of methylene blue on the acetylcholine-induced relaxation of guinea-pig uterine artery allowed the supposition that the vasodilatation elicited by acetylcholine in this vessel is possibly mediated by endothelial NO formation. In order to further analyze this possibility, the inhibitor of NO synthesis, N^G-monomethyl-L-arginine (for review see Moncada, 1992), was tested. N^G-Monomethyl-L-arginine antagonized the effects of acetylcholine on uterine artery in a concentration-dependent manner. The concentrations of N^G-monomethyl-L-arginine used brought about a non-competitive inhibition of acetylcholine-induced vasodilatation as shown by the depression of the maximum response and the rightward shift of the concentration-response curve. In addition, L-arginine but not D-arginine antagonized the effects of N^G-monomethyl-L-arginine on acetylcholine-induced relaxation. These results suggests that both N^G-monomethyl-L-arginine and L-arginine are competing specifically for the same mechanisms. Since this effect was enantiomerically specific, it is probably due to an effect of NO synthesis. On the basis of these results, it seems reasonable to suggest that endothelial NO production is involved in the acetylcholine-induced relaxation of guinea-pig uterine artery. These experimental data and conclusion would be in agreement with previous findings obtained with the same preparation (Tare et al., 1990).

It has been hypothesized that acetylcholine hyperpolarizes guinea-pig vascular smooth muscle by releasing a NO itself (Tare et al., 1990), as opposed to some other arteries in which the involvement of an endothelial factor independent of the NO, termed endothelium-derived hyperpolarizing factor (EDHF), has been suggested (for review see Vanhoutte, 1993). The mechanism proposed for the endothelium-dependent hyperpolarization is an increase in K^+ conductance by the opening of K^+ channels (Tare et al., 1990; Vanhoutte, 1993). Studies of the rabbit middle cerebral artery have suggested that endothelium-dependent hyperpolarization may involve the opening of adenosine 5′-triphosphate (ATP)-dependent K^+ channels (Brayden, 1990). It is believed that glibenclamide is one of the most selective blockers of ATP-dependent K^+ channels (for review see Cook and Quast, 1990). In the present study, glibenclamide in a concentration clearly sufficient to block ATP-dependent K^+ channels (Wilson, 1989) did not affect relaxation of guinea-pig uterine artery evoked by acetylcholine. Eckmen et al. (1992) hypothesized that large conductance Ca^{2+}-activated K^+ channels may be involved in the pathways by which acetylcholine produces relaxation in coronary arteries. It has been suggested that tetraethylammonium blocks several types of K^+ channels (for review see Cook and Quast, 1990), but that it shows some selectivity in its blockade of large conductance Ca^{2+}-activated K^+ channels (for review see Cook and Quast, 1990). The concentration of tetraethylammonium used in our study was sufficient to block large conductance Ca^{2+}-activated K^+ channels (for review see Cook and Quast, 1990), but tetraethylammonium did not alter the relaxation of uterine artery induced by acetylcholine. Furthermore, Adeagbo and Triggle (1993) reported that the opening of small conductance Ca^{2+}-activated K^+ channels is involved in acetylcholine-induced endothelium-dependent relaxation of the rat mesenteric arterial bed. Apamin has been reported to block small conductance Ca^{2+}-activated K^+ channels (for review see Cook and Quast, 1990). In quiescent preparations, we found that a high concentration of apamin (Eckmen et al., 1992) was without effect on the relaxant action of acetylcholine. On the basis of this result, it is likely that small conductance Ca^{2+}-activated K^+ channels are not involved in the pathway by which acetylcholine produces relaxation of guinea-pig uterine artery. Similar results were obtained with a high concentration of 4-aminopyridine, a potential and non-selective K^+ channel blocker (for review see Cook and Quast, 1990).

Moreover, all four K^+ channel blockers did not affect the N^G-monomethyl-L-arginine-resistant component of acetylcholine-induced relaxation. Finally, smooth muscle relaxation in the uterine artery was also unaffected by precontraction with a K^+-rich Krebs-Ringer-bicarbonate solution, which is known to inhibit smooth muscle hyperpolarization to acetylcholine (Plane and Garland, 1993).

If guinea-pig uterine artery vascular smooth muscle hyperpolarization is involved in acetylcholine-evoked relaxation, it seems logical that the blockade of K^+ channels would alter the relaxation of this preparation in response to acetylcholine. The experimental data from the present study are in accord with our results obtained with human uterine artery (Jovanović et al., 1994b), but in a disagreement with the findings reported by Tare et al. (1990). It should be mentioned that Tare et al. (1990) observed hyperpolarization of guinea-pig uterine artery vascular smooth muscle using only one, supramaximal, concentration of acetylcholine (10 μM, about 400 times the observed EC_{50} value for acetylcholine in this preparation). Consequently, it is not clear yet whether application of a lower concentration of acetylcholine can evoke hyperpolarization of this blood vessel. Is it possible that hyperpolarization in guinea-pig uterine artery occurs during acetylcholine action but does not have a significant role in acetylcholine-induced relaxation, as has been already described in some other blood vessels (Plane and Garland, 1993). Nevertheless, our experimental data do not exclude the existence of hyperpolarization during acetylcholine action in guinea-pig uterine artery, but also it seems reasonable, on the basis of these results, to conclude that K^+ channel activation is not necessary for the acetylcholine-evoked relaxation of this vessel. Certainly, further investigations are required to resolve this dilemma.

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