Inhibitory Effect of Ammonium Chloride on Acetylcholine-Induced Relaxation

Katsuyuki Ando, Toshiro Fujita

Abstract We designed the present study to clarify whether the intracellular pH change by ammonium chloride influences endothelium-dependent relaxation in thoracic aorta of 9-week-old Sprague-Dawley rats. Intracellular alkalinization with 3 mmol/L ammonium chloride, which did not affect resting vascular tone, attenuated acetylcholine-induced relaxation but not nitroglycerin vasodilation. Acetylcholine relaxation was more inhibited by a shorter duration of treatment. Thus, change in intracellular pH may be important in the effect because the alkalinizing effect of ammonium chloride disappears gradually. In support of this, the proton ionophore nigericin abolished the effect. Also, amiloride shortened the effect of ammonium chloride, suggesting that intracellular pH plays a role: sodium-proton antiport antagonizes the disappearance of ammonium chloride–induced intracellular alkalinization. The synthesis of vasoconstrictor prostaglandins, such as thromboxane A2, may be stimulated during acetylcholine treatment, resulting in the attenuation of acetylcholine relaxation, because the relaxation was abolished by treatment with the phospholipase A2 inhibitor quinacrine, cyclooxygenase inhibitor indomethacin, prostaglandin H2/thromboxane A2 receptor antagonist S1452, and thromboxane A2 synthase inhibitor dazmegrel. Phospholipase A2 may contribute to the effect of intracellular alkalinization, which is compatible with the fact that the optimal pH of phospholipase A2 is neutral to alkaline. In addition, superoxide dismutase attenuated the effect of ammonium chloride. In conclusion, intracellular alkalinization by ammonium chloride attenuated acetylcholine-induced relaxation, possibly through the interrelated production of both thromboxane A2 and superoxide radicals. (Hypertension. 1994;24:189–194.)

Key Words • endothelium • proton concentration • prostaglandins • superoxide • ion channels • rats, Sprague-Dawley

Moreover, some vasoconstrictors, which caused intracellular alkalinization, have been reported to produce prostaglandins. Also, phospholipase A2 (PLA2), which produces arachidonate and facilitates prostaglandin generation, is activated by intracellular alkalinization, because the optimal pH of this enzyme is neutral to alkaline. In addition, increased pH stimulated the generation of superoxide radicals in human neutrophils. Thus, vasoconstrictor prostaglandins and/or superoxide may be involved in alkalinization-induced vasoconstriction through endothelial cells. To clarify this hypothesis, we examined the effect of NH4Cl on acetylcholine-induced relaxation and its involvement with prostaglandins and superoxide in rat thoracic aorta.

Methods

Preparation of Aortic Rings

Aortic rings of 9-week-old male Sprague-Dawley rats (Charles River Japan; approximately 280 to 320 g) were prepared for isometric tension recording with a previously reported standard organ bath method. Briefly, rats were killed with light ether anesthesia, and the thoracic aorta was removed. In Krebs' bicarbonate solution, the aorta was divided into four segments 4 to 5 mm long. The rings were carefully handled to avoid damage to the inner surface. In some rings, endothelium was removed mechanically by rubbing with cotton. The rings were suspended in organ bath chambers containing 8 mL Krebs' bicarbonate buffer of the following composition (mmol/L): NaCl 112, NaHCO3 25.2, KCl 4.73, MgCl2 1.19, KH2PO4 1.19, CaCl2 0.9, EDTA 0.026, and dextrose 11.0. The medium was equilibrated to pH 7.40 by continuous aeration with a mixture of 95% O2/5% CO2 and maintained at 37°C. The upper wire supporting each ring was attached to a force-displacement transducer (TB-651T, Nihon-Kohden), and changes in isometric force were displayed on a chart recorder (WT-685G, Nihon-Kohden). Resting force of aortic rings was 1.0 g; rings were allowed to equilibrate for 90 minutes. Then rings were exposed...
to a depolarizing concentration of KCl (60 mmol/L) and washed with Krebs' solution. After 20 minutes, the aortic rings were contracted by 3×10^{-7} mol/L norepinephrine, and, when the contractile response was stabilized, 10^{-4} mol/L acetylcholine was applied to the rings for examination of whether functional endothelium was present. For the following experiment, we used rings with intact endothelium, in which a sufficient relaxation (>80% of norepinephrine contraction) was obtained by acetylcholine application, and rings without endothelium, which had no relaxation by acetylcholine. Twenty minutes after the chambers were washed at least three times with Krebs' solution, the experiments were started. Endothelium-derived relaxation was evaluated by cumulative addition of acetylcholine (10^{-9} to 10^{-4} mol/L) in precontracted aortic rings by 3×10^{-7} mol/L norepinephrine. In some rings, endothelium-unrelated relaxation was examined by nitroglycerin (10^{-9} to 10^{-4} mol/L) after norepinephrine precontraction.

Experimental Protocols

Experiment 1: Effect of Changes in pH, on Acetylcholine Relaxation

First, we examined the effect of the different NH_{4}Cl doses (3, 5, 10, and 20 mmol/L; 10 minutes) on norepinephrine contraction (3×10^{-7} mol/L). Intracellular alkalinization was induced by a small dose (3 mmol/L) of NH_{4}Cl (10 minutes) because a high dose (>10 mmol/L) affected vascular tone (see “Results”) and vascular reactivity (Fig 1) in rat aorta, similar to previous reports.15,16 Acidification was induced by the removal of 3 mmol/L NH_{4}Cl (10 minutes after its administration) and treatment (10 minutes) with 10^{-6} mol/L nigericin. After these treatments, cumulative acetylcholine relaxation was examined. Nitroglycerin relaxation was also examined after NH_{4}Cl treatment, as was acetylcholine relaxation in NH_{4}Cl-treated aorta without intact endothelium.

Experiment 2: Time-Dependent Effect of NH_{4}Cl Treatment on Acetylcholine Relaxation

Intracellular alkalinization by NH_{4}Cl gradually disappeared,14,15 so we examined the effects of different treatment periods of NH_{4}Cl (0-minute and 10-minute treatments) on acetylcholine relaxation. Norepinephrine precontraction was started immediately after NH_{4}Cl administration in the 0-minute treatment and 10 minutes after NH_{4}Cl administration in the 10-minute treatment. Because accurate treatment time is critical in experiment 2, acetylcholine relaxation began 3 minutes after norepinephrine precontraction, and cumulative acetylcholine application was done each 1 minute. Acetylcholine relaxation was also examined after inhibition of NH_{4}Cl (0-minute and 10-minute treatments)—induced alkalinization by 10^{-4} mol/L nigericin. Moreover, we tested the influence of Na^{-}H^{+} antiport inhibition with 10^{-9} mol/L amiloride on the action of NH_{4}Cl.

Experiment 3: Possible Contribution of Vasocostrictive Prostanoids and Superoxide to the Effect of NH_{4}Cl

We examined the effect of treatments (20 minutes) with the PLA_{2} inhibitor quinacrine (10^{-3} mol/L), cyclooxygenase inhibitor indomethacin (10^{-5} mol/L), prostaglandin H_{2} (PGH_{2}), thromboxane A_{2} (TXA_{2}) receptor antagonist S1452 (3×10^{-4} mol/L), TXA_{2} synthase inhibitor dazmegrel (10^{-7} mol/L), and 5-lipoxygenase inhibitor AA861 (10^{-5} mol/L) on the action of NH_{4}Cl. Acetylcholine relaxation was also accomplished with treatment (10 minutes) with the superoxide scavenger superoxide dismutase (SOD, 100 U/mL). In addition, we examined the effect of the combination of SOD and indomethacin on acetylcholine relaxation.

Experiment 4: Effect of Inhibited Nitric Oxide Generation on the Action of NH_{4}Cl

Nitric oxide generation was abolished by 3×10^{-4} mol/L N^{0}-nitro-L-arginine methyl ester (L-NAME). Ten minutes after L-NAME treatment, acetylcholine relaxation was accomplished in rat aorta with or without NH_{4}Cl. Also, we examined the effect of L-NAME plus 10^{-7} mol/L indomethacin.

Drugs

NH_{4}Cl, nigericin, amiloride, quinacrine, and L-NAME were purchased from Sigma Chemical Co; norepinephrine from Sankyo Pharmaceutical Co; acetylcholine and indomethacin from Wako Chemical Industries; and nitroglycerin from Nihon-Kayaku. The PGH_{2}/TXA_{2} receptor antagonist S1452 was a gift from Shinogi, the TXA_{2} synthase inhibitor dazmegrel from Pfizer, and the 5-lipoxygenase inhibitor AA861 from Takeda. Nigericin, amiloride, quinacrine, indomethacin, S1452, and AA861 were dissolved in ethanol. Dazmegrel was dissolved in 0.1 mol/L NaOH, and pH was adjusted to 8.5 with 0.1 mol/L HCl. Other drugs were dissolved in saline. Five microliters of each solution was added to treated aorta, and 5 μL of each vehicle (ethanol, 0.1 mol/L NaOH/0.1 mol/L HCl [pH 8.5], or saline) to untreated aorta (each vehicle did not affect acetylcholine relaxation; see “Results”).

Statistical Analysis

Data are expressed as mean±SEM. The maximal relaxation by acetylcholine (E_{max}, expressed as percentage of the level of norepinephrine-precontracted tension) and the acetylcholine concentration that produced a half-maximal relaxation (pD_{2}, expressed as minus logarithm of the concentration [mol/L]) were used for statistical comparison. Statistical analysis for E_{max} and pD_{2} was done by Student's t test (experiment 1; comparison between two [control and treated] groups) and by one-way (experiments 1 through 4) ANOVA and subsequent Tukey method. Values of P<.05 were considered significant.

Results

Experiment 1: Effect of Changes in pH, on Acetylcholine Relaxation

Ten and 20 mmol/L NH_{4}Cl increased resting vascular tone slightly (3, 5, 10, and 20 mmol/L: not determined, 3±2%, 9±3%, and 13±4% of KCl contraction) and potentiated norepinephrine contraction (Fig 1). In contrast, 3 and 5 mmol/L NH_{4}Cl did not significantly change resting vascular tone and norepinephrine contraction. As shown in Table 1 and Fig 2, 3 mmol/L NH_{4}Cl treatment attenuated acetylcholine relaxation. The removal of NH_{4}Cl restored acetylcholine relaxation to control levels. Also, nigericin alone did not affect acetylcholine relaxation. In contrast to acetylcholine relaxation, NH_{4}Cl did not change nitroglycerin relaxation. Acetylcholine did not relax aortic rings without endothelium in control or NH_{4}Cl-added media.
TABLE 1. Effect of Changes in Intracellular pH on Acetylcholine Relaxation in Rat Aorta Precontracted by 3 x 10^{-7} mol/L Norepinephrine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Without Treatment</th>
<th>With Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh relaxation</td>
<td>n</td>
<td>E_max</td>
</tr>
<tr>
<td>NH_4Cl (3 mmol/L)</td>
<td>8</td>
<td>92.7±2.0</td>
</tr>
<tr>
<td>Removal of NH_4Cl</td>
<td>5</td>
<td>93.4±0.8</td>
</tr>
<tr>
<td>Nigericin (10^{-6} mol/L)</td>
<td>7</td>
<td>96.8±1.3</td>
</tr>
<tr>
<td>NTG relaxation</td>
<td>Nh_4Q (3 mmol/L)</td>
<td>6</td>
</tr>
</tbody>
</table>

E_max indicates maximal relaxation by acetylcholine, expressed as percentage of precontracted levels of norepinephrine; pD_2, acetylcholine concentration that produced half-maximal relaxation, expressed as negative logarithm of concentration (mol/L); ACh, acetylcholine; and NTG, nitroglycerin. Values are mean±SEM.

*p < 0.05 compared with control.

Experiment 2: Time-Dependent Effect of NH_4Cl Treatment on Acetylcholine Relaxation

Acetylcholine relaxation was attenuated more by the 0-minute treatment of NH_4Cl than by the 10-minute treatment (Table 2). Nigericin inhibited the attenuating effect of both 0- and 10-minute treatments of NH_4Cl similarly (Fig 3). Acetylcholine relaxation was not affected by amiloride in control medium. Amiloride abolished the effect of the 10-minute treatment of NH_4Cl, but did not significantly change that of the 0-minute NH_4Cl treatment.

Experiment 3: Possible Contribution of Vasoconstrictive Prostanoids and Superoxide to the Effect of NH_4Cl

Quinacrine, indomethacin, S1452, dazmegrel, and AA861 did not change acetylcholine relaxation in control medium (Table 3). However, the impaired acetylcholine relaxation by NH_4Cl was reversed by quinacrine, indomethacin, S1452, and dazmegrel. In contrast, AA861 did not normalize but rather slightly enhanced the inhibitory effect of NH_4Cl. SOD did not affect E_max of acetylcholine relaxation in control medium but slightly enhanced pD_2. SOD inhibited the attenuation of acetylcholine relaxation by NH_4Cl. Indomethacin plus SOD also normalized the effect of NH_4Cl. In aortic rings with NH_4Cl plus the combined treatments, acetylcholine relaxation did not differ from that in rings with NH_4Cl plus either SOD or indomethacin.

Experiment 4: Effect of Inhibited Nitric Oxide Generation on the Action of NH_4Cl

L-NAME abolished acetylcholine relaxation in aorta with NH_4Cl treatment (Fig 4). In contrast, acetylcholine constricted aortic rings treated with NH_4Cl plus L-NAME (E_max, 19.6±5.1%; pD_2, 7.41±0.30). Indomethacin reversed the effect of NH_4Cl in L-NAME-treated aorta.

Discussion

In the present study, NH_4Cl treatment, which increases pH_4, attenuated acetylcholine-induced relaxation in rat aorta. The H^+ ionophore nigericin, which increases intracellular H^+ concentration and blocks NH_4Cl-induced intracellular alkalinization, normalized attenuation of acetylcholine relaxation. In addition, the removal of NH_4Cl from medium, which causes abrupt intracellular acidification despite slow extrusion of NH_4^+ from cells, did not affect acetylcholine relaxation. Thus, intracellular H^+ rather than intracellular NH_4^+ may be critical in the attenuating effect of NH_4Cl in acetylcholine relaxation. This hypothesis is supported by the present finding that the effect of NH_4Cl disappeared in a time-dependent fashion (Table 2). When NH_4Cl was added extracellularly, NH_4 rapidly moves into intracellular spaces and binds H^+, resulting in intracellular alkalinization, in vascular smooth muscle and endothelial cells. On the other hand, NH_4 slowly enters into the cell and releases H^+, leading to a gradual normalization of pH. Thus, NH_4Cl-induced attenua-
acetylcholine relaxation was performed after 10 minutes of treatment on acetylcholine relaxation. This hypothesis is supported by the fact that acetylcholine contractions of KC1 and phenylephrine in rat aorta and that a high dose (>20 mmol/L) of NH4Cl produced relaxation. In contrast, 3 mmol/L NH4Cl did not affect basal tension of aorta and norepinephrine-induced contraction (see "Results" and Fig 1). Moreover, nitroglycerin relaxation was not affected by NH4Cl treatment. Acetylcholine did not relax denuded aorta in control medium or medium to which NH4Cl had been added. Thus, vascular smooth muscle cells may not participate in the attenuation of acetylcholine relaxation by small doses (3 mmol/L) of NH4Cl in rat aorta. High CO2 tension suppressed norepinephrine-induced contraction in rat aorta, possibly with endothelial modulation. Our recent study suggested that acetylcholine relaxation was modified by altered activity of Na\(^{+}\)-H\(^{+}\) antiport, NA\(^{+}\)-H\(^{+}\) antiport, which extrudes H\(^{+}\), may delay the amelioration of acetylcholine relaxation by NH4Cl-induced alkalinization. Thus, the blockade of Na\(^{+}\)-H\(^{+}\) antiport, which extrudes H\(^{+}\), may delay the amelioration of acetylcholine relaxation by NH4Cl-induced alkalinization. Thus, the blockade of Na\(^{+}\)-H\(^{+}\) antiport may facilitate the return of pH, to basal levels and accelerate the reversal of acetylcholine relaxation from NH4Cl-induced attenuation.

Although intracellular alkalinization induced by a high dose (>10 mmol/L) of NH4Cl affected vascular tone directly, the direct vascular effects may not contribute to our results. Previous studies indicated that a relatively high dose (>10 mmol/L) potentiated vasoconstriction. Recent studies have reported that a high dose (20 mmol/L) of NH4Cl produced relaxation.

### Table 2. Effect of Different Treatment Periods of 3 mmol/L NH4Cl on Acetylcholine Relaxation in Rat Aorta Precontracted by 3\(\times\)10\(^{-7}\) mol/L Norepinephrine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=6)</th>
<th>0-Minute (n=6)</th>
<th>10-Minute (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E_{\text{max}})</td>
<td>93.5±2.1</td>
<td>86.1±5.5 (\ast)</td>
<td>79.1±2.8 (\ast)</td>
</tr>
<tr>
<td>(pD_2)</td>
<td>7.35±0.04</td>
<td>6.50±0.17 (\ast)</td>
<td>6.90±0.20 (\dagger)</td>
</tr>
</tbody>
</table>

\(E_{\text{max}}\) indicates maximal relaxation by acetylcholine, expressed as percentage of precontracted levels of norepinephrine; \(pD_2\), acetylcholine concentration that produced half-maximal relaxation, expressed as negative logarithm of concentration (mol/L).

*P<.05 compared with control.
†P<.05 compared with 0-minute treatment.
TABLE 3. Changes in NH$_4$Cl-Induced Attenuation of Acetylcholine-Induced Relaxation by Several Drugs in Rat Aorta Precontracted by 3×10$^{-7}$ mol/L Norepinephrine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control Without Treatment</th>
<th>Control With Treatment</th>
<th>NH$_4$Cl Without Treatment</th>
<th>NH$_4$Cl With Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinacrine (10$^{-5}$ mol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>99.0±0.9</td>
<td>91.5±3.0</td>
<td>69.2±3.3*</td>
<td>96.3±2.1†</td>
</tr>
<tr>
<td>pD$_2$</td>
<td>7.56±0.06</td>
<td>7.09±0.27</td>
<td>6.39±0.05*</td>
<td>7.39±0.03†</td>
</tr>
<tr>
<td>Indomethacin (10$^{-5}$ mol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>93.9±2.3</td>
<td>93.3±2.2</td>
<td>71.9±4.0*</td>
<td>93.8±2.5†</td>
</tr>
<tr>
<td>pD$_2$</td>
<td>7.42±0.13</td>
<td>7.28±0.07</td>
<td>6.47±0.08*</td>
<td>7.49±0.05†</td>
</tr>
<tr>
<td>S145 (3×10$^{-8}$ mol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>97.6±1.3</td>
<td>91.8±2.8</td>
<td>74.4±3.5*</td>
<td>91.7±2.3†</td>
</tr>
<tr>
<td>pD$_2$</td>
<td>7.44±0.06</td>
<td>7.24±0.11</td>
<td>6.49±0.09*</td>
<td>7.05±0.07†</td>
</tr>
<tr>
<td>Dazmegrel (10$^{-6}$ mol/L)</td>
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<tr>
<td>n</td>
<td>7</td>
<td>5</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>96.8±1.5</td>
<td>94.3±1.4</td>
<td>74.5±4.0*</td>
<td>89.5±3.3†</td>
</tr>
<tr>
<td>pD$_2$</td>
<td>7.41±0.10</td>
<td>7.77±0.10</td>
<td>6.52±0.08*</td>
<td>7.43±0.33†</td>
</tr>
<tr>
<td>AA861 (10$^{-5}$ mol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>95.7±0.4</td>
<td>89.3±0.6</td>
<td>72.2±1.5*</td>
<td>55.3±2.0†</td>
</tr>
<tr>
<td>pD$_2$</td>
<td>7.48±0.02</td>
<td>7.09±0.27</td>
<td>6.52±0.03*</td>
<td>6.47±0.04*</td>
</tr>
<tr>
<td>SOD (100 U/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>91.2±1.9</td>
<td>92.9±0.8</td>
<td>73.8±3.5*</td>
<td>95.2±1.9†</td>
</tr>
<tr>
<td>pD$_2$</td>
<td>7.38±0.07</td>
<td>7.71±0.09*</td>
<td>6.49±0.08*</td>
<td>7.34±0.02†</td>
</tr>
<tr>
<td>Indomethacin+SOD (10$^{-5}$ mol/L+100 U/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>93.5±1.9</td>
<td>94.7±3.6</td>
<td>74.0±5.2*</td>
<td>94.1±2.8†</td>
</tr>
<tr>
<td>pD$_2$</td>
<td>7.19±0.09</td>
<td>7.41±0.11</td>
<td>6.60±0.09*</td>
<td>7.27±0.11†</td>
</tr>
</tbody>
</table>

$E_{\text{max}}$ indicates maximal relaxation by acetylcholine, expressed as percentage of precontracted levels of norepinephrine; pD$_2$, acetylcholine concentration that produced half-maximal relaxation, expressed as negative logarithm of concentration (mol/L); and SOD, superoxide dismutase. Values are mean±SEM.

*P<.05 compared with control.
†P<.05 compared with NH$_4$Cl treatment alone.

NH$_4$Cl-treated aorta with the blockade of nitric oxide production by L-NAME and that simultaneous indomethacin treatment abolished this contraction. In contrast, the blockade of 5-lipoxygenase (AA861) did not ameliorate the attenuating effect of NH$_4$Cl on acetylcholine relaxation but rather enhanced it. Thus, leukotrienes may not contribute to the attenuating effect of NH$_4$Cl on acetylcholine relaxation but compensate it, because leukotriene D$_4$ relaxed blood vessels.

Increased pH, has been indicated to be accompanied by increased intracellular Ca$^{2+}$ (Ca$^{2+}$), which PLA$_2$ requires in synthesizing arachidonic acid. In bovine aortic endothelial cells, intracellular alkalization by NH$_4$Cl leads to increased Ca$^{2+}$, also. Intracellular alkalization by NH$_4$Cl or monensin, in human endothelial cells with alkalization by either NH$_4$Cl or monensin. Thus, in aorta, intracellular alkalization may not only enhance the affinity of PLA$_2$ to Ca$^{2+}$ but also increase Ca$^{2+}$ itself. Both enhanced affinity and increased Ca$^{2+}$ facilitate the production of arachidonic acid, resulting in increased vasoconstrictor prostaglandins. In fact, the role of both pH, and Ca$^{2+}$, was suggested in prostaglandin release from Kupffer cells after hypoxia-reoxygenation. Intracellular alkalization induced by 3 mmol/L NH$_4$Cl might not elevate Ca$^{2+}$ sufficiently to stimulate basal activity of PLA$_2$ but may increase the affinity of PLA$_2$ to enhance the response to acetylcholine-induced rise in Ca$^{2+}$, because it affected vascular tone only in the presence of acetylcholine. In addition to TXA$_2$, the present results suggest that superoxide radicals may mediate the effect of NH$_4$Cl because SOD restored the effect. The combination of extracellular alkalization and N-formyl-methionyl-leucyl-phenylalanine increased both pH, and the generation of superoxide in human neutrophils, but extracellular alkalization alone did not affect either, suggesting that the intracellular alkalization should be
Thus, the attenuation of acetylcholine-induced relax-
generation, its receptor antagonist, or SOD. TXA
further investigations are required to clarify the precise
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