Vol. 177, No. 1, 1991
May 31, 1991

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 407-413

POSSIBLE ROLE OF SODIUM-HYDROGEN ANTIPORT IN ACETYLCHOLINE-INDUCED RELAXATION OF RAT AORTA

Katsuyuki Ando, Katsutoshi Takahashi, Ayumu Ono, Tatsuo Shimosawa, Etsuro Ogata, and Toshiro Fujita

Fourth Department of Internal Medicine, University of Tokyo School of Medicine, Tokyo 112, Japan

Received April 22, 1991

The decreased extracellular Na concentration (25mM) attenuated vasodilator effect of acetylcholine (ACh) in norepinephrine-treated aortic ring. This attenuation was greater in the low Na medium substituted by Li, which can exchange intracellular H through Na-H antiport, as compared with that substituted by choline, which cannot. 10μM amiloride canceled the difference between the two low Na mediums. Thus the inhibition of Na-H antiport may counteract the suppressive effect of decreased intracellular Na on ACh vasodilation, suggesting a possible role of Na-H antiport in a release of vasoactive substance(s) from endothelial cells.

The amiloride analog dichlorobenzamil blocked the relaxation by acetylcholine (ACh) and A23187 in rat aorta (1). Either low extracellular Na or amiloride attenuated ACh-induced increment in cyclic GMP in rat aorta (2). Then, it has been proposed an important role of Na-Ca exchange in the endothelium-dependent vascular relaxation. There may be an intimate relationship between Na-Ca exchange and Na-H antiport through changing intracellular Na concentration (3). Na-H antiport contributes to intracellular pH regulation (4). Intracellular pH change may affect the release of vasoactive factor(s) from endothelial cells: intracellular acidosis by high CO_2 tension suppressed vasoconstrictor effect of norepinephrine (NE) in rat aorta, which was partially abolished by hemoglobin or rubbing of endothelium (5). Amiloride analogs itself relaxed dog coronary artery (6). This amiloride-induced

0006-291X/91 $1.50

vasodilation was greatly attenuated by removal of endothelium. To evaluate a possible role of Na-H antiport in endothelium-related vasodilation, we examined the effect of low extracellular Na concentration (substituted by Li or choline) and amiloride on ACh-induced relaxation in rat aorta.

MATERIALS AND METHODS

Nine week-old male Sprague-Dawley rats (approximately 280-320g) were killed. The thoracic aorta was immediately removed, care being taken to avoid damage to the endothelium. It was placed in Krebs-bicarbonate solution with the following millimolar composition: NaCl 112, $NaHCO_3$ 25.2, KCl 4.73, $MgCl_2$ 1.19, KH_2PO_4 1.19, $CaCl_2$ 0.9, EDTA 0.026, Dextrose 11.0. The medium was equilibrated to pH 7.4 by aeration with a mixture of 95% O_2 and 5% CO_2. Each aorta was divided into four segments of 3-4 millimeter length. In some rings, the endothelium was removed by rubbing endothelial layer with cotton. The tissue rings were mounted on stainless-steel wires and suspended in 8-ml organ bath chambers containing Krebs solution. They were maintained at 37°C and aerated continuously with 95% O_2 and 5% CO_2. The upper wire of each ring was attached to an force-displacement transducer (TB-651T; Nihon-Koden, Tokyo, Japan). Changes in isometric force were displayed on a chart recorder (WT-685G; Nihon-Koden). Resting force of aortic rings were 1.0g and tissue were allowed to equilibrate for 90 min. And then, the tissue were exposed to a deporalizing KCl (60mM) and washed by Krebs solution. After waiting for 20 min, the experiments were started.

Endothelium-related vasodilation was evaluated by acetylcholine chloride in pre-contracted aortic rings with 300nM of NE. The experiment was done after 10 min of incubation with low Na (25mM) medium, 10μM amiloride, and these combination. In low Na medium, sodium chloride was substituted by lithium or choline chloride (Li- or choline-low Na). In addition, we examined the effect of endothelium-unrelated nitrovasodilator nitroglycerin (NTG; Nihon-Kayaku, Tokyo, Japan) in Li- or choline-low Na. All drugs except for NTG were purchased from Sigma Chemical Co., St Louis, MO.

Since NE-induced vasodilation was not different between control (102±5% of contraction with 60mM KCl) and the treatments (Li-low Na: 98±9%, choline-low Na: 82±10%, amiloride: 92±5%, Li-low Na plus amiloride: 86±8%), relaxation of aorta was expressed as percent decrement of NE-induced contraction immediately before an addition of ACh in each treatment. Results are presented as means±SEM. Data were evaluated by two-way analysis of variance and subsequent multiple comparison by Tukey's or Scheffe's method (7). Significance was accepted at the $p<0.05$ level.

RESULTS

ACh caused dose-related relaxation in the rat aortic ring with endothelium but not without endothelium (Figure 1). Either low Na

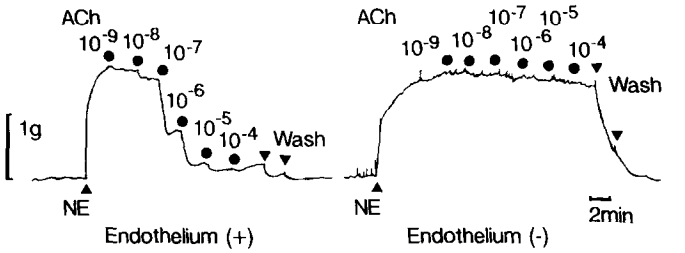

Figure 1. Effects of acetylcholine (ACh) on pre-contracted rat aorta by 300nM norepinephrine (NE) with (left panel) and without (right) endothelium. ACh, 1nM to 100μM, was applied.

medium significantly inhibited ACh-induced vasorelaxation ($p<0.01$ by Scheffe's method, Li- and choline-low Na, respectively) (Figure 2, panel A). However, the inhibition was greater in Li-low Na than choline-low Na ($p<0.01$). 10μM amiloride did not affect ACh-induced relaxation of aorta in normal Na (Figure 2, panel B). When 10μM amiloride was added in Li-low Na, the inhib-

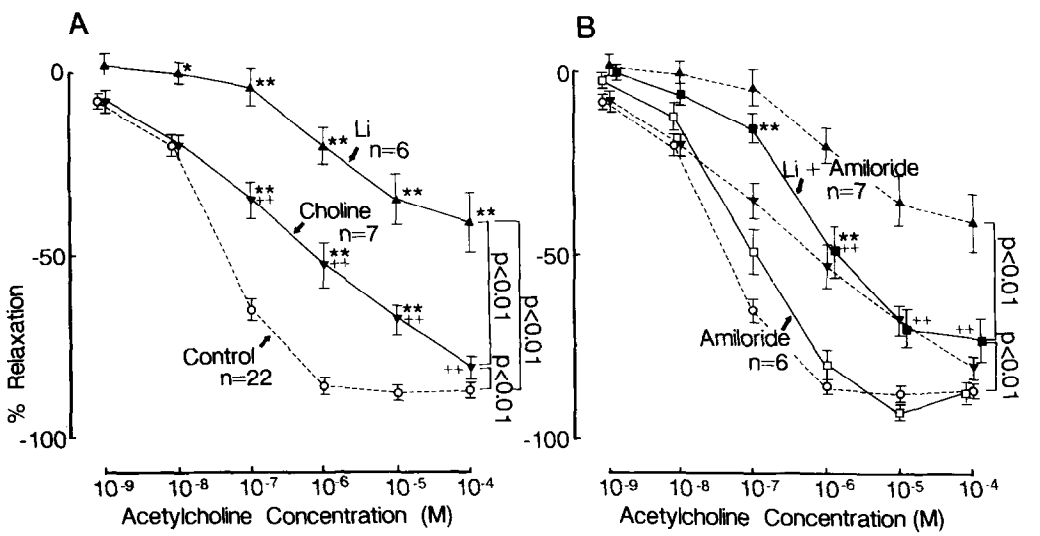

Figure 2. Effects of acetylcholine (ACh) on pre-contracted rat aorta by 300nM norepinephrine in the treatments. Panel A: Influences of low (25mM) sodium (Na) medium substituted by lithium (Li; solid triangle) or choline (solid inversed triangle). Control (ACh-induced relaxation in normal Na medium) is expressed as open circle. Panel B: Influences of amiloride in normal Na (Amiloride; open square) and in low Na substituted by Li (Li+Amiloride; solid square). Each value of each dose of ACh was compared by Tukey's method (* $p<0.05$, ** $p<0.01$ as compared to control and ++ $p<0.01$ as compared to Li-substituted low Na medi-μm) and comparison between the treatments was done by Scheffe's method (p values are shown at the right side of each panel).

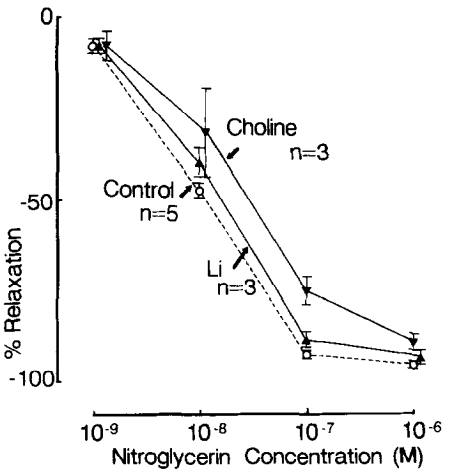

Figure 3. Effect of nitroglycerin (NTG) on pre-contracted rat aorta by 300nM norepinephrine in low (25mM) sodium medium substituted by lithium (Li; solid triangle) or choline (solid inverse triangle). Control (NTG-induced relaxation in normal Na medium) is presented as open circle. There are no differences between the treatments.

ited response was partially restored ($p<0.01$). As a result, there was no difference between response in Li-low Na plus amiloride and in choline-low Na. NTG-induced dilation was not changed with Li- and choline-low Na (Figure 3). In aortic rings without endothelium, ACh did not change vascular tone even in Li- or choline-low Na (n=2, each; data are not shown).

DISCUSSION

In the present study, low Na medium inhibited ACh-induced relaxation probably via the interaction of Na-Ca exchange through decreased intracellular Na, as previously reported (1,2). Although either Li- or choline-low Na attenuated vasodilation by ACh, the extent of the attenuation was greater in Li-low Na. The difference between two low Na medium may be due to the fact that external Li can be exchanged for internal H through Na-H antiport but that external choline cannot (4). In Li-low Na, low extracellular Na causes decreased intracellular Na but intracellular acidosis was prevented by exchange between extracellular Li and intracellular H. On the other hand, choline-low Na decreased

internal Na and inhibited H efflux by decreased exchangeable ion in the medium.

The different response between the two low Na medium might not be due to different intracellular Na concentration: the $K_{1/2}$ for interaction of H ion with Na-H antiport is in the range 10 to 100nM, far lower than the reported $K_{1/2}$ value (1-10mM) for Li and Na (4,8) so external Li may be mainly exchanged internal H. Our concept is supported by the fact that Na-H antiport inhibitor amiloride restored the ACh-induced relaxation in Li-low Na to the same extent as the response in choline-low Na. Thus the change in intracellular pH, as well as that in intracellular Na, may modulate ACh-induced vasodilation. In fact, intracellular acidosis by high CO_2 tension suppressed vasoconstrictor effect of NE through endothelium-related mechanism in rat aorta (5). Amiloride analogs itself caused relaxation in dog coronary artery, which was greatly attenuated by removal of endothelium (6). However, amiloride and its analogs inhibited ACh-induced relaxation (4) or increment in cyclic GMP (5). Thus, Na-H antiport may have two opposite directional effect on vascular tone: enhanced Na influx may facilitate vasodilation through EDRF release (1,2) but increased H efflux might result in suppression in endothelium-related relaxation. And then, the apparent response may be determined by the balance between the two factors (intracellular Na and H).

Li has been reported to inhibit phosphatidyl inositol turnover, which is activated by cholinergic agonists in bovine and rat brain (9,10). However, an inhibitory effect of Li on phosphatidyl inositol turnover in endothelial cells may not cause the different responses between the two low Na medium because amiloride, which has not been reported to affect phosphatidyl inositol turnover, reversed the attenuated ACh-relaxation in Li-low Na.

Moreover, in rat aorta, low Na medium substituted by Li suppressed ACh-induced increment of cyclic GMP to the almost same extent of that in choline-low Na (2). In addition, effects of Li on smooth muscle cells may hardly contribute to our results because NTG-induced vasodilation was not affected by Li-low Na.

Amiloride and its analogs do not influence only the Na channel and Na-H antiport (4,11,12). In the physiological Na concentration, the 50% inhibition of Na influx (IC_{50}) of amiloride for Na-H antiport is as high as 1mM (11). Such a large dose of amiloride cause the additional effects, for example, inhibiting Na-K-adenosine triphosphatase (13), inhibiting Na-Ca exchange (11), and acting as a weak base (14). In contrast, the IC_{50} for Na-H antiport is as low as 6μM when measured in the presence of a low extracellular Na concentration (25mM) (15). We used 10μM amiloride in normal and Li-low Na medium and this small dose has little additional effects (4). However, further studies are necessary to clarify the influence of the additional effects of amiloride because it is not denied the possibility that they might partially modulate the effect of amiloride of Na-H antiport in low extracellular Na concentration.

In the present study, vasodilator effect of NTG was not affected by Li- or choline-low Na medium. ACh did not cause vasodiation in aorta without endothelium in two low Na as well as in normal Na medium. Thus the different effect of ACh between rat aorta in Li- and choline-low Na may not be due to direct effect of the treatment on smooth muscle cells.

In summary, the attenuated effect of low Na on ACh-induced relaxation of rat aorta was greater in Li substitution than in choline substitution probably because Li can be exchanged for intracellular H but choline cannot. In fact, amiloride restored the inhib-

ited response to ACh in Li-low Na. Thus it is suggested that Na-H antiport may play an important role of endothelium-related vasodilation through changes in both intracellular Na and pH.

ACKNOWLEDGMENTS

The authors thank Miss Chisato Kitajima and Miss Mieko Hosogoe for excellent technical assistances.

REFERENCES

1. Winquist, R.J., Bunting, P.B., and Schofield, T.L. (1985) J. Pharmacol. Exp. Ther. 235,644-650.
2. Schoeffter, P., and Miller, R.C. (1986) Mol. Pharmacol. 30,53-57.
3. Siffert, W., and Akkerman, W.N. (1989) FEBS lett. 259,1-4.
4. Mahnensmith, R.L., and Aronson, P.S. (1985) Circ. Res. 56,773-778.
5. Fukuda, S., Matsumoto, M., Nishimura, N., Fujiwara, N., Shimoji, K., Takeshita, H., and Lee, T.J.-F. (1990) Am. J. Physiol. 258,H1049-H1054.
6. Cocks, T.M., Little, P.J., Angus, J.A., and Cragoe, E.J. Jr. (1988) Br. J. Pharmacol. 95,67-76.
7. Wallenstein, S., Zucker, C.L., and Fleiss, J.L. (1980) Circ. Res. 47,1-9.
8. Aronson, P.S. (1985) Ann. Rev. Physiol. 47,545-560.
9. Hallcher, L.M., and Sherman, W.R. (1980) J. Biol. Chem. 255,10896-10901.
10. Avissar, S., Schreiber, G., Danon, A., and Belmaker, R.H. (1988) Nature 331,440-442.
11. Kleyman, T.R., and Cragoe, E.J. Jr. (1988) J. Membrane. Biol. 105,1-21.
12. Izzard, A.S., and Heagerty, A.M. (1990) J. Hypertens. 8,389-391.
13. Soltoff, S.P., and Mandel, L.J. (1983) Science 220,957-959.
14. Dubinsky, W.P. Jr., and Frizzell, R.A. (1983) Am. J. Physiol. 245,C157-C159.
15. L'Alleman, G., Franchi, A., Cragoe, E. Jr., Pouyssegur, J. (1984) J. Biol. Chem. 259,4313-4319.