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Biochemical Pharmacology 61 (2001) 109–118

Biochemical Pharmacology

Potentiation by aminoethylisothiourea of the extra-cellular Ca^{2+} component of norepinephrine-induced contraction in rat femoral arteries

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Received 29 February 2000; 2 June 2000

Abstract

Aminoethylisothiourea (AET) is a potent inhibitor of inducible nitric oxide synthase (NOS). The present study was performed to investigate whether AET and its rearrangement products might modulate vascular contraction independently of its effects as a NOS inhibitor in rat small femoral arteries. AET caused an endothelium-independent increase in contraction induced by norepinephrine (NE). This effect was not affected by either N^{ω}-nitro-L-arginine methyl ester, nitro-L-arginine, indomethacin or propanolol, but it was suppressed in Ca^{2+}-free medium. AET enhanced extracellular Ca^{2+} component of NE-induced contraction, and this effect was prevented by the receptor-mediated Ca^{2+} entry blocker, 1-{β-[3-(*p*-methoxyphenyl)-propyloxyl]-*p*-methoxyphenetyl}-1*H*-imidazole hydrochloride (SK&F 96365), but not by the voltage-dependent Ca^{2+} channel blocker, nitrendipine. AET did not alter the response to $CaCl_2$ in vessels exposed to KCl depolarization. The protein kinase C (PKC) inhibitor, 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) (GF 109203X), prevented the potentiating effect of AET on the NE response. AET failed to produce an increase in tone in the presence of NE and GTP in permeabilized arteries. Among the AET rearrangement products, mercaptoethylguanidine produced an endothelium-independent increase in the NE response. 2-aminothiazoline had no effect, and guanidinoethyldisulphide produced relaxation. The effect of mercaptoethylguanidine was dependent on extracellular Ca^{+} and was prevented by GF 109203X. These results indicate that AET is able to potentiate the contraction to NE in rat femoral resistance arteries independently of its inhibitory effect on either NOS or cyclo-oxygenase. Its effect occurs via an enhancement of SK&F 96365-sensitive Ca^{2+} entry. A PKC inhibitor-sensitive mechanism also appears to be involved in the AET effect. Mercaptoethylguanidine potentiates NE response through a mechanism similar to AET.

Keywords: Aminoethylisothiurea; Mercaptoethylguanidine; Femoral artery; Nitric oxide synthase; Protein kinase C

1. Introduction

S-substituted isothioureas represent a new class of molecules reported to be potent inhibitors of NOS. They do not affect the activity of other enzymes such as xanthine oxidase, catalase, cytochrome P450 or superoxide dismutase [1, 2], but they have been reported to reduce activities of lactate dehydrogenase and liver transaminase [3]. Despite their limited selectivity on the three human recombinant NOSs, these drugs have been described as selectively inhibiting inducible NOS activity *in vivo*, as well as protecting animals against mortality and the multiple organ dysfunction syndrome caused by LPS [4–7] and by hemorrhagic shock [8] without producing any changes in hemodynamic in control animals. By contrast, AET has been reported in some cases to aggravate LPS effects on the liver microcirculation [9, 10] by impairing hepatic blood flow and by producing a potent vasopressor effect [11]. It has been reported that AET in aqueous solution, can rearrange to ATZ and MEG with the latter, after dimerization in an oxidative medium, being transformed to GED. It is thought that these compounds may be responsible for the pharma-

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Abbreviatoins: AET, aminoethylisothourea; ATZ, aminothiazoline; CHAPS, 3-((3-cholamidopropyl)-dimethylammonio)-1-propane-sulfonate; COX, cyclo-oxygenase; GED, guanidinoethyldisulphide; GF, 109203X, 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl); LPS, lipopolysaccharide; MEG, mercaptoethylguanidine; L-NA, nitro-L-arginine; L-NAME, N^{ω}-nitro-L-arginine methyl ester; NOS, nitric oxide synthase; NE, norepinephrine; PKC, protein kinase C; PSS, physiological salt solution; and SK&F 96365, 1{β-[3-(*p*-methoxyphenyl)-propyloxyl]-*p*-methoxyphenetyl}-1*H*-imidazole hydrochloride.

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PII: S0006-2952(00)00527-X

cological effects of AET in inhibiting inducible NOS [12]. However, isothioureas or related compounds (mercaptoalkylisothioureas) possess other activities which are independent of NOS inhibition. In macrophages and in rat lung homogenates, AET can prevent the expression of inducible NOS after LPS treatment [13]. Furthermore, MEG can inhibit both constitutive and inducible COX activities [14] and can scavenge peroxynitrite [15]. These additional effects of isothioureas and their related compounds may contribute to their beneficial effects in counterbalancing the deleterious consequences of inflammatory pathologies.

The present work was aimed at studying the effects of AET on vascular contraction in femoral arteries of the rat, independently of its inhibitory effect on NOS and COX. It is reported that AET is able to potentiate the contractile response to NE in femoral resistance arteries of the rat by enhancing Ca^{2+} entry through an SK&F 96365- sensitive pathway, with this potentiation also involving a PKC inhibitor-sensitive mechanism in this resistance artery. Among the three compounds resulting from rearrangement of AET in solution, only MEG potentiated the NE-induced response through a mechanism similar to that of AET.

2. Materials and methods

2.1. Arterial preparation and mounting

Male Wistar rats (10–12 weeks old) bred in our institute were killed by cervical dislocation and exsanguinated by carotid artery transection. Distal femoral arteries (length 2 mm, internal diameter 150 μm) were removed and cleaned of fat and connective tissue. The segment was mounted in a myograph filled with PSS of the following composition (in mM): 119 NaCl, 4.7 KCl, 0.4 KH_2PO_4, 14.9 $NaHCO_3$, 1.17 $MgSO_4$, 2.5 $CaCl_2$ and 5.5 glucose, bubbled with 95% O_2-5% CO_2 (pH 7.4) at 37°. Briefly, two tungsten wires (30 μm in diameter) were inserted through the lumen of the vessels. Mechanical activity was recorded isometrically by a force transducer (model DSG BE4, Kistler-Morse) connected to one of the two tungsten wires; the other wire was attached to a support carried by a micromanipulator. In some arteries, the vascular endothelium was removed by infusion of 5% CHAPS. After setup, the vessels were equilibrated for 30 min before they were passively stretched to an internal diameter that yields a circumference equivalent to 90% of that given by an internal pressure of 100 mmHg; this required a load of 700 mg. The presence of functional endothelium was then routinely assessed by the ability of 10 μM acetylcholine to induce $>$ 40% relaxation of vessels precontracted with 1 μM NE. In all experiments, the concentration of NE was adjusted for each preparation to reach the same level of precontraction, corresponding to 80% of the maximal response to NE. This contraction obtained with NE was 7.4 $\pm$ 0.9 mN/mm (N = 39), being not significantly different between arteries with or without endothelium.

Preliminary studies showed that AET up to 300 μM did not produce contraction under basal conditions. Cumulative concentration-response curves for AET (1–300 μM) were constructed on vessels with and without endothelium, precontracted with a submaximal concentration of NE (1–3 μM). To study the NOS-independent effects of AET, cumulative concentration-response curves to AET were performed in vessels without functional endothelium in the presence of either of the NOS inhibitors, L-NAME (100 μM) or L-NA (100 μM). In addition, the COX- or β-adrenoceptor-independent effects of AET were investigated in vessels with functional endothelium in the presence of the COX inhibitor, indomethacin (10 μM) or the β-adrenoceptor antagonist, propranolol (0.3 μM). All inhibitors were incubated with the tissue for 30 min before the precontraction with NE. Additionally, cumulative concentration-response curves to ATZ, MEG or GED (1–300 μM) were performed in vessels without functional endothelium, precontracted with a submaximal concentration of NE.

All the experiments designed to investigate the mechanism by which AET modulates the response to NE in respect to Ca^{2+} handling were performed in vessels without functional endothelium. In this set of experiments, the vessels were challenged with 10 μM NE in Ca^{2+}-free solution (PSS without $CaCl_2$ containing 0.5 mM EGTA). When the tension reached a steady state, $CaCl_2$ (1 mM) was added to the bath in the continuous presence of NE. The same experiment was repeated in the presence of AET (100 μM) alone, in the presence of the receptor-mediated Ca^{2+} entry blocker, SK&F 96365 (30 μM) [16] alone, or in the presence of both compounds. The same protocol was also performed in the presence of MEG (100 μM).

To investigate Ca^{2+} entry through voltage-operated channels, the vessels were challenged with KCl (40 mM) in Ca^{2+}-free solution, and $CaCl_2$ (0.01–1 mM) was added in a cumulative manner to the bath in the continuous presence of KCl (40 mM). The same experiment was repeated in the presence of AET (100 μM). In another set of experiments, three concentration-response curves to NE (0.1–30 μM) were successively constructed, separated by washout periods of 30 min. Control experiments had shown that there was no significant difference between the three curves in the absence of any other treatment, and the first curve constructed in the absence of inhibitors was then taken as control. The second curve was constructed in the presence of AET. The third curve was established in the presence of AET plus either nitrendipine, a blocker of voltage-operated Ca^{2+} channels (1 μM), or an inhibitor of PKC (GF 109203X, 3 μM). All inhibitors were incubated for 30 min prior to addition of NE. The same protocol was performed in the presence of MEG (100 μM).

2.2. Permeabilization of the small femoral arteries

Arteries were permeabilized using β-escin using the method previously described by Kitazawa et al. [17], with

minor modifications. The permeabilization process was achieved by incubating the arteries in the relaxing solution containing 50 μM β-escin at room temperature for 45 min. They were then mounted and passively stretched at 700 mg as described above, and were equilibrated for 20 min in the relaxing solution. The experiment was carried out at room temperature. For contraction experiments, the concentration of EGTA was decreased from 4 to 2 mM. The tension developed in response to a high concentration of Ca^{2+} using Ca^{2+} methanesulfonate (pCa 5.0) was recorded. After re-equilibration in relaxing solutions, a submaximal Ca^{2+} concentration (pCa 6.0) was added. When the contraction reached a steady-state level, NE was added in the presence of GTP (50 μM). In parallel experiments, the same experimental protocol was performed on vessels that had been preincubated for 30 min with AET or GF 109203X.

2.3. Expression of results and statistical analysis

Results were expressed as mN/mm. pD_2 values (-log $EC_{50,}$ EC_{50} being the molar concentration of the agonist that produced 50% of the effect obtained with the highest concentration of the agonist used) were calculated by logit-log regression. All results are expressed as means ± SEM of n separate experiments, where n represents the number of animals studied. Analysis of variance (ANOVA) was used for statistical analysis. $P < 0.05$ was taken as statistically significant.

2.4. Drugs and solutions

AET, nitrendipine, and SK&F 96365 were a generous gift from Dr. C. Thiemermann (The William Harvey Research Institute, London), from Bayer AG, and from Smith-Kline Beecham Pharmaceuticals, respectively. GF 109203X was purchased from Interchim, MEG from A.G. Scientific, and GED from Calbiochem. All other chemicals were purchased from Sigma Chemical. Nitrendipine was dissolved in ethanol, indomethacin in 5% $NaHCO_{3,}$ and GF 109203X in dimethyl sulphoxide (1 mg/mL). The final concentration of all solvents in the bath was < 0.1%. All other drugs were diluted in deionized water.

3. Results

3.1. Influence of endothelium, NOS, and COX pathways on the effect of AET in NE precontracted vessels

As shown in Fig. 1A, AET induced a concentration-dependent increase in tension of vessels precontracted with a submaximal concentration of NE. The concentration-response curves to AET and the contraction obtained with the highest concentration of AET used (300 μM) were not significantly different in vessels with and without functional endothelium, although there was a tendency for a greater response in vessels with endothelium (5.81 ± 0.44 and 3.95 ± 1.05 mN/mm, respectively) (Fig. 1B). It should be noted that in vessels with functional endothelium, L-NAME (1–300 μM) increased tension in a concentration-dependent manner that was not significantly different from that produced by AET under the same experimental conditions (results not shown). However, in vessels without functional endothelium, L-NAME had no effect (not shown), and it did not affect the concentration-response curve of AET (Fig. 1B). In addition, the concentration-effect curve of AET was not modified after pretreatment of the vessels without endothelium with another NOS inhibitor, L-NA (100 μM) (not shown).

In vessels with functional endothelium, indomethacin did not significantly affect the concentration-response curve of AET (the effect obtained with AET [300 μM] being 4.17 ± 0.98 mN/mm) (Fig. 1C). Additionally, in vessels with functional endothelium, propranolol did not significantly modify the concentration-response curve of AET (Fig. 1C). Thus, the enhancement by AET of the contractile response of NE was not due to an effect at the level of β-adrenoceptors. All of the following experiments were performed in vessels without functional endothelium to study the endothelium-independent effects of AET and the products formed when it is placed in aqueous solution.

3.2. Effect of ATZ, MEG, and GED on NE-induced contraction

All three compounds showed marked differences in their effects on vessels without functional endothelium, precontracted with a submaximal concentration of NE (Fig. 2). ATZ did not cause any changes in tension. As previously shown [12], GED (1–300 μM) induced endothelium-independent relaxation. By contrast, MEG (1–300 μM) increased NE-induced tension, and the concentration-response curve of MEG was superimposable on that of AET. The effect obtained with the highest concentration of MEG used (300 μM) was 4.35 ± 0.27 mN/mm compared to 3.95 ± 1.05 mN/mm for AET at the same concentration.

3.3. Effect of AET and MEG on the extra-cellular Ca^{2+} component of NE-induced contraction

To test the effect of AET and MEG on Ca^{2+} release and Ca^{2+} entry induced by NE, the vessels were exposed to the agonist in Ca^{2+}-free medium. Under these conditions NE elicited a transient contraction, which probably reflects the release of Ca^{2+} from intracellular stores (Fig. 3A). Further, addition of extracellular $CaCl_2$ in the continuous presence of NE restored tension to the same level as previously reached in normal PSS. Neither AET nor MEG affected the transient contraction induced by NE in Ca^{2+}-free medium (Fig. 3B), but both significantly enhanced the contraction

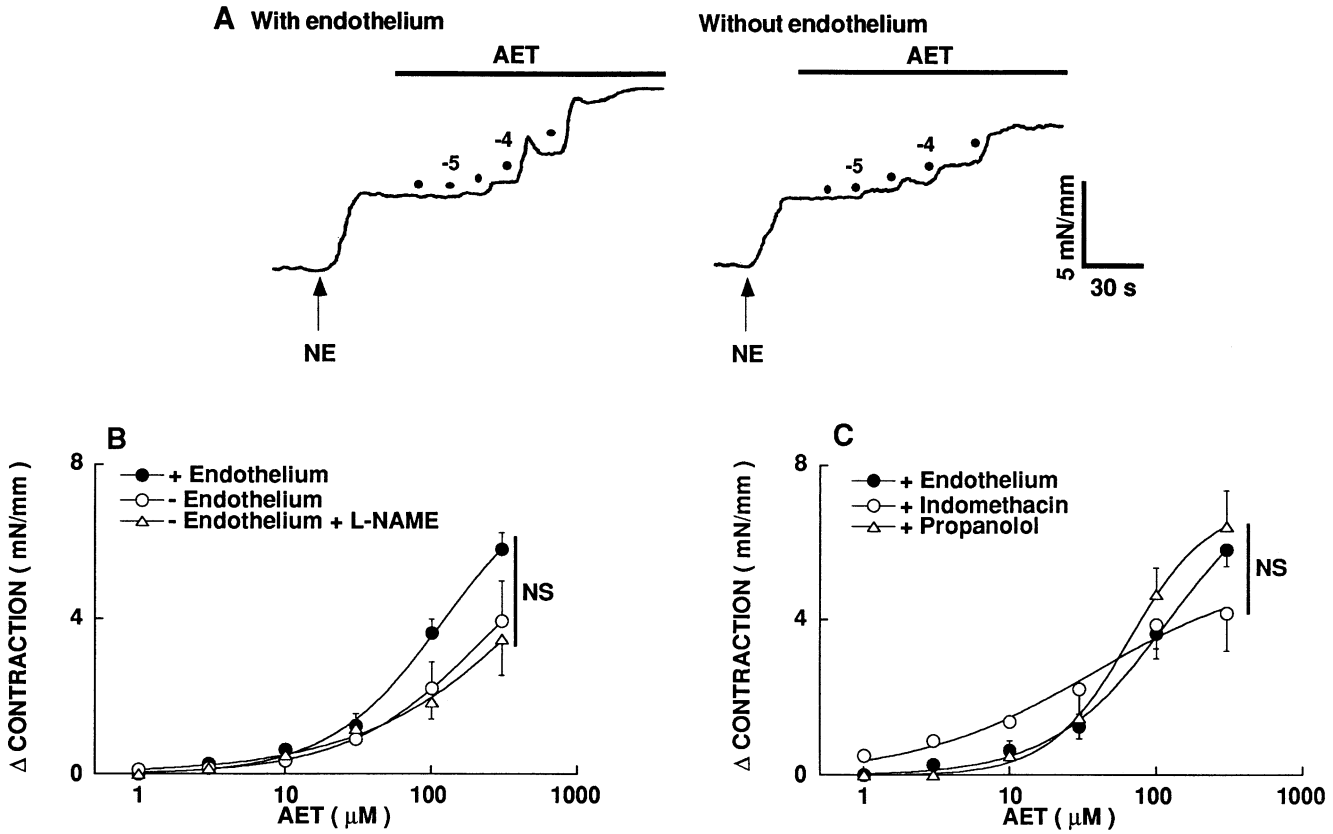

Fig. 1. Representative traces showing the effect of AET (A). Concentration-response curves to AET in the presence and absence of endothelium and in the presence of L-NAME (100 μM) (B) in rat femoral arteries precontracted with NE (1–3 μM). Concentration-response curves to AET in vessels with endothelium, in the absence and presence of indomethacin (10 μM), or in the presence of propanolol (0.3 μM) (C) in rat femoral arteries precontracted with NE. The level of precontraction induced by NE was (mN/mm) 3.99 ± 0.37 (with endothelium), 4.01 ± 0.12 (without endothelium), 3.92 ± 0.41 (without endothelium plus L-NAME), 4.12 ± 0.31 (with indomethacin), and 3.87 ± 0.23 (with propanolol). Values are expressed as the increase in the contraction in mN/mm. Each point is the mean ± SEM of 5 experiments. NS = the curves are not significantly different.

produced by $CaCl_2$ ($P < 0.01$) (Fig. 3C). These results suggest that AET and MEG are able to potentiate the extracellular Ca^{2+} component of NE-induced contraction.

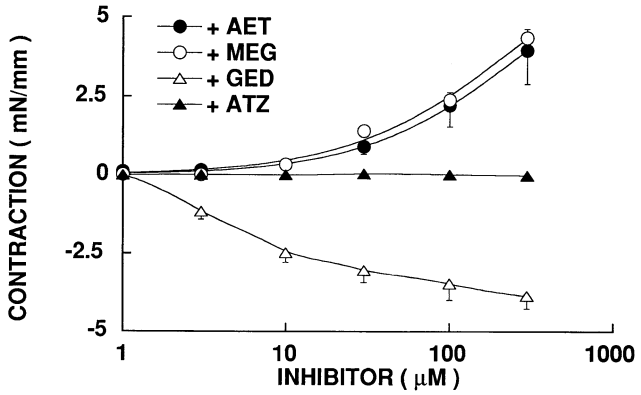

Fig. 2. Concentration-response curves to 2 ATZ, MEG, and GED in vessels without endothelium precontracted with NE (1–3 μM). Values are expressed as the Δcontraction in mN/mm. Each point is the mean ± SEM of 5 experiments.

3.4. Mechanism of the effect of AET on the extra-cellular Ca^{2+} component of NE-induced contraction

SK&F 96365 did not affect the transient contraction induced by NE in Ca^{2+}-free medium (not shown), but did significantly reduce the tension produced by $CaCl_2$ in the continuous presence of the agonist (Fig. 4). In the presence of SK&F 96365, AET failed to potentiate the response to $CaCl_2$ in the continuous presence of NE (Fig. 4). These results suggest that AET is able to potentiate the SK&F 96365-sensitive Ca^{2+} entry induced by NE.

SK&F 96365 has been reported to have a dual effect on the Ca^{2+} entry through inhibition of both receptor-operated and voltage-dependent Ca^{2+} channels. Therefore, to test whether AET enhanced Ca^{2+} entry through voltage-dependent Ca^{2+} channels, two different experimental protocols were used. First, the effect of the dihydropyridine Ca^{2+} channel blocker, nitrendipine, on the potentiating effect of AET on NE-induced contraction was tested. Treatment with nitrendipine shifted the concentration-response curves to

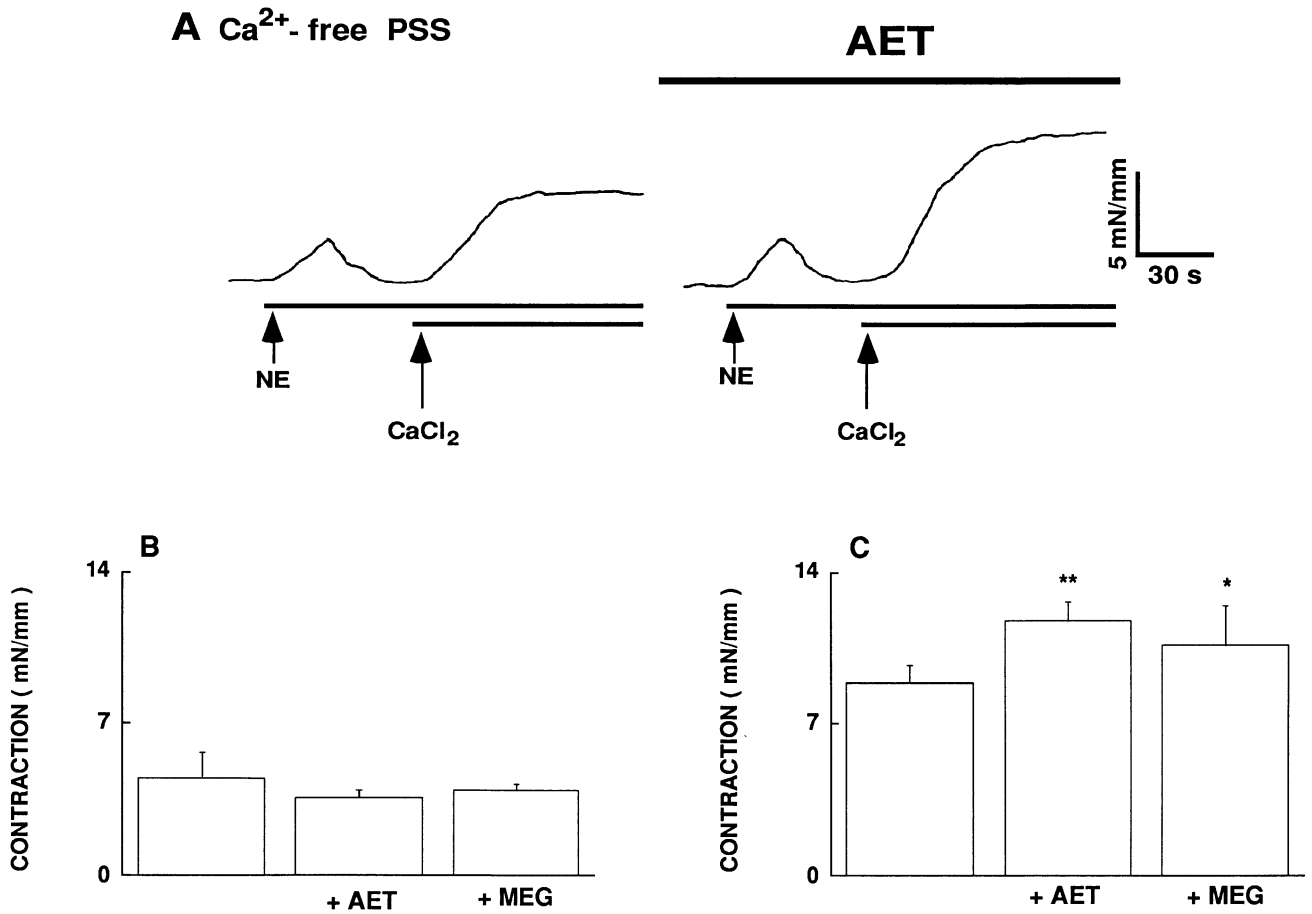

Fig. 3. (A) Representative traces showing the effect of AET on the responses induced by NE (10 μM) in Ca^{2+}-free medium and after subsequent addition of $CaCl_2$ (1 mM) in the continuous presence of NE. Histograms showing the effect of NE in Ca^{2+}-free medium (B) and subsequent addition of $CaCl_2$ (1 mM) (C) in the absence and the presence of AET (100 μM) or MEG (100 μM). Values are expressed in mN/mm. Each point is the mean ± SEM of 5 experiments. *$P < 0.05$, **$P < 0.01$ significantly different from the contraction induced by $CaCl_2$ in the absence of AET or MEG.

NE slightly but significantly to the right and significantly reduced its maximal effect ($P < 0.05$) (Fig. 5A, Table 1).

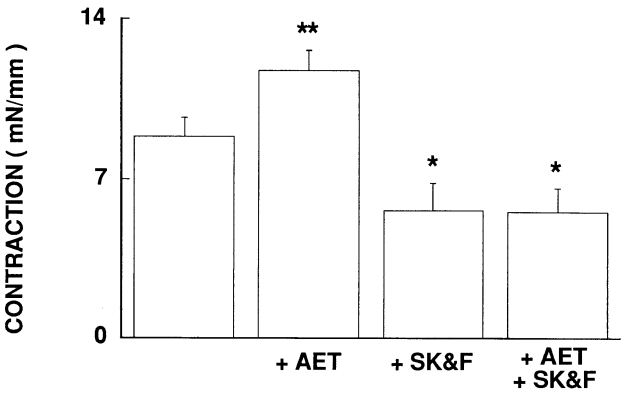

Fig. 4. Histograms showing the effect of $CaCl_2$ (1 mM) in NE-exposed vessels in Ca^{2+}-free medium, in the absence and presence of SK&F 96365 (30 μM) alone or plus AET (100 μM). Values are expressed in mN/mm. Each point is the mean ± SEM of 5 experiments. *$P < 0.05$, **$P < 0.01$ significantly different from the contraction induced by $CaCl_2$ in the absence of AET.

Exposure to AET produced a leftward shift of the control curve to NE without any modification in the maximal contraction (Table 1). In the presence of nitrendipine, AET was still able to potentiate NE-induced contraction without any change in maximal effect (Fig. 5A, Table 1). Second, in vessels exposed to KCl (40 mM) in Ca^{2+}-free medium, cumulative addition of $CaCl_2$ produced concentration-dependent contraction. Under these conditions, AET did not affect the contractile response to $CaCl_2$ (pD_2 values 3.6 ± 0.03 and 3.7 ± 0.03) (Fig. 4B). Taken together, these results suggest that AET-induced potentiation is not mediated through voltage-dependent Ca^{2+} channels in small femoral arteries.

To test the possible involvement of PKC in the potentiating effect of AET, GF 109203X, a potent inhibitor of PKCα over PKCδ and μ [18, 19], was used at a concentration at which it did not significantly affect the response to NE (Fig. 6, Table 1). Under these conditions, AET-induced potentiation was prevented by the PKC inhibitor (Fig. 6, Table 1). Also, the concentration-effect curve obtained with NE was significantly enhanced ($P < 0.05$) in vessels ex-

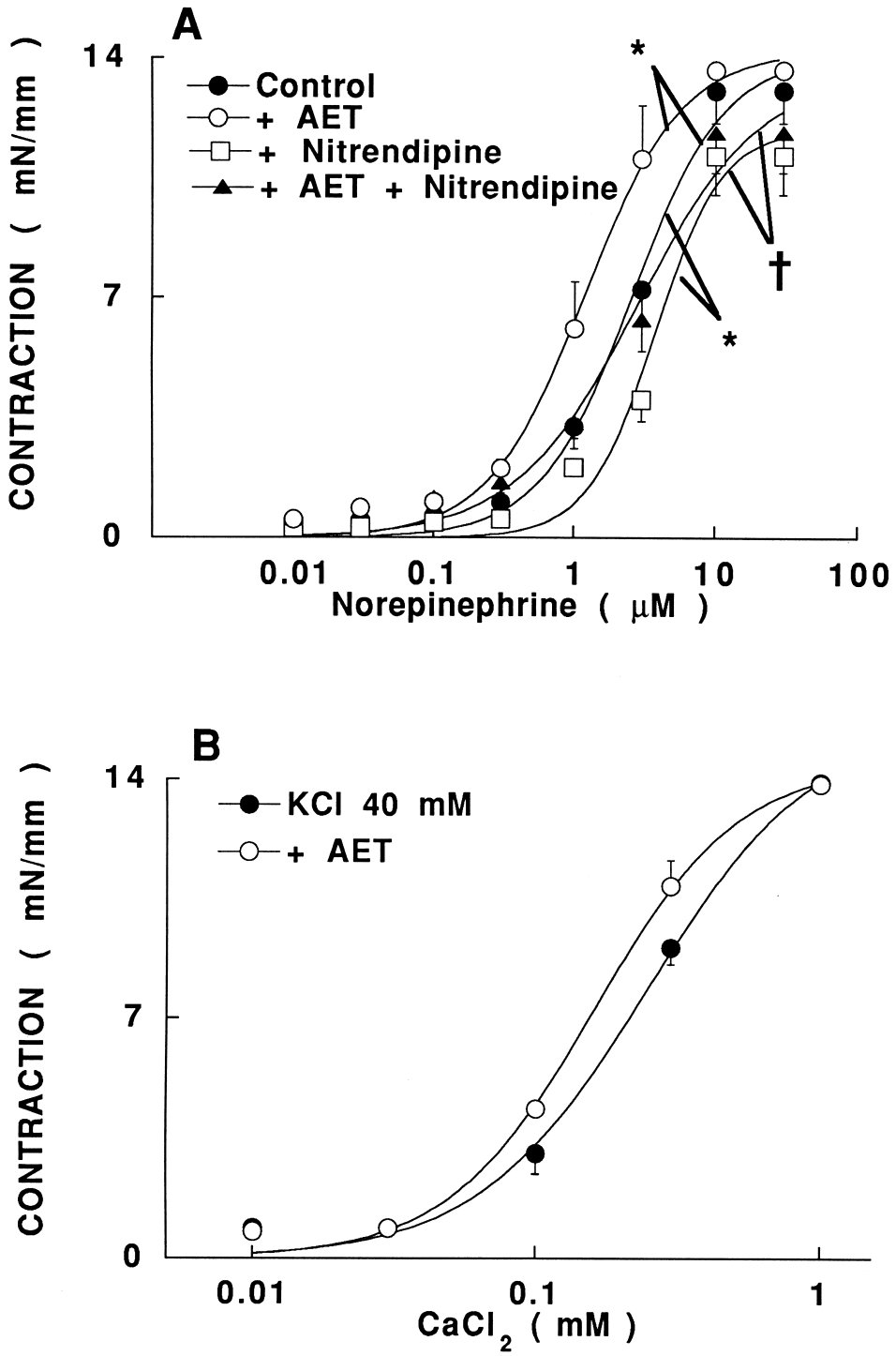

Fig. 5. (A) Concentration-response curves to NE in the absence and presence of AET (100 μM), nitrendipine (1 μM), or nitrendipine plus AET in rat femoral arteries. *$P < 0.05$ significantly different from the contraction induced by NE in the absence of AET. †$P < 0.05$ significantly different from the contraction induced by NE in the presence of nitrendipine alone. (B) Concentration-response curves to $CaCl_2$ in the absence and in the presence of AET (100 μM) in PSS containing 40 mM KCl in rat femoral arteries. Values are expressed in mN/mm. Each point is the mean ± SEM of 6–7 experiments.

Table 1
pD_2 values and maximal effects (E_{max}) of NE in rat small femoral arteries

Treatment	pD_2	E_{max} (mN/mm)
NE	5.3 ± 0.04	12.7 ± 1.1
AET + NE	5.9 ± 0.02*	13.7 ± 0.11
Nitrendipine + NE	5.1 ± 0.06*	11.1 ± 0.4*
Nitrendipine + AET + NE	5.6 ± 0.08**	11.9 ± 1.1
GF 109203X + NE	5.3 ± 0.06	12.4 ± 1.05
GF 109203X + AET + NE	5.4 ± 0.01	12.1 ± 0.9
MEG + NE	5.7 ± 0.03*	11.7 ± 0.8
GF 109203X + MED + NE	5.4 ± 0.01***	11.8 ± 0.9

Values are means ± SEM of 6–8 experiments.
* $P < 0.05$ versus NE alone.
* $P < 0.05$ versus nitrendipine + NE.
*** $P < 0.05$ versus MEG + NE.

posed to MEG (Table 1), and this potentiating effect of MEG was prevented by GF 109203X.

3.5. Lack of effect of AET on Ca^{2+} sensitization

Ca^{2+} sensitization was studied in β-escin-permeabilized arteries. As illustrated in Fig. 7A and B, neither GTP alone nor NE alone had influenced tone in vessels precontracted with a constant concentration of Ca^{2+} (pCa 6). However, NE in the presence of GTP was able to produce a further increase in tone (Fig. 7B). Under these conditions, GF 109203X did not affect either responses to Ca^{2+} or to NE plus GTP (Fig. 7C). Also, AET failed to potentiate the increase in tone produced by both Ca^{2+} and NE plus GTP (Fig. 7D).

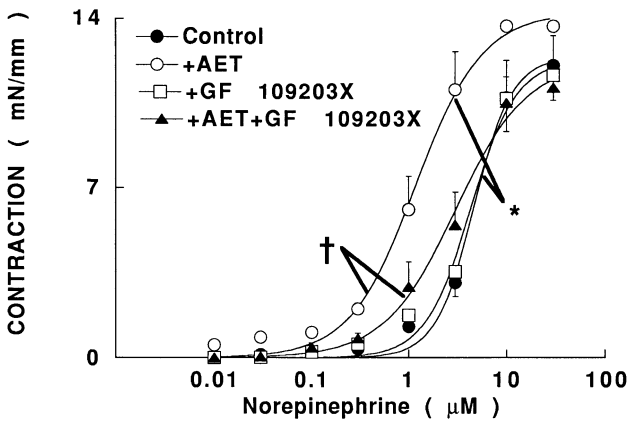

Fig. 6. Concentration-response curves to NE in the absence and presence of AET, GF 109203X (3 μM), and AET plus GF 109203X in rat femoral arteries. Values are expressed in mN/mm. Each point is the mean ± SEM of 8 experiments. *$P < 0.05$ significantly different from the contraction induced by NE in the absence of AET. †$P < 0.05$ significantly different from the contraction induced by NE in the presence of AET.

4. Discussion

Here, we report that AET potentiates NE-induced contraction via SK&F 96365-sensitive Ca^{2+} entry. In addition, this potentiation involves a PKC inhibitor-sensitive mechanism in the small femoral artery of the rat. As MEG, but not ATZ or GED, potentiates the response to NE through a mechanism similar to AET, it may contribute at least in part to the observed pharmacological effect of AET. AET was reported as a selective inhibitor of inducible NOS *in vitro* and *in vivo* [2], its effects being explained by its intramolecular rearrangement to generate mercaptoalkylguanidines, which are also selective inhibitors of inducible NOS [12]. Very recently, other effects of AET and its related compounds, such as direct inhibition of constitutive and inducible COX [14], have been reported. The authors suggested that these additional effects may contribute to the beneficial effects of the compounds in inflammatory states. However, it has not yet been tested whether AET might affect vascular contractility by other mechanisms.

The results of the present study show that AET is able to increase NE-induced contraction at concentrations at which it has been reported to inhibit the activity of both NOS and COX. In vessels with functional endothelium, it cannot be excluded that part of AET-induced contraction is due to inhibition of endothelial NOS. The maximal contraction obtained at the highest concentration of AET shows a tendency to be greater in vessels with endothelium than in those without endothelium, even although the response is not statistically different. However, the contraction obtained in vessels functionally denuded of endothelium strongly suggests that AET also has direct effects on smooth muscle cells. AET might mediate its action through inhibition of inducible NOS that might be expressed under the experimental conditions used in the present study. However, two inhibitors of NOS (L-NAME and L-NA) did not affect the response of AET in vessels without functional endothelium. Finally, the increase in tension produced by AET was not affected by the COX inhibitor, indomethacin, in these arteries. Taken together, these results suggest that inhibition of either NOS or COX seems unlikely to be responsible for the increase in tension produced by AET in rat small femoral arteries.

AET in aqueous solution can rearrange to ATZ and MEG with the latter, after dimerization in an oxidative medium, being transformed to GED [12]. The present study clearly shows that MEG, but not ATZ or GED, can produce endothelium-independent contraction of rat femoral arteries. Thus, MEG could be responsible for the pharmacological effects of AET observed in the present study. As MEG and AET were equipotent in producing contraction, it seems that the relaxing effect of GED did not influence the increase in tone produced by AET. Thus, rearrangement of AET probably did not result in the generation of a significant amount of GED under the experimental conditions. MEG has been proposed as a novel anti-inflammatory agent [20–23]. MEG

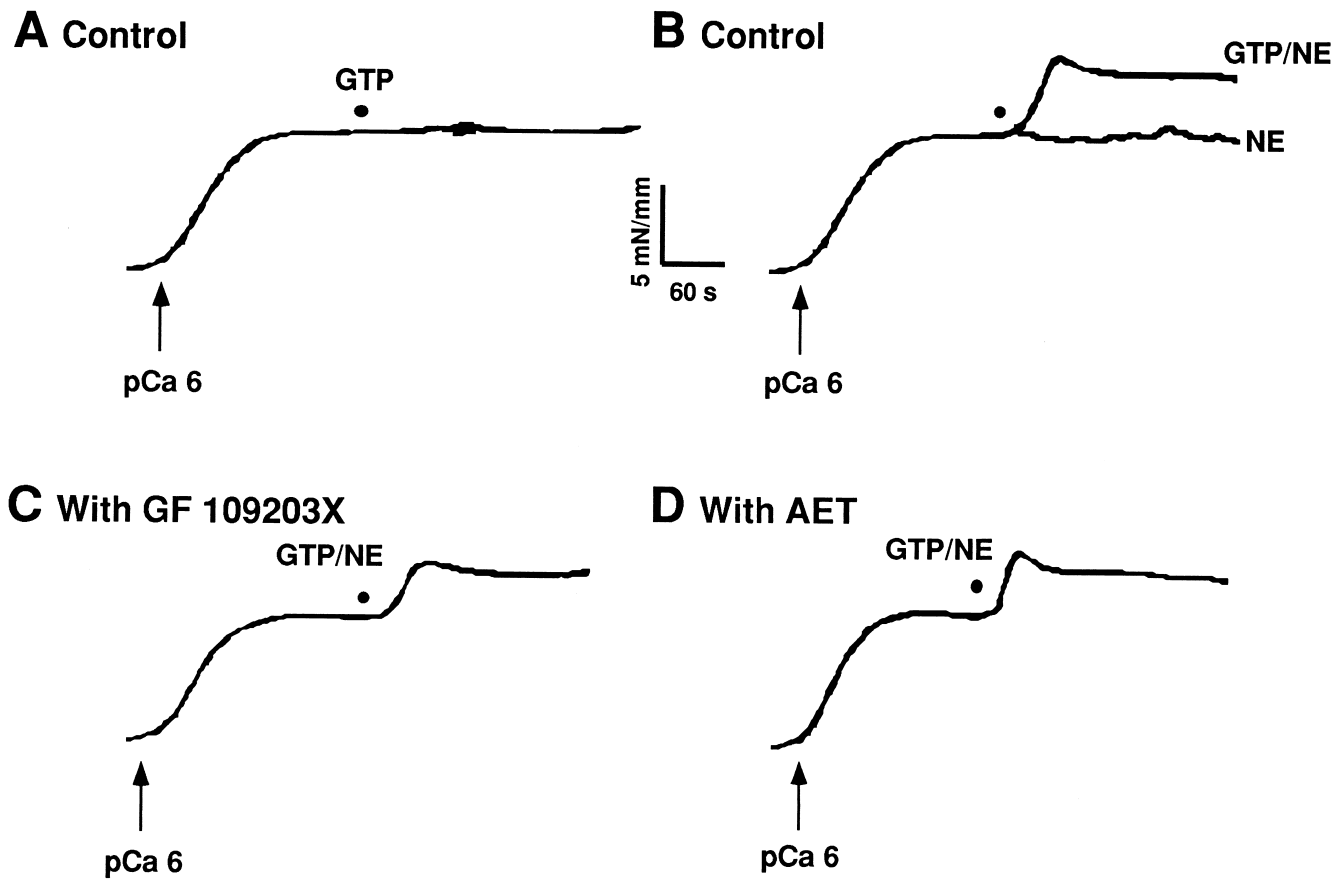

Fig. 7. Representative traces showing the effects of GTP (50 μM) alone (A), NE (10 μM) alone (B), and the NE plus GTP-induced Ca^{2+} sensitization at pCa 6 in the absence (B) and presence of GF 109203X (3 μM) (C) and AET (100 μM) (D) in β-escin-permeabilized arteries. Traces are representative of 3 experiments.

did not affect the vascular response to NE in control rats several hours after administration *in vivo* [20]. In contrast, in the present study, MEG, like AET, produced a very rapid increase in NE-induced contraction (Fig. 1, representative trace). These discrepancies could be explained by the dimerization *in vivo* of MEG to GED, which produces relaxation, or it can form other species with unknown properties in physiological media [12].

In the present study, neither AET nor MEG affected the contraction to NE in Ca^{2+}-free medium, suggesting that the mechanism by which the two compounds potentiate NE-induced contraction might not involve the release of Ca^{2+} from the intracellular sources. However, both AET and MEG were able to enhance the contractile response to $CaCl_2$ in the continuous presence of NE. These results suggest that AET and MEG might potentiate NE-induced contraction linked to either Ca^{2+} entry, Ca^{2+} sensitization of contractile myofilaments, or both.

With respect to Ca^{2+} entry, we have studied the involvement of voltage-dependent Ca^{2+} channels in the effect of AET. Nitrendipine alone produced a rightward shift of curve to NE, indicating the involvement of voltage-dependent Ca^{2+} channels in its mechanism leading to contraction. However, AET was still able to potentiate the NE response even in the presence of nitrendipine. Interestingly, AET also did not affect the response to $CaCl_2$ in vessels exposed to KCl depolarization. The above data strongly suggest that voltage-dependent Ca^{2+} channels are not involved in AET-induced potentiation. However, it cannot be ruled out that AET might affect Ca^{2+} entry through another pathway. To test this hypothesis, the so called receptor-mediated Ca^{2+} entry blocker, SK&F 96365, was used. SK&F 96365 was found to reduce the response to $CaCl_2$ in the continuous presence of NE. In addition, it prevented the potentiation induced by AET. SK&F 96365 has been reported to possess dual effects on both receptor-mediated Ca^{2+} entry and voltage-dependent Ca^{2+} channels in response to agonists in different smooth muscle [24, 25]. As discussed above, voltage-dependent Ca^{2+} channels are not involved in AET-induced potentiation; therefore, it is likely that the inhibitory effect of SK&F 96365 on AET-induced potentiation of NE contraction takes place at the level of receptor-operated

Ca^{2+} channels, the nature of which remains to be characterized.

The hypothesis that AET increases NE-induced tone through an increase in Ca^{2+} sensitization was also tested. One of the pathways implicated in the mechanism leading to Ca^{2+} sensitization produced by α-adrenoceptor stimulation in femoral arteries from different species is the activation of PKC [26, 27]. In the present study, NE produced myofilament Ca^{2+} sensitization in small femoral artery of the rat, as shown in arteries permeabilized with β-escin. The requirement of GTP for NE-induced Ca^{2+} sensitization is consistent with the activation of G-protein coupled receptor accounting for this effect of the agonist. Neither the PKC inhibitor GF 109203X nor AET altered the response to Ca^{2+} in permeabilized arteries suggesting that neither drug directly affected the myosin light chain kinase. In addition, both GF 109203X and AET failed to inhibit the increase in tone produced by NE. These results suggest that the PKC pathway is not likely to be involved in agonist-induced Ca^{2+} sensitization and that AET does not enhance NE-induced Ca^{2+} sensitization.

Interestingly, GF 109203X prevented the potentiating effect of both AET and MEG on NE-induced contraction in intact arteries. Thus, these results provide pharmacological evidence for the involvement of a PKC inhibitor-sensitive pathway in the effect of AET and MEG in small femoral arteries. The mechanism by which the two compounds stimulate the latter pathway may not be linked to NE-induced Ca^{2+} sensitization, Ca^{2+} release from intracellular sources, or Ca^{2+} entry through voltage-dependent Ca^{2+} entry, as discussed above. Since the potentiating effect of AET was sensitive to SK&F 96365, activation of PKC by NE may affect the pathway leading to Ca^{2+} entry through the receptor-operated Ca^{2+} channels in this resistance artery. The nature of receptor-operated Ca^{2+} channels involved in NE-induced Ca^{2+} entry remains to be elucidated using other methodology such as electrophysiological and molecular biology. Nevertheless, the present work shows that PKC inhibitor-sensitive pathway is involved in the potentiating effect of both AET and MEG in femoral resistance arteries.

In summary, the present study indicates that AET potentiates NE-induced contraction independently of its effect on NOS, COX, or both, by enhancing SK&F 96365-sensitive Ca^{2+} entry. Furthermore, a PKC inhibitor-sensitive pathway appears to be involved in its action. Finally, among the products resulting from the rearrangement of AET in aqueous solution, MEG could be responsible for the pharmacological effects of AET observed in small femoral resistance arteries. Whether the enhancement of the effects of NE contributes to the beneficial and detrimental effects of AET in shock or inflammation needs further studies. Nevertheless, the latter effects of AET may partially contribute to the hemodynamic effect of this compound in animal models of shock [12].

Acknowledgment

We thank Dr. Gillian A. Gray for careful review of the manuscript.

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