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Effects of Na^+, K^+-Pump Inhibitors on Acetylcholine-Induced Relaxation in the Rabbit Aorta

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The purpose of this study was to investigate the effects of inhibitors of the Na^+, K^+-pump and membrane depolarizing agents on endothelium-dependent acetylcholine-induced relaxation in the rabbit thoracic aorta. Aortic rings were prepared from the rabbit descending thoracic aorta and the contractility of the ring was measured in various conditions such as application of ouabain, exposure to K^+-free Krebs-Henseleit solution and high K^+. Ouabain or exposure to K^+-free Krebs-Henseleit solution inhibited acetylcholine or sodium nitroprusside-induced relaxation. KCl also inhibited the acetylcholine or sodium nitroprusside-induced relaxation. These results suggest that the Na^+, K^+-pump may play a role in endothelium-dependent acetylcholine-induced relaxation.

Key Words : Vascular smooth muscle, endothelium-dependent relaxation, Na^+, K^+-pump

Since Furchgott and Zawadzki (1980) first reported the obligatory role of the endothelium in the regulation of vascular tone, it soon became obvious that endothelial cells control the degree of contraction of the underlying vascular smooth muscle in part, or mainly, by releasing a potent relaxing substances (endothelium-derived relaxing factor, EDRF; endothelium-derived hyperpolarizing factor, EDHF).

Rapoport & Murad (1983b) reported that relaxation induced by the endothelium-dependent vasodilators and the nitrovasodilators may be mediated through the formation of cyclic guanosine monophosphate (cyclic GMP). There fore the vascular smooth muscle relaxation induced by either EDRF or by the nitrovasodilators is associated with increased in intracellular levels of cyclic GMP (Katsuki *et al.* 1977; Ignarro *et al.* 1981; Rapoport *et al.* 1983; Rapoport & Murad, 1983b; Griffith *et al.* 1985), through activation of soluble guanylate cyclase (Rapoport *et al.* 1983; Forstermann *et al.* 1986).

The mechanism of endothelium-dependent vasodilation and nitrocompound-induced vasodilation by cyclic GMP elevation is not clear yet, but it is likely to be by reducing the availability of cytosolic free calcium within the vascular smooth muscle cell, through multiple actions which include inhibition of calcium influx and of intracellular calcium release (Collins *et al.* 1986; Malta *et al.*) 1986; Meisheri *et al.* 1986).

On the other hand, Rapoport *et al.* (1985) reported that inhibitors of the Na^+, K^+-pump inhibited nitrovasodilator-induced relaxation as well as relaxations by 8-bromo cyclic GMP, and elevated cyclic GMP levels due to a high concentration of sodium nitroprusside were decreased by the Na^+, K^+-pump inhibitors. These observations suggest that nitrovasodilator-induced relaxation may be due to the activation of Na^+, K^+-pump by cyclic GMP and the increasing nitrovasodilator concentrations, the formation of cyclic GMP may be dependent upon membrane properties including the activity of the Na^+, K^+-pump (Rapoport *et al.* 1985).

Thus, the present experiments were designed to

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analyse the effects of various Na^+, K^+-pump inhibitors and membrane depolarizing agents on relaxation caused by the endothelium-dependent vasodilators, acetylcholine, in order to investigate the role of the Na^+, K^+-pump in endothelium-dependent relaxation.

MATERIALS AND METHODS

Preparation of aortic ring and tension recording

The preparation of aortic ring was similar to that originally described by Furchgott & Zawadzki (1980). Briefly, adult rabbits weighing 2~3 kg were killed by stunning and exsanguination. The descending thoracic aorta was removed, After excision, the aorta were immersed in Krebs-Henseleit solution [mM: NaCl, 118; KCl, 4.8; $CaCl_2$, 2.5; $MgSO_4$ 1.2; KH_2PO_4; 1.2; $NaHCO_3$, 24; glucose, 11; disodium ethylenediaminetetraacetic acid (EDTA), 0, 03 pH 7.4]. The aorta was then cleaned of adhering fat and connective tissue and cut into rign's 2~3 mm in width. Special care was taken to avoid damage to the endothelial layer for endothelium intact preparation. In some preparations, the endothelium were removed mechanically by gently rubbing the intimal surface with a moist wooden stick for endothelium free preparation. The intagrity of endothelial cells was checked by the method of Furchgott & Zawadzki (1980).

The aortic rings were mounted for recording of isometric tension in a 20 ml organ baths filled with Krebs-Henseleit solution (KH solution) at 37°C. The bath solution was continusly aerated with 95% O and 5% CO_2. The preparations were attached to a force transducer (Grass FT03) and isometric tension was recorded (Model 7 Grass Polygrapy). A resting tension of 2 g was maintained throughout the experiments. Tissues were allowed to equilibrate for 90 min before experimentation.

Each aortic ring was made contracted isometrically with norepinephrine (10^{-7} M). This challenge was repeated to insure that the response was stable. Thereafter, when the contraction reached a plateau at the third or fourth challenge, acetylcholine was added to the bath in a cummulative manner (10^{-8}~10^{-6} M).

Some segments were preincubated with 1 mM ouabain for 15 min or exposed to KH solution without KCl and KH_2PO_4 (K^+-free Krebs-Henseleit solution; K^+-free KH solution).

Drugs

Acetylcholine-hydrochloride, ouabain octahydrate, and l-arterenol bitartrate (norepinephrine) were obtained from Sigma and sodium nitroprusside was obatined by Merck.

RESULTS

Endothelium-dependent relaxation

Recordings of typical patterns of acetylcholine-induced relaxation in aortic rings with and without endothelial cells are shown in Figure 1A and B. With endothelium, acetylcholine relaxed norepinephrine pre-contracted rings in a concentration-dependent manner (Fig. 1A). On the other hand, acetylcholine did not relax norepinephrine pre-contracted rings without endothelium (Fig. 1B).

Effects of Na^+, K^+-pump inhibitors and membrane depolarizing agent on acetylcholine-induced relaxation

Effects of agents and procedure known to inhibit

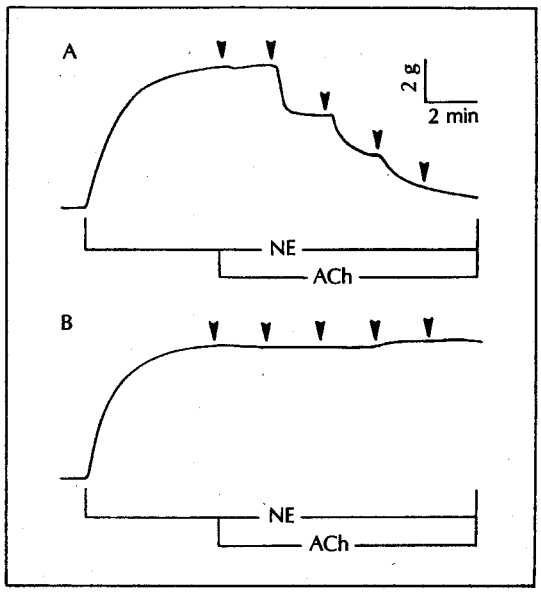

Fig. 1. *Representative tracings of acetylcholine-induced relaxation with (A) and without (B) endothelial cell in norepinephrine (10^{-7} M) pre-contracted rings. Arrows indicate concentration of acetylcholine (10^{-8}, 5×10^{-8}, 10^{-7}, 5×10^{-7}, 10^{-6} M). NE: norepinephrine ACh: acetylcholine*

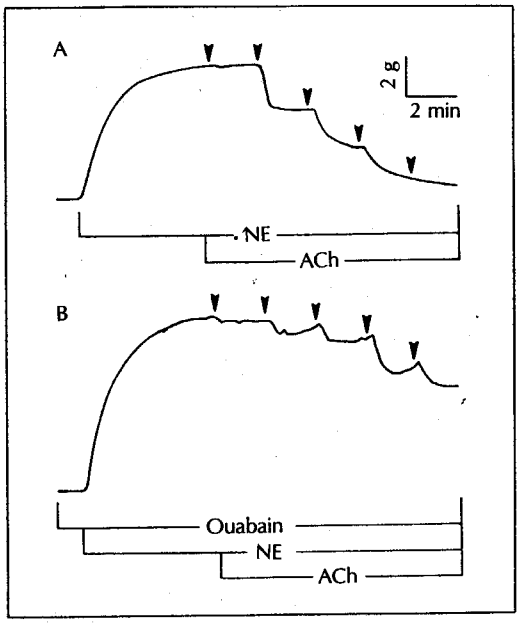

Fig. 2. *Effect of ouabaine on relaxation by acetylcholine in rabbit thoracic aorta with endothelium. Aorta was exposed to 1 mM ouabain for 15 min (B) or remained unexposed (A). Contraction were then elicited by 10^{-7} M norepinephrine followed by cummulative addition of acetylcholine (10^{-8}, 5×10^{-8}, 10^{-7}, 5×10^{-7}, 10^{-6} M). Exposure to ouabain inhibited acetylcholine-induced relaxation. NE: norepinephrine ACh: acetylcholine*

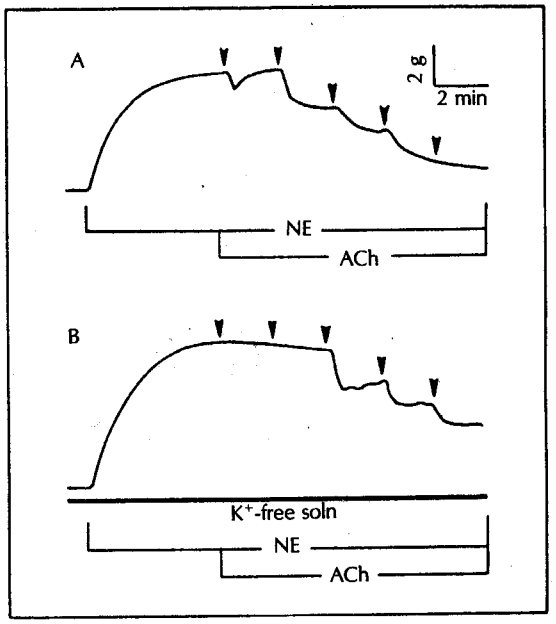

Fig. 3. *Effect of K^+-free Krebs-Henseleit solution on reaxation by acetylcholine in rabbit thoracic aorta with endothelium. Aorta was exposed to K^+-free Krebs-Henseleit solution for 30 min (B) or remained normal Krebs-Henseleit solution (A). After contraction of aortic ring were elicited by 10^{-7} M norepinephrine, cummulative relaxation by acetylcholine were constructed as in Fig. 2. Exposure to K^+-free Krebs-Henseleit solution inhibited acetylcholined relaxation. NE: norepinephrine ACh: acetylcholine*

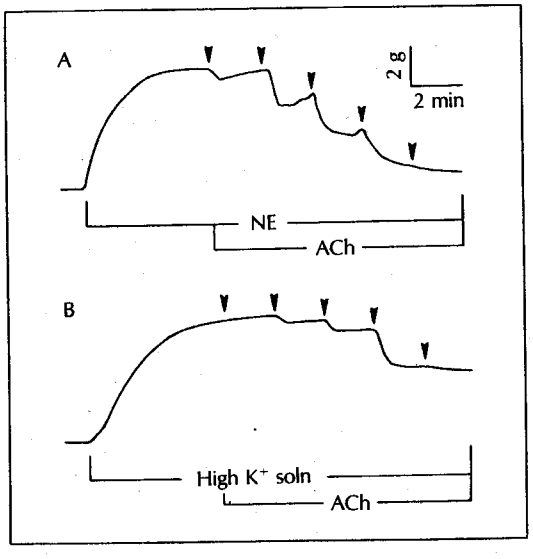

Fig. 4. *Effect of high K^+ Krebs-Henseleit solution on relaxation by acetylcholine in rabbit thoracic aorta with endothelium. Aorta was contracted with 10^{-7} M norepinephrine (A) and high K^+ Krebs-Henseleit solution (40 mM, B), followed by cummulative addition of acetylcholine (10^{-8}, 5×10^{-8}, 10^{-7}, 5×10^{-7}, 10^{-6} M). The relaxant effect of acetylcholine in norepinephrine pre-contracted rings was greater than in high K^+ Krebs-Henseleit solution pre-contracted ones. NE: norepinephrine ACh: acetylcholine*

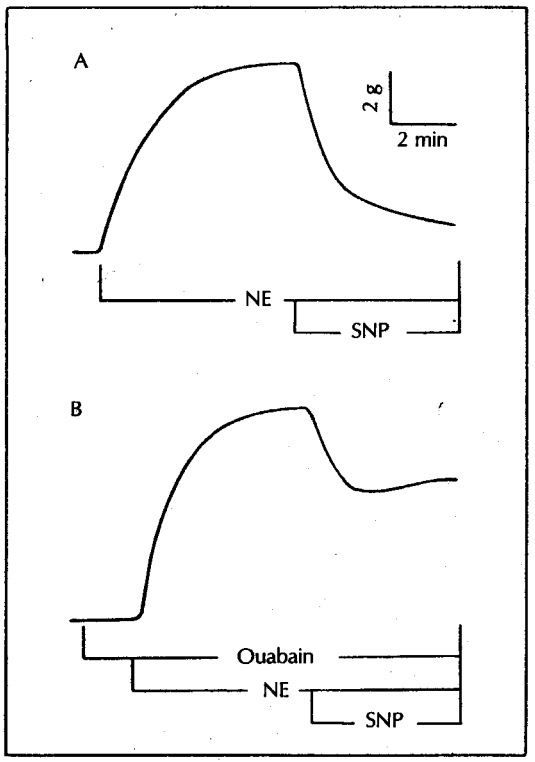

Fig. 5. *Effect of ouabain on relaxation by sodium nitroprusside in rabbit thoracic aorta with endothelium. Aorta was exposed to 1 mM ouabain for 15 min (B) or remained unexposed (A). contraction were then elicited by 10^{-7} M norepinephrine followed by addition of sodium nitroprusside (25 μM). Exposure to ouabain inhibited sodium nitroprusside-induced relaxation. NE: norepinephrine SNP: sodium nitroprusside*

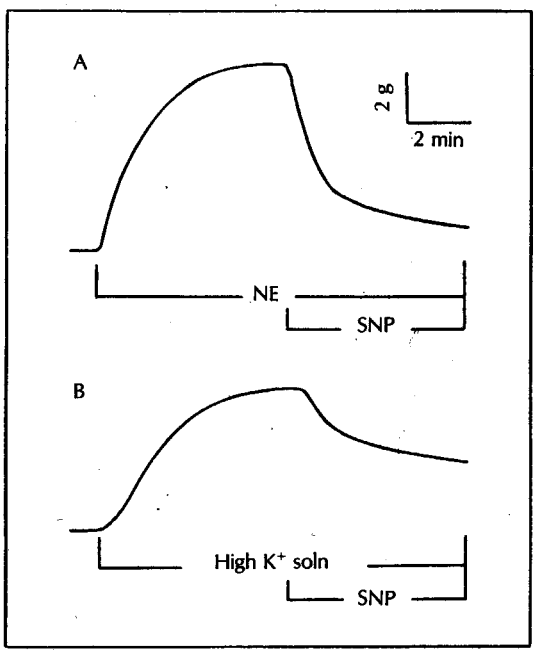

Fig. 6. *The effect of high K^+ Krebs-Henseleit solution on relaxation by sodium nitroprusside in the rabbit thoracic aorta with endothelium. The aorta was contracted with 10^{-7} M norepinephrine (A) or high K^+ Krebs-Henseleit solution (40 mM, B), followed by addition of sodium nitroprusside (25 μM). The relaxant effect of sodium nitroprusside in norepinephrine pre-contracted rings was greater than in high K^+ Krebs-Henseleit solution pre-contracted ones. NE: norepinephrine SNP: sodium nitroprussdie*

the Na^+, K^+-pump on acetylcholine-induced relaxation were shown in Figure 2 and 3, respectively. After control dose-responses to acetylcholine were obtained, aortic rings were rested for 30 min and were then preincubated in 1 mM ouabain for 15 min and K^+-free KH solution for 30 min prior to obtaining a dose-response to acetylcholine. Preincubation to 1 mM ouabain for 15 min (Fig. 2) or exposure to K^+-free KH solution (Fig. 3) all inhibited acetylcholine-induced relaxation of the thoracic aorta.

The effect of membrane depolarizing agent of acetylcholine-induced relaxation were shown in Figure. 4, After acetychloine-induced relaxation in norepinephrine pre-contracted rings wer obatined, aortic rings were rested for 30 min. and then, acetylcholine-induced relaxation in high-K^+ (40 mM KCl) pre-contracted rings was obtained. The endothelium-dependent relaxant effect of acetylcholine in norepinephrine pre-contracted rings was greater than in high-K^+ pre-contracted ones.

Effects of Na^+, K^+-pump inhibitor and membrane depolarizing agents on sodium nitroprusside-induced relaxation

The effects of Na^+, K^+-pump inhibitor and membrane depolarizing agents on sodium nitroprusside-induced relaxation are shown in Figure 5 and 6, respectively. Preincubation to 1 mM ouabain for 15 min inhibited sodium nitroprusside-induced relaxa-

tion (Fig. 5). The relaxant effect of sodium nitroprusside in norepinephrine pre-contracted rings was greater than in high-K^+ pre-contracted ones (Fig. 6).

DISCUSSION

It has been suggested that nitrovasodilators-induced relaxation by elevation of cyclic GMP may be due, in part, to activation of the Na^+, K^+-pump (Rapoport and Murad, 1983c). Rapoport and Murad (1983c) has shown that exposure to ouabain, Mg^{2+}-free or K^+-free Krebs-Ringer bicarbonate solution, which are conditions known to inhibit the Na^+, K^+-pump (Webb and Bohr, 1979; Fleming, 1980), inhibited relaxation by sodium nitroprusside. The present studies indicate that ouabain inhibited sodium nitroprusside-induced relaxation (Fig. 5) and the these conditions also inhibited endothelium-dependent relaxation by acetylcholine (Fig. 2 and 3, respectively). The inhibitory effect of ouabain may suggest that activation of the Na^+, K^+-pump of smooth muscle may play a role in the endothelium-dependent acetylcholine-induced relaxation (De May and Vanhoutte, 1980).

The cause by which the inhibition of relaxation occurs may be the effects of membrane depolarization which presumably follow Na^+, K^+-pump inhibition (Fleming, 1980), since KCl and tetraethylammonium, agents withch induce membrane depolarization (Somlyo and Somlyo, 1968; Bolton, 1979), also inhibited sodium nitroprusside-induced relaxation (Rapoport *et al.* 1985). Others have also shown that relaxations due to the endothelium-dependent vasodilators (De May and Vanhoutte, 1980; Furchgott and Zawadzki, 1980; Chand and Altura, 1981; Furchgott, 1984) and nitrovasodilators (Kreye *et al.* 1975; Verhaehe and Shephard, 1976; Hester *et al.* 1979; Karaki *et al.* 1980; Lincoln, 1983) were inhibited in tissues contracted with KCl. The present studies demonstrated that the relaxant effect of sodium nitroprusside in norepinephrine pre-contracted rings was greater than in high K^+ pre-contracted rings (Fig. 6) and the endothelium-dependent relaxant effect of acetylcholine exhibited the same results.

It has also been suggested that relaxation induced by the nitrovasodilators and endothelium-dependent vasodilators may be mediated through a common mechanism within the smooth muscle via the formation of cyclic GMP (Rapoport *et al.* 1983; Rapoport and Murad, 1983a, b). At a relatively low concentration of sodium nitroprussdie (0.1μM), elevated cyclic GMP levels were only slightly decreased or remained unaffected by Na^+, K^+-pump inhibitors and membrane depolarizing agents (Rapoport and Murad, 1983c; Rapoport *et al.* 1985). Thus, the formation of cycli GMP by the nitrovasodilators is, in some manner, coupled to membrane events due to the Na^+, K^+-pump inhibition and to Na^+-K^+-pump activity itself.

In the present study, an inhibitory effect of this agent and procedures on the ability of acetylcholine to release the endothelium-derived relaxing factors as well as on the smooth muscle cannot be ruled out. The elevated cyclic GMP levels due to the relatively low concentration of acetylcholine, in contrast to the low concerntration of sodium nitroprusside, were significantly reduced by the Na^+-K^+-pump inhibitors and membrane depolarizing agents (Rapoprort and Murad, 1983c; Rapoport *et al.* 1985). Furthermore, these agents and procedures caused greater shifts of the concentration-relzxation curves and decrease in maximum relaxations due to acetylcholine than to sodium nitorprusside (Rapoport and Murad, 1983c; Rapoport *et al.* 1985). The ability of this agent and procedures to inhibit the formation of cyclic GMP does not appear to be due to removal of the endothelium, since prior exposure to tetraethylammonium, KCl or K^+-free Krebs-Ringer bicarbonate solution had no effect on subsequent elevations in cyclic GMP levels due to acetylcholine.

In conclusion, the present study confirms that ouabatin or exposure to K^+-free KH solution, which is agent and procedure known to inhibit the Na^+, K^+-pump, inhibited acetylcholine-induced relaxation. In addition, high K^+, membrane depolarizing agent, also inhibited the acetylcholine-induced relaxation. Therefore, the Na^+, K^+-pump may play a role in endothelium-dependent acetylcholine-induced relaxation.

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