to electrical stimulation was largest at 30°C. As the temperature was lowered further the response to the higher frequencies (4 to 16 Hz) decreased. As compared to the control response (37°C) 2 Hz was depressed at 10°C. At 5°C, no response to electrical impulses could be obtained.

At all temperatures, cooling caused a shift to the left of the dose-response curves to noradrenaline at 5°C the maximal response to the catecholamine was depressed. The response to K^+ decreased progressively as the temperature was lowered, at 5°C only minimal increases in tension could be evoked with K^+.

These experiments suggest that: (1) progressive cooling causes a progressive depression of the contractile process, as evidenced by the inhibition of the K^+-induced responses; (2) the increased affinity of the alpha-adrenoceptors of the vascular smooth muscle cells, evidenced by moderate cooling (Janssens & Vanhoutte, 1978), persists at very low temperatures; (3) at very low temperatures the release of noradrenaline by nerve activation is inhibited since the vessels still respond to exogenous noradrenaline but not to electrical stimulation; and (4) the intracellular depressant effect and the inhibition of noradrenaline release may combine, in the intact organism, to explain 'cold vasodilatation' occurring at very low temperatures.

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Is the direct relaxing effect of acetylcholine on vascular smooth muscle due to activation of Na^+/K^+ ATP-ase?

J.G. DE MEY & P.M. VANHOUTTE

Department of Medicine, Universitaire Instelling Antwerpen, Wilrijk, Belgium

Experiments were performed to investigate the mechanism by which acetylcholine inhibits the contraction of isolated vascular smooth muscle (Vanhoutte, 1974; De Mey, Rusch & Vanhoutte, 1978).

Rings of dog femoral arteries were mounted for isometric tension recording in an organ chamber filled with aerated (95% O_2 – 5% CO_2) Krebs-Ringer bicarbonate solution maintained at 37°C. Prior to the experimentation, the arterial segments were placed at the optimal point of their length-tension relationship using a standard concentration (5×10^{-7}M) of noradrenaline. The same concentration of noradrenaline was used throughout the study to evoke the background of contraction necessary to demonstrate the direct relaxing effect of acetylcholine.

Acetylcholine (10^{-9} to 10^{-6}M) caused concentration dependent relaxations of femoral rings (made to contract with 5×10^{-7}M noradrenaline). Ouabain (2×10^{-6} and 10^{-5}M), cooling (from 37° to 22°C), and high potassium concentrations (from 6.2 to 45 mEq/l) significantly inhibited the relaxations caused by acetylcholine, without significantly depressing the contractile response to noradrenaline. In rings incubated in 1.2 mEq/l K^+ and made to contract with noradrenaline, increasing the K^+ concentration to 5.9 mEq/l caused a transient relaxation (potassium-relaxation), which was inhibited by ouabain (2×10^{-6}M). Acetylcholine (10^{-7}M) significantly decreased the contractile response to noradrenaline in 1.2 mEq/l K^+, but augmented the relative amplitude and the rate of the relaxation induced by reintroducing a higher K^+-concentration.

The Na^+/K^+ ATP-ase in vascular smooth muscle is inhibited by cooling, ouabain and increases in K^+ concentration (e.g. Webb & Bohr, 1978). Potassium-relaxation is due to activation of Na^+/K^+ ATP-ase (Bonaccorsi, Hermsmeyer, Aprigliano, Smith & Bohr, 1977). Thus, the present experiments support the hypothesis that activation of Na^+/K^+ ATP-ase plays an important role in the direct inhibitory effect of acetylcholine on vascular smooth muscle cells.

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