Alpha-adrenergic subsensitivity of isolated femoral arteria following short-term cold acclimatisation in rats[1]

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Summary. Cold acclimatisation lasting 2 and 4 days lowered the response of isolated femoral arteria to noradrenaline and phenylephrine in rat.

In isolated atria of rats, several stress conditions, such as cold exposure[2] and physical excercise[3] as well as repeated injections of ACTH[3], phenylephrine (PHE) or isoprenaline[4], induce subsensitivity to an alpha-adrenergic drug, PHE. So far, there are only a few reports on adrenergic subsensitivity induced in other organs. Kuzmicheva et al.[5] observed subsensitivity of intestinal smooth muscles to noradrenaline (NA) caused by sympathetic hyperinnervation. Contradictory results have been found in the vascular system of animals exposured to cold; an increased adrenergic sensitivity[6,7], decreased sensitivity[8] or no change in sensitivity[9]. On the other hand, subsensitivity to cholinergic agonists was observed in the heart after cold exposure[10] and after repeated treatment with disulfoton[11], and also in ileum[11,12] and in iris tissues[13]. However, there are many examples of supersensitivity induced in various organs after denervation or after drug treatment (e.g. reserpine[14]) or in a hypothyroidism state[15]. According to Fleming et al.[14] the most common types of sensitivity change, postjunctional supersensitivity and postjunctional subsensitivity, are probably opposite expressions of the same phenomenon.

In the present work isolated femoral artieries were used to study whether the adrenergic subsensitivity could be induced by cold exposure in the cardio-vascular system outside the heart.

Material and methods. A total of 95 male Spraque-Dawley rats, 200–300 g in weight, were used. Half of them were arranged in 6 groups and exposed to a cold environment of 4 °C for 1, 2, 4 or 14 days; 2 animals in each cage. The other half served as controls and were kept at 24 °C. The number of animals in each group is shown in the graphs. Using isolated femoral arteries from both legs, the concentration-response curves for the perfusion flow responses to NA and PHE were determined. The animals were killed by decapitation, and after removing the nerves and connective tissues, the femoral arteria dissected above the knee proximally to the pelvic branch (about 1.5 cm long). A blunt 22 gauge injection needle was inserted and tied into the proximal end of the isolated vessel in Tyrode's solution at 24 °C. Both arteries were used at the same time. Tyrode's solution at 35 °C, gassed with 95% O_2–5% CO_2, was allowed to flow through the arteries under pressure of 50 mm Hg. Flow rates were measured by counting the drops using a force transducer attached to a Mingograf 34-recorder. NA and PHE were added in increasing concentration into perfusion solution. To test the results, an additional experimental series was made with a constant perfusion rate (100 ml/h) and the pressure changes were recorded with a pressure transducer (Statham P23DC) on the Grass Polygraph.

Results and discussion. The graphs in figure 1 show that the log concentration-response curve in rats kept in the cold for 1 day did not differ significantly from that of the control animals, but after 2 days of cold exposure the sensitivity of femoral arteries to NA was strongly lowered. The curve was shifted to the right but the EC_{50}-values did not differ in the control and experimental groups because the cold exposure also lowered the maximum response (at –log 4 $p < 0.005$). After 4 days of exposure, the lowering in sensitivity was even greater (at –log 4 $p < 0.001$), and this subsensitivity was also observed when the response was measured from the perfusion pressure (figure 2). However, after prolonged exposure of 14 days, the sensitivity returned to the control level (figure 1 D).

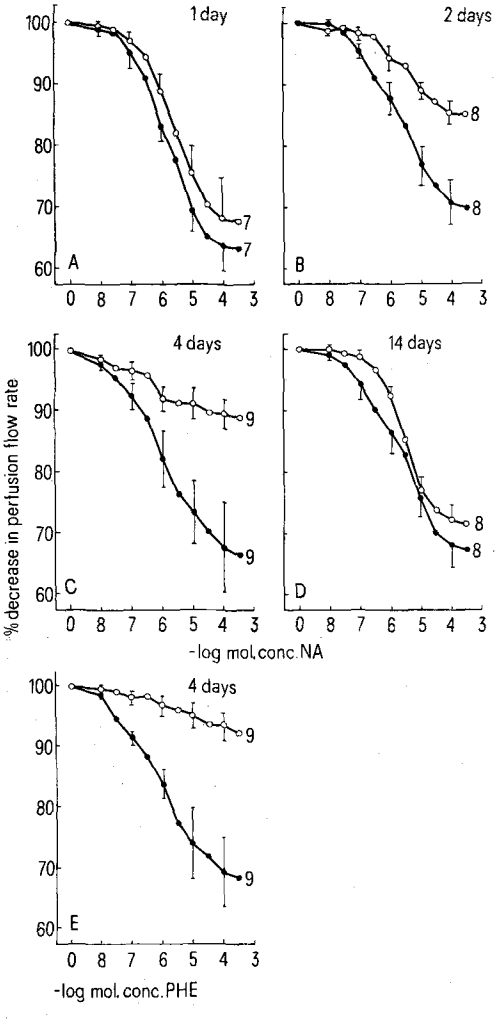

Fig. 1. Log concentration-response curves for the rate of perfusion flow responses to noradrenaline (A, B, C, D) and to phenylephrine (E) in isolated femoral arteries from control rats (●) and from rats exposed to a cold environment for 1, 2, 4 and 14 days (○). 100% varied from 1.75–2.2 ml/mim and did not differ significantly between the control and experimental groups. The number of animals in each group is given at the end of the graphs. Both femoral arteries were used. Vertical bars indicate ± SE.

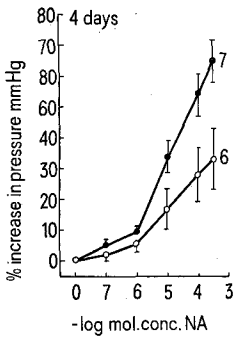

Fig. 2. Log concentration-response curves for the perfusion pressure response to noradrenaline in isolated femoral arteries from control rats (●) and from rats exposured to cold environment for 4 days (○). Initial pressure is 30.7 mm Hg for the cold exposed group and 29.7 mm Hg for the control group. Further explanation as in figure 1.

A comparison between the figure 1 C and E shows that the subsensitivity to NA induced in rats by the cold environment was exactly as great as response ro PHE, thus indicating lowered alpha-adrenergic response in femoral arteries. This result is similar to that found in isolated atria from cold-exposed rats, where the temporary alpha-adrenergic subsensitivity was developed in the cold within 4 days[2]. This induction time is also in agreement with the results concerning supersensitivity. The nonspecific postjunctional supersensitivity to NA was shown to develop in the dog heart in 1–3 days of treatment with reserpine[13,14]. In rabbit aortic strip, significant supersensitivity was found 24 h after a single dose of reserpine and was maximal after 3 daily doses[16]. Contradiction among the previous results[6–9] on vascular sensitivity induced in cold are probably due to the differences of acclimatisation time used. This type of adaptive change seems to be temporary, becoming compensated later by other factors.

Earlier[2–4] we had assumed that the enhanced adrenergic activity in the rat could be a reason for the subsensitivity in the heart. The present results indicate that this inductive effect is not only limited to the heart but is also present in the vascular system and perhaps in all adrenergically innervated effectors.

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The action of ouabain in promoting the release of catecholamines

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Summary. It is suggested that ouabain promotes catecholamine release by causing a rise in intracellular Na^+ which, in turn, causes an elevated steady-state level of intracellular Ca^{2+}. It is suggested that the Na^+-K^+-ATPase is not directly involved in exocytosis at either adrenergic or cholinergic synapses.

Katsuragi and Suzuki[1] in a recent paper in Experientia have shown clearly that ouabain (the inhibitor of the Na^+-K^+-ATPase) at a concentration of 10^{-5}M is effective in releasing extraneuronal catecholamine in the guinea-pig vas deferens. This study complements their earlier findings[2] in which they suggested that ouabain was also able to facilitate catecholamine release from the neuronal site. Ouabain has been known to promote catecholamine release since the earlier studies of Banks[3] on spontaneous release from bovine adrenal gland. Katsuragi and Suzuki have suggested that the Na^+-K^+-ATPase is essential for storage at both extraneuronal[1] and neuronal sites[2], catecholamine release taking place when the ATPase is inhibited by ouabain. This hypothesis is similar to that proposed by Garcia and Kirpekar[4,5] who have shown that ouabain causes a dose-dependent release of noradrenaline from cat spleen slices and suggest[4] that the Na^+-K^+-ATPase serves to maintain the integrity of the axonal membrane; procedures that depress enzyme activity (e.g. intracellular accumulation of Ca^{2+}, Garcia et al.[5]) would cause transmitter release by producing a temporary disturbance in the membrane.

Comparable suggestions concerning the involvement of the Na^+-K^+-ATPase in exocytosis have been advanced for the release of transmitters from synaptosomes[6] and of acetylcholine in the myenteric plexus of the longitudinal

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