turne de la quantité filtrée qui accroit le flux transglomérulaire des substances dissoutes et explique, en partie du moins, la nette augmentation de l'excrétion urinaire par unité de temps de l'eau et des solutés. Ces variations circadiennes de la filtration glomérulaire sont à rapprocher de celles de l'excrétion urinaire de l'eau, des électrolytes et des protéines[2,10,11]. L'injection d'une substance vasodilatatrice, comme la théophylline, qui entraîne une augmentation de la filtration glomérulaire, s'accompagne également d'un accroissement de l'excrétion de l'eau, des électrolytes et des solutés[12]. Toutefois, l'exactitude de ces conceptions ne pourra être définitivement assurée que par des expériences de microponction capsulaire et tubulaire, qui montreront les relations existantes entre les variations circadiennes de la composition de l'ultrafiltrat et celles de l'urine définitive.

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Acute stress reduces the sensitivity of the vasculature to sympathetic control[1]

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Summary. Vascular smooth muscle from rabbits subjected to acute severe stress exhibits decreased sensitivity to sympathetic regulation. Stimulation of the sympathetic innervation of isolated vascular segments resulted in a similar subsensitivity as did exposure to norepinephrine (NE) but not histamine. Periodic contraction of these segments caused an increase in their maximum ability to contract independent of the constrictor procedure used. These results suggest that the increase in sympathetically mediated NE release that occurs in stress and some other pathological conditions may result in a blunting of neural control and possibly resistance to certain therapeutic agents.

One of the immediate physiological responses to stress is an increased activity of the sympathetic nervous system[2]. Increased levels of sympathetic tone revealed by elevated levels of circulating norepinephrine have been shown to occur in animals after short-term immobilization, handling, exercise and cold exposure[3]. Increased efferent sympathetic activity also occurs in acute heart failure[4], asthma[5], and shock[6]. We present evidence in this paper that acute stress results in subsensitivity of the vasculature to sympathetic control, in part at any rate, due to an increase in sympathetic neural discharge. This may be a phenomenon of some medical import, related to the decreased reactivity and resistance to certain therapeutic sympathetic agents observed in heart failure[7], asthma[8] and shock[9].

The level of sympathetic efferent discharge immediately prior to death varies with the mode of sacrifice. We hypothesized that the consequences of such differences may be reflected in the sensitivity to sympathetic activation of blood vessels subsequently removed and studied in vitro. To test this idea, blood vessels were removed from rabbits sacrificed by exsanguination, when there is a large increase[10], or by injection of pentobarbital, when there is a decrease in sympathetic tone[11].

In order to investigate whether the level of sympathetic activity, per se, could be responsible for alteration in sensitivity of the vascular smooth muscle - the influence of sympathetic nerve stimulation in vitro on the subsequent response of ear arteries to NE was examined. The possibility that NE was responsible for any of the observed sensitivity change was investigated by determining the influence of NE exposure in vitro on subsequent NE responsiveness.

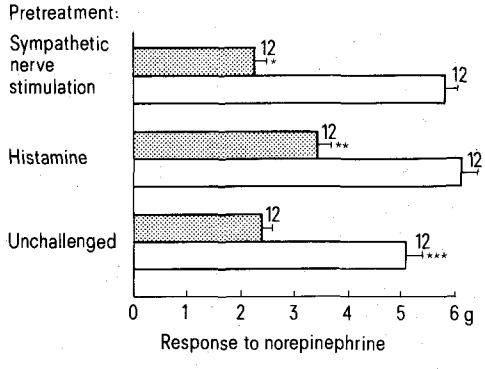
Materials and methods. Rabbits were sacrificed either by stunning followed by rapid exsanguination or by i.p. injection of anesthetic or lethal doses of pentobarbital (approximately 50 and 150 mg/kg, respectively). 4 mm ear artery ring segments were removed rapidly and mounted in tissue baths at 1715 dynes resting tension, which is optimal for both groups of arteries. The method has been previously described[12]. From cumulative concentration-response curves determined in the presence of desmethylimipramine (DMI 10^{-7} M), the NE EC_{50s} were calculated. In order to investigate the influence of nerve stimulation on vascular sensitivity, nerve terminals in the wall of isolated ear artery segments from exsanguinated animals were selectively field stimulated for 8 periods of 2 min each over a period of 2.5 h (stimulus pulse duration 0.3 msec, frequency 16 Hz, supramaximal voltage). The size and duration of the contractile responses to transmural nerve stimulation (TNS) were matched in paired segments by contractions elicited by periodic additions of histamine (approximately 2×10^{-6} M). Additional control segments were allowed to remain unchallenged throughout the 2.5 h pretreatment period.

In order to investigate the effect of prior NE exposure on subsequent NE sensitivity, segments were exposed to NE (10^{-6} M) for 8 periods of 5 min each over a period of 2.5 h. As described above, in paired segments the NE responses were matched with histamine (approximately 5×10^{-6} M). In a separate series of experiments, the NE sensitivities of segments pretreated with NE were compared to those of unchallenged controls.

All vessels were washed repeatedly. Responses to NE in the presence of DMI (10^{-7} M), and desoxycorticosterone

(DOC, 4×10^{-5} M) and propranolol (3×10^{-7} M) were determined simultaneously 30 min following the last period of TNS, exposure to histamine or NE. Paired t-test was used to determine significance of differences between groups.

Results and discussion. NE concentrations that caused a contraction which was 50% of the maximum response (EC_{50}) of ear arteries from stressed and unstressed animals were 6.1×10^{-8} M (3.6-10.3) and 4.1×10^{-8} M (2.1-8.3), respectively, (geometric mean, 95% confidence interval; n = 7 in each case). Thus, vessels from stressed animals were 49% less sensitive to NE than those from controls ($p < 0.05$ paired t-test). Maximum responses to NE were identical in both instances. This significant diminution in sensitivity of response lasted for at least 6 h. The NE sensitivity and maximal response of arteries removed from exsanguinated animals and incubated for 90 min in pentobarbital (10^{-4} M) could not be distinguished from those of control, untreated vessels. Thus, pentobarbital itself was not responsible for the observed change in NE sensitivity. Nerve stimulation in vitro resulted in blood vessels less sensitive to NE than the histamine pretreated controls. However, nerve stimulated tissues responded to a NE test dose the same as unchallenged control tissues. It is of interest to note that the maximum responses of unchallenged control tissues were significantly less than those of TNS vessels as well as those exposed to histamine (figure). Since tetrodotoxin (3×10^{-7} g/ml) completely blocked the response to TNS and abolished any differences between groups, such field stimulation did not exert a direct effect on vascular smooth muscle. Incubation in NE in vitro resulted in vessels less sensitive to NE than those pretreated with histamine [1.8×10^{-7} M (1.6-2.0) and 1.3×10^{-7} M (1.1-1.6), respectively, (geometric mean EC_{50}, 95% confidence interval; N = 5 in each case; $p < 0.05$)]. The maximum responses were identical. However, NE EC_{50s} of tissues incubated with NE were not significantly different from those of unchallenged (rested) controls. However, the maximum responses of NE incubated tissues were significantly greater than these unchallenged controls [6.70 g ± 0.43 and 5.72 g ± 0.28, respectively, (mean ± SE; N = 12 in each case; $p < 0.05$)].



Mean responses of isolated rabbit ear arteries to NE (4.6×10^{-8} M; stippled bar) or maximal NE concentration (open bar) in the presence of desmethylimipramine (10^{-7} M) desoxycorticosterone (4×10^{-5} M) and propranolol (3×10^{-7} M) after previous periods of sympathetic nerve activity or exposure to equieffective concentrations of histamine (approximately 2×10^{-6} M) are shown in comparison to rested controls. Numbers in parentheses represent number of vessels tested. Responses to the NE test dose were expressed as a percentage of the maximal NE response when statistical significance was determined. *Sympathetic nerve stimulated tissues were significantly less sensitive to NE than the histamine controls ($p < 0.005$). **Histamine controls were significantly more sensitive than unchallenged controls ($p < 0.005$). ***Maximum responses of unchallenged controls were significantly less than those of sympathetic nerve stimulated tissues and histamine controls ($p < 0.01$).

These results suggest that increased sympathetic nerve activity in vivo results in a subsensitivity of vascular smooth muscle to NE. This is supported by the observations that TNS or NE activation in vitro induced subsensitivity of the ear artery to NE in comparison to the case when contractions were induced by histamine. This change must reflect an alteration in the reaction of NE with the alpha-adrenoreceptor or on one or more of the subsequent series of initiated cellular events. The observation that the vascular smooth muscle of the vessels previously contracted by histamine were more sensitive to NE than the rested controls (in spite of the concomitant increase in maximum response of the histamine pretreated tissues) suggests that a periodic increase in isometric tone, per se, leads to a supersensitivity of the alpha-adrenoceptor mediated response of vascular smooth muscle. Vascular smooth muscle of vessels previously contracted by KCl or serotonin showed increases in NE sensitivity (as well as maximum responses) similar to those observed when contracted by histamine (R. Rapoport and J.A. Bevan, unpublished observation). Thus, the subsensitivity to NE seen after sympathetic activity develops despite the opposite consequences of an increase with isometric tone development.

The observation that the vascular smooth muscle of vessels previously contracted by TNS, histamine or NE achieved increased maximum responses is consistent with the increased maximum responses observed in the pulmonary artery following exposure to high concentrations of various agonists[13]. Furthermore, although the maximum responses were increased, the lower portions of the concentration-response curves (though not the EC_{50s}) were shifted to the right for that agonist which produced the contraction[13]. This is again consistent with our observations that TNS and NE increased the maxima and decreased the sensitivities to NE. Subsensitivity of the pineal gland and iris sphincter have been shown after acute changes in the level of nerve traffic in vivo[14]. The aorta and vas deferens have been shown to develop subsensitivity to NE after brief exposure to NE in vitro[15]. This report, however, to our knowledge is the first demonstration that nerve stimulation in vitro can lead to vascular subsensitivity.

Vascular smooth muscle of animals sacrificed by stunning followed by rapid exsanguination exhibited a decreased sensitivity to sympathetic regulation. A similar change was observed when the increased sympathetic activity associated with stress of this type was mimicked in vitro by nerve stimulation or NE. In contrast, a periodic increase in muscle tension resulting from nonsympathetic causes, increased vascular sensitivity and maximum responses to its normal neurotransmitter. This may be one mechanism by which vasoactive circulating substances are able to modify vascular responses to neurogenically released and/or circulating catecholamine. It raises the possibility that the greater levels of sympathetic activity resulting from stress whether exogenous or endogenous of even comparatively short duration can depress normal homeostatic regulation of the vasculature. This may limit the magnitude of sympathomimetic vasoconstriction during prolonged stress. This consequence may be disadvantageous in certain diseases in that it may possibly result in resistance to drugs used in their therapeutic control.

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Selective destruction of intestinal nervous elements by local application of benzalkonium solution in the rat

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Summary. Intestinal aganglionosis produced by serosal application of 0.1% benzalkonium solution to the colon of the rat was studied electronmicroscopically, and it was concluded that a higher susceptibility to the agent and a lower recovering ability of the nerve elements might be responsible for the phenomenon.

It has previously been reported by us that local serosal application of 0.1% benzalkonium chloride (BC) normal saline solution to the colon of the rat for 30 min produces selective destruction of intestinal nervous elements, and that the aganglionic colonic segment, produced by this method, is histologically and physiologically completely denervated, whereas the smooth muscles per se remain normal, morphologically and functionally[1]. Benzalkonium chloride or Osvan, a product of Takeda Pharmaceutical Co., Osaka, is dimethylalkylbenzylammonium chloride, or $[C_6H_5CH_2N(CH_3)_2R]Cl$, in which R ranges from C_8H_{17} to $C_{18}H_{37}$, about 60% being $C_{12}H_{25}$, about 35% being $C_{14}H_{29}$, less than 1% being $C_{10}H_{21}$ and less than 1% being $C_{16}H_{33}$. In this report, electron microscopic observation was performed to obtain sequential ultrastructural findings following local application of the solution, and to investigate the mechanism of action of the solution on the intestinal structures.

Materials and methods. 27 adult Wistar rats, weighing about 200 g, were used. Under nembutal anesthesia, the rat was laparotomized, and a gauze stick, which was 1.5–2 cm wide and had been soaked in 0.1% BC solution, was rolled around a segment of the descending colon, and was maintained for 30 min, followed thereafter by flushing with copious saline solution and abdominal closure. The animals were sacrificed at intervals ranging from 1 week to 14 months after the procedure and the treated intestinal segment was observed by light and electron microscopy. Specimens for light microscopy were stained by hematoxylin and eosin, Nissl, and Bodian stains. Specimens for electron microscopy were pre-fixed with 1%-glutaraldehyde-4%-paraformaldehyde, post-fixed with 1% osmium tetraoxide, dehydrated through graded acetones, embedded in Epon 812, cut into ultrathin sections with Porter-Blum M-1 ultramicrotome, doubly stained with uranyl acetate and lead acetate, and observed with JEM-100U electron microscope.

Results. Light microscopically the intestinal segment, 1–4 week(s) after the BC treatment, showed more or less manifest findings of inflammation mainly adjacent to the serosa at the early stage. Changes in nervous elements were not remarkable at the early stage, but at 4 weeks after the procedure complete disappearance of intestinal nervous elements, either sparing or not sparing Schwann cells, was observed (figure 1). Smooth muscle cells showed no marked change except for some partial reduction in stainability of outer layer muscles. More than 4 weeks after the procedure, there was no inflammatory change nor smooth muscle abnormality, and the intestinal nervous elements, including nervous networks in the intestinal wall, were found to have disappeared completely.

Electron microscopic findings were as follows. At 1 to 2 week(s) after the BC treatment, inflammatory changes, such as granulocytic infiltration, were observed. At this stage intermuscular nerve plexi showed either such changes as constriction of ganglia as a whole, increase in cytoplasmic electron density of nerve cells and swelling of mitochondria, or, at places, only minor changes. Smooth muscle cells generally tended to show contraction, and diffuse reduction in cytoplasmic electron density, decrease in amount of myofilament and formation of intra- and extracellular vacuoles were observed at places (figure 2, a). At 3 to 6 weeks after the BC treatment, inflammatory changes were found to have subsided, and there was an increase in collagen fibrils in the widened intermuscular spaces and intramuscular intercellular spaces, where nerve cells and nerve fibres had disappeared or degenerated. From 7th week after the BC treatment, degeneration and disappearance of nervous elements continued, while smooth muscle cells became normal, although markedly contracting (figure 2, b). In the widened intermuscular and intercellular spaces, an increase in collagen fibrils was observed. At places Schwann units containing vacuoles, presumably corresponding to degenerative axons, were observed.