We attempted to demonstrate that anoxic HPa lines require HCO_3^- (figure 2). Whatever the initial pressure of carbon dioxide in the nitrogen atmosphere (0–23 torr), the final acidosis of the culture medium was nearly the same, the proton concentration becoming stabilized at around 180 $neq \cdot l^{-1}$ (pH about 6.75). The release of end products reached maximal values for carbon dioxide pressures ranging between 0.2 and 1 torr. The cells failed to synthesize malate in the absence of HCO_3^- in the culture and they did not survive. Malate plays the rôle lactate does in vertebrates, but unlike lactate, it is not accumulated[8]. It easily enters the mitochondrion and there supports anaerobic production of ATP during its conversion to succinate. The generation of $NADH^+$ requires the linked dehydrogenation of the ketoacids produced by transamination. Mitochondrial particles isolated from parasitic worms catalyze an electron transport of this type[11]. Flavine protein and coenzyme Q are necessary, but it is not yet established whether a cytochrome b is involved. Cockroach hemocytes appeared to be very sensitive to rotenone and antimycine (figure 1), known to act as stoichiometric inhibitors of flavoproteins and cytochrome b respectively.

Nutritional requirements were easily characterized using anoxic cultures in chemically defined media. HPa cells need riboflavin and phenylalanine, both precursors of flavoproteins and coenzyme Q. Vitamin B12 is not required by the hemocytes under normoxia or hypoxia when nucleotide precursors are supplied, but becomes essential under anoxia. Cyanocobalamin-deficient cells begin to degenerate during the second week of culture: they fail to synthesize propionate, but release more acetate (table).

Thus, the biosynthetic pathways leading to propionate appear to be essential for cell survival. Among the by-products of anoxic metabolism, the less abundant, malate and propionate, are perhaps the most significant for the anaerobic production of energy.

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Presynaptic sympathetic supersensitivity following long-term preganglionic denervation

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Summary. After chronic preganglionic denervation of the rabbit ear artery, the contractile response to low frequency stimulation of the postganglionic innervation is increased. A preliminary analysis suggests that this cannot be accounted for by effector cell changes, but is due to an increase in adrenergic transmitter release.

Denervation supersensitivity of excitable tissues is a well documented occurrence[2,3]. We report here that long-term preganglionic denervation (decentralization) of adrenergic neurons results in increased responsiveness of the rabbit ear artery to electrical stimulation of sympathetic nerve terminals and to tyramine, an effect not accounted for by vascular smooth muscle hypersensitivity.

Unilateral preganglionic axotomy, by removal of about 3 cm of preganglionic nerve proximal to the superior cervical ganglion, under sterile conditions, was performed in young adult New Zealand white rabbits of 2.0–2.3 kg b.wt. 8 weeks after surgery the animals were sacrificed by a blow on the head and exsanguinated. Ring segments 3 mm long, dissected in situ from both the decentralized and control ear arteries were mounted in organ baths at 37.5 °C. Rings were suspended from stainless steel wires held in place by machined Lucite holders. One of the Lucite holders was stationary, the other was connected to a Statham G10b force-displacement transducer, which was coupled to a Grass polygraph chart recorder (model 79D) for displaying changes in vessel tension[4]. After equilibration for 1 h, the nerve terminals in the blood vessel wall were stimulated using biphasic pulses of 0.2 msec duration, at a supramaximal voltage from an electronic stimulator (Grass model S4). This mode of electrical stimulation, transmural nerve stimulation (TNS), elicited a response due solely to the release of norepinephrine (NE) from sympathetic nerve terminals[5], since it was abolished by tetrodotoxin (9×10^{-7} M).

The density and distribution of adrenergic nerve terminals, demonstrated by specific catecholamine fluorescence in the decentralized and control ear arteries, were similar. This contrasts with the disappearance of nerve terminals after postganglionic sympathetic denervation[6]. TNS caused frequency-dependent increases in contraction of the artery wall (figures 1 and 2). As seen in figure 1, a, the decentralized artery showed a contractile response to stimulation at 0.1 Hz. The phasic contractions seen in the figure correspond to each stimulus. In contrast, the threshold frequency for the control artery was 0.4 Hz (figure 1, b). The frequency-mean response curves of the decentralized and control ear arteries are shown in figure 2. At frequencies of up to 1.6 Hz, the contractile force of the decentralized artery was significantly greater than that of the control when compared using the paired t-test ($p < 0.05$). When the cells in a control artery were exposed to K^+ (10 mM), a twitch-like contraction was observed in response to TNS at 0.1 Hz in 2 out of 5 animals (figure 1, b). The concentration-response curve of the decentralized ear artery to tyramine, which is known to act by releasing catecholamines from sympathetic nerve terminals, was displaced to the left of the control artery curve. At the EC_{50} (concentration to elicit a response

one half of maximum), the shift was approximately times 8.4.

According to Girling[7], who made a quantitative study in situ of the effect of TNS on the rabbit ear artery, constrictor responses are frequency-dependent and the range of effective frequency is 0.5–25 Hz. However, Folkow[8] concluded that most physiological adjustments were made in the lower range of frequencies. Our findings show that the greatest differences between the contractile responses of the decentralized and control arteries occurred at frequencies of nerve stimulation from 0.1–1.6 Hz. The reason for this frequency dependence is not clear.

A shift to the left of the norepinephrine (NE) concentration-response curve of decentralized arteries compared with their innervated controls has been shown in other dissues[2,3]. However, the displacement following decentralization is less than after denervation[11]. Decentralized car arteries were 2.0–3.0-fold more sensitive than controls when compared at the NE EC_{50} level[9].

The mean contractile forces of the decentralized and control arteries induced by approximately threshold concentration NE (3×10^{-9} M) were 0.16 ± 0.04 g (SEM, n=6; this is 7.9% of the NE-induced maximum response) and 0.12 ± 0.08 g (n=6; 4.9% of the maximum response), respectively. There was no significant difference between these values[10] (figure 3). The maximum force development produced by high concentration of NE (10^{-4}M) was approximately 20% less in the decentralized vessel compared with the control (figure 3).

It has been reported that decentralization is associated with increases in transmitter release in cat spleen[12] and rat iris[13]. The phenomenon of increased presynaptic excitability to TNS may be due to subsensitivity of presynaptic α-receptors to NE. The superior cervical ganglion has been shown to be less sensitive to cholinomimetic agents and inorganic ions after denervation[14,15]. This would result in a net increase in the amount of NE released per nerve impulse. It has been shown that chronic interruption of innervation leads to a partial depolarization in some smooth and cardiac muscles[16,17]. This may be the case in the preganglionically denervated sympathetic neuron, leading to an increased release of transmitter per pulse.

In conclusion, increased responsiveness to electrical stimulation and tyramine has been found after chronic preganglionic denervation of the rabbit ear artery. This cannot be accounted for by postsynaptic effector cell changes. The presynaptic mechanism underlying the increased sensitivity remains to be elucidated.

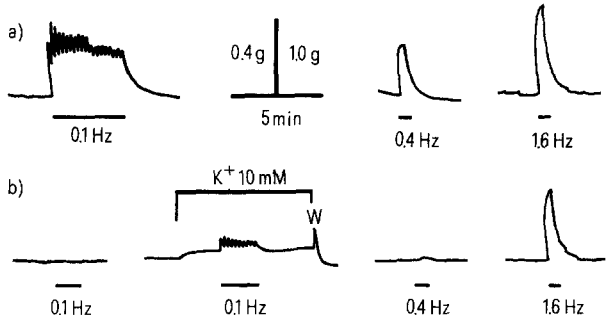

Fig. 1. Typical responses of segment of rabbit ear arteries to transmural electrical nerve stimulation (0.2 msec duration, supramaximal voltage, biphasic pulses) from decentralized ear artery (a) and innervated control artery (b). A pair of decentralized and control arterial segments were examined 8 weeks after unilateral preganglionic axotomy.

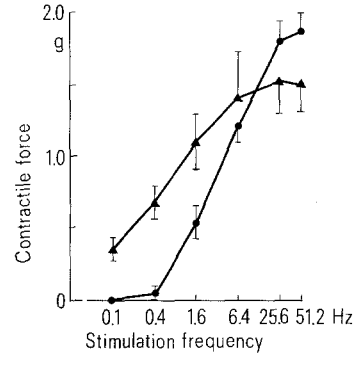

Fig. 2. Frequency-mean response curves of decentralized (▲) and control (●) ear artery segments to transmural nerve stimulation obtained from rabbits 8 weeks after unilateral preganglionic axotomy. Mean values of 6 observations and standard errors are shown.

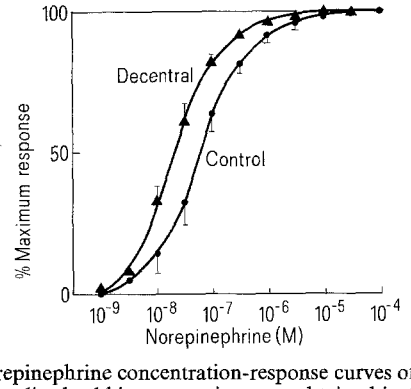

Fig. 3. Norepinephrine concentration-response curves of the control and decentralized rabbit ear arteries were obtained in the presence of propranolol 3×10^{-7} M, desmethylimipramine 3×10^{-7} M and hydrocortisone 8.6×10^{-6} M in order to eliminate β-adrenoceptors, neuronal and extraneuronal uptakes. Abscissa: norepinephrine concentration in the tissue bath. Ordinate: percentile response of the maximum contraction; each point represents a mean value of 8 experiments and vertical bars show the SEM.

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