Role of neuronal uptake in the disposition of released [^{3}H]-noradrenaline in the dog's saphenous vein

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The present experiments were designed to obtain more information on the relative importance of neuronal uptake and enzymatic degradation (by monoamine oxidase and catechol-O-methyltransferase) in the disposition of noradrenaline released by nerve impulses.

Helical strips of dogs' saphenous veins were incubated in Krebs-Ringer solution containing [^{3}H]-noradrenaline. They were then mounted for isometric tension recording, and superfusion at constant flow with aerated Krebs-Ringer solution at 37°C (Vanhoutte, Lorenz & Tyce, 1973). The amounts of [^{3}H]-noradrenaline and its major metabolites (3,4-dihydroxyphenylglycol, DOPEG; 3,4-dihydroxymandelic acid, DOMA; normetanephrine, NMN; 3-methoxy-4-hydroxyphenylglycol, MOPEG; and 3-methoxy-4-hydroxymandelic acid, VMA) in the superfusate were determined by column chromatography (Verbeuren, Coen & Vanhoutte, 1977). In certain experiments, the tissue uptake of [^{3}H]-noradrenaline was determined by extraction (Muldoon, Vanhoutte & Tyce, 1978).

Electrical stimulation caused an increase in efflux of [^{3}H]-noradrenaline and its metabolites, except for the VMA fraction which decreased. Cocaine (3×10^{-5}M) given during continuous electrical stimulation significantly augmented all fractions, except for DOPEG which decreased. Desimipramine (DMI; 10^{-6}M) significantly augmented the noradrenaline and VMA fraction, reduced the DOPEG and MOPEG fraction, but did not affect the DOMA and NMN efflux. In strips treated with DMI, cocaine reduced all fractions except VMA which was augmented. In veins treated with phentolamine (3×10^{-5}M), the only significant effect of cocaine and DMI was to augment the overflow of intact [^{3}H]-noradrenaline. At the concentrations used, cocaine, DMI and phentolamine inhibited tissue uptake of [^{3}H]-noradrenaline by 84%, 80% and 34%, respectively.

The present experiments indicate that in the canine saphenous vein: (1) cocaine, besides blocking neuronal uptake, inhibits the evoked release of noradrenaline, and displaces the transmitter and its metabolites from non-neurogenic binding sites; (2) DMI, besides blocking neuronal uptake, has alpha-blocking properties; (3) phentolamine, besides blocking alpha-adrenoceptors, inhibits neuronal uptake; (4) the neuronal uptake process is the rate limiting step in the chain of events leading to intraneuronal deamination of noradrenaline, which leads to the formation of both DOPEG and MOPEG; and (5) blockade of neuronal uptake increases the overflow of [^{3}H]-noradrenaline by less than 50%.

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Effect of profound cooling on adrenergic neuroeffector interaction in the blood vessel wall

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Moderate cooling (from 37° to 20°C) augments the response of cutaneous vessels to sympathetic stimulation and exogenous vasoconstrictor agents (see Vanhoutte, 1979). The present study was performed to investigate the effect of profound cooling (from 37° to 5°C) on the adrenergic neuroeffector interaction in the blood vessel wall. Rings of canine lateral saphenous veins and anterior tibial arteries were mounted for isometric tension recording in an organ chamber filled with Krebs-Ringer bicarbonate solution aerated with 95% O_2 and 5% CO_2. The temperature of the solution could be altered (37, 30, 20, 15, 10 and 5°C) by adjusting the temperature of the water jacket surrounding the organ chamber. Frequency-response curves to electrical stimulation (10V, 2 msec, 0.5 to 16 Hz) or cumulative dose-response curves to either noradrenaline (5×10^{-9} to 10^{-5}M) or potassium ions (K^+) (5.9 to 80 mEq/l) were obtained in different segments of the same vessels, at the different temperatures tested. The responses to K^+ were obtained in solutions containing phentolamine (10^{-6}M), to rule out the evoked release of noradrenaline caused by high concentrations of the ion (Vanhoutte & Verbeuren, 1976).

In unstimulated arteries and veins cooling to 5°C caused a slight decrease in basal tension. The response

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?

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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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