Release of Sympathetic Neurotransmitter Evoked by Electrical Stimulation Is Increased in the Chronically Decentralized Artery

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ABSTRACT—The present study was performed to confirm our previous proposal that the increase in neurotransmitter release is responsible for the supersensitivity of chronically decentralized artery to transmural nerve stimulation (TNS) in the rabbit. The ear artery was decentralized unilaterally by removing the preganglionic fiber proximal to the superior cervical ganglion (SCG), and the ear arteries and SCG were dissected 8 weeks after the operation. The increase in the tritium overflow induced by TNS from the chronically decentralized artery, which had been incubated with ³H-noradrenaline (NA) for 1 hr, was markedly increased at lower frequencies (0.1 and 0.2 Hz) than that from the control artery, whereas there was no difference at higher frequencies (>0.5 Hz). No difference was observed in the neuronal uptake of ³H-NA during incubation for 1 hr between the control and decentralized arteries. There was also no change in the contents of catecholamines in both the artery and SCG after chronic decentralization, when assayed by a radioenzymatic procedure. In conclusion, the results obtained indicate that the supersensitivity to TNS after chronic decentralization is not due to the deranged catecholamine uptake and storage mechanisms in adrenergic nerve terminals and augmented transmitter biosynthesis, but due to the increased release of transmitter in response to low frequencies of TNS.

Keywords: Chronic decentralization, Prejunctional supersensitivity, Ear artery (rabbit), Sympathetic neurotransmission, Transmural nerve stimulation

Postjunctional supersensitivity following interruption of impulse traffic in various smooth muscles has been well documented (see reviews, Refs. 1 and 2). We have previously demonstrated that the degrees of postjunctional supersensitivities to potassium (K⁺) and noradrenaline (NA) after denervation and decentralization are similar in the rabbit ear artery (3, 4). Denervation was performed by removing the superior cervical ganglion (SCG), and decentralization was performed by removing the preganglionic sympathetic fiber to the SCG. After chronic denervation for 8 weeks, catecholamine fluorescence disappeared from the adventitial-medial junction of the ear artery (5), but in contrast, after chronic decentralization for 8 weeks, there was no change in catecholamine fluorescence, suggesting that the postganglionic sympathetic nerves survived in the artery (3). Reflecting these differences in the presence or absence of postganglionic nerve terminals in the artery between after denervation and decentralization, the responses of the denervated and decentralized arteries to electrical stimulation (transmural nerve stimulation, TNS) were quite different. Namely, whereas the denervated artery lost the ability to respond to the stimulation, the decentralized artery exhibited higher sensitivity to TNS than the control. This supersensitivity to TNS has a different time course from that of the postjunctional supersensitivities to NA and K⁺. Therefore, we assumed that after chronic decentralization, prejunctional sympathetic supersensitivity might develop more slowly than the postjunctional one (6).

Little is known about the prejunctional changes of the postganglionic neuron after decentralization, i.e., preganglionic denervation. Possible mechanisms involved in the supersensitivity to electrical stimulation after decentralization are: (i) increase in the neurotransmitter release, (ii) increase in transmitter content in nerve terminals, (iii) decrease in the destruction of transmitter, and (iv) postjunctional supersensitivity to transmitters. The last mechanism is indeed involved in the phenomenon and has been studied previously (6). Thus, the present study was undertaken (a) to confirm the increase in transmitter release, using ³H-NA as an indicator, from the chronically decentralized artery evoked by TNS, (b) to measure
whether there is a change in catecholamine contents in the chronically decentralized artery and denervated SCG, and (c) to evaluate whether there is a change in neuronal uptake, which is thought to be a main route of destruction or dissipation of the transmitter, after chronic decentralization for 8 weeks in the rabbit ear artery.

The results obtained support the notion that neurotransmitter release in responses to low frequencies of TNS is increased in the chronically decentralized artery, i.e., chronically denervated nerve endings become supersensitive to TNS.

MATERIALS AND METHODS

This study was approved by the Animal Welfare Committee of Hiroshima University School of Medicine.

Decentralization and denervation of ear artery
The central artery of the rabbit ear was decentralized or denervated as described previously (5, 6). Briefly, male White Japanese rabbits, weighing 2.0–2.3 kg, were anesthetized, following sedation with chlorpromazine (10 mg/kg, i.m.), by giving an intraperitoneal injection of a mixture containing per 100 ml: 4.25 g chloral hydrate, 0.97 g pentobarbital sodium, 2.13 g magnesium sulfate, 42.8% propylene glycol and 11.5% alcohol. The dose used was about 2 ml/kg (5). The central artery of the ear was either decentralized or denervated unilaterally by dissecting the preganglionic fiber 2–3 cm in length up to the SCG or by removing the SCG together with appropriate lengths of the pre- and postganglionic fibers, respectively. In both operations the contralateral side was left intact and served as the control. The operation was performed under sterile conditions.

Tissue isolation and preparation
Eight weeks after the operation, the animal was killed by a blow on the head and rapid exsanguination. The control and either decentralized or denervated ear arteries as well as the control and denervated SCG were isolated and immediately placed in ice-cold Krebs' bicarbonate solution which had been previously bubbled with a gas mixture of 95% O₂ + 5% CO₂. The composition of the modified Krebs' bicarbonate solution was: 119.0 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25.0 mM NaHCO₃ and 11.1 mM glucose. Ascorbic acid (0.57 mM) and disodium ethylenediamine tetraacetate (EDTA) (0.03 mM) were also included in the solution throughout the experiment to inhibit catecholamine oxidation. The isolated tissues were prepared for the following studies.

Measurement of ³H-NA overflow
The method of measuring ³H-NA overflow from the arterial preparation was a modification of the original method of Su and Bevan (7). Briefly, the arterial segments were cleaned under a dissecting microscope and cylindrical preparations, 3 cm in length, were made. Two short pieces of polyethylene tubing were inserted into both ends of the arterial preparation, one being anchored to the bottom of the electrical stimulation apparatus and another being attached with a piece of silk thread for connection with a force displacement transducer (Fig. 1). The preparation was then immersed in, without load, 20 ml of modified Krebs' bicarbonate solution contained in a tissue bath. The solution was aerated with the gas mixture and maintained at 37°C.

After equilibration for 30 min and subsequent incubation with 10⁻⁷ M l-[7,8-³H]-noradrenaline (³H-NA, specific activity of 1.11 and 1.24 TBq/mmol, or 30 and 33.5 Ci/mmol, respectively; Amersham, Arlington Heights, IL, USA) for 1 hr at 37°C, the arterial preparation was appropriately sandwiched by a pair of parallel platinum-wire electrodes, 1 mm in diameter, separated by 2 mm along the whole length of the strip. Then the preparation was perfused and superfused with warmed and oxygenated Krebs' solution at a constant flow rate of 2
ml/min by means of microtube peristaltic pump (Tokyo Rika Co., Tokyo; model MP-201). The gaps between the electrodes and artery were filled consistently by the superfusate retained by capillary action. The arterial preparation was stretched vertically for a resting tension of 0.5 g, and the changes in tension and intraluminal pressure were monitored by means of a force-displacement transducer (Nihon Kohden Kogyo, Tokyo; model AP-601G) and a pressure transducer (Nihon Kohden Kogyo, model TP-200T), respectively, and recorded on an ink-writing oscillograph (Nihon Kohden Kogyo, model RJG-4004). The superfusate was collected every 2 min by a fraction collector (LKB Produkter AB, Bromma, Sweden; model Redi-Rac 2112).

After equilibration for 30 min, TNS was performed by means of an electronic stimulator (Nihon Kohden Kogyo, model SEN-3301) at an interval of about 16 min by giving a train of 120 pulses at each frequency, except for 0.1 Hz when a total of 60 pulses was given. Parameters of TNS were 0.3-msec duration and supramaximal intensity of 25 V. An aliquot of the collected solution was assayed for total tritium activity by liquid scintillation spectrometry with the counting solution ACS II (Amersham) and an Aloka (Tokyo) LSC-3500 counter at an efficiency of about 42%.

Measurement of $^3$H-NA uptake

The uptake experiment was conducted according to the procedure of Su et al. (8) with minor modifications. Briefly, small segments, 5 mm in length, were cut from isolated control, decentralized and denervated ear arteries under a dissecting microscope. The arterial segments were separately incubated for 1 hr in tissue baths containing modified Krebs' solution equilibrated with 95% O$_2$ and 5% CO$_2$ at 37°C. Then the segments from each artery were divided into two groups: one was treated with $10^{-4}$ M cocaine, a neuronal uptake inhibitor, and the other was not. Thirty minutes later, $10^{-8}$ M $^3$H-NA was added to the baths containing both groups of arteries. After 60 min of incubation, each segment was quickly rinsed in another bath of modified Krebs' solution at 37°C containing unlabeled $10^{-8}$ M NA, blotted between two sheets of Whatman No. 1 filter paper, weighed and digested for 1 day with 1 ml of the tissue solubilizer NCS (Amersham). The experiment was performed in duplicate, and the tritium activities of both tissue and an aliquot of incubation mixture were assayed as described above.

Assay for catecholamine contents

Catecholamines in the vascular tissue and SCG were assayed by the modified radioenzymatic thin layer chromatographic method (9), and the following catecholamine assay used in the present study was essentially based on the procedure described in the CAT-A-KIT™ (Amersham International plc, Amersham, England). Briefly, isolated arteries and SCG were cleaned macroscopically in the ice-cold Krebs' solution, and an arterial preparation of about 4 cm in length and only spindle-like SCG were obtained by cutting off the remaining portions. Then the preparations were blotted on a filter paper (Whatmann #100), weighed and minced with fine scissors. The tissues were homogenized in an all-glass homogenizer in about 100 vol. of ice cold 0.4 N perchloric acid containing 5 mM reduced glutathione. These procedures were performed as quickly as possible.

After centrifugation at 27,000×g for 5 min (<0°C), an aliquot of the supernatant was measured and reacted with $S$-adenosyl-$L$-[methyl-$^3$H]-methionine ($[^3$H]-SAM; specific activity of 9.25 MBq, i.e., 250 µCi in HCl : ethanol (9 : 1), pH 2.1; Amersham) and catechol-O-methyltransferase (COMT). In the case of SCG, due to the high catecholamine concentrations, the protein-free supernatant was diluted with distilled deionized water 1 : 5. After 90 min of incubation at 37°C, O-methylation was stopped by adding 1 M borate buffer, pH 8, and cold carriers. $^3$H-O-Methylated catecholamines were then extracted and separated chromatographically (10). The assay was performed in duplicate, and the reagents used in this experiment including $^3$H-SAM and COMT were provided by the CAT-A-KIT™ assay system (Amersham). The tritium activity was assayed by liquid scintillation spectrometry with the following counting solutions: toluene/isoamyl alcohol/Liquifluor (New England Nuclear Research Products, Boston, MA, USA) for dopamine and toluene/Liquifluor for NA and adrenaline and counted in an Aloka LSC-3500 counter.

Drugs and chemical agents

Drugs used were noradrenaline bitartrate (l-Arterenol bitartrate; Sigma, St. Louis, MO, USA), tetrodotoxin (TTX; Sankyo, Tokyo), cocaine hydrochloride (Takeda, Osaka), chlorpromazine (Wintermin; Shionogi, Osaka), pentobarbital sodium (Tokyo Kasei, Tokyo), ascorbic acid (Katayama Chemical, Osaka), disodium ethylenediamine tetraacetate (EDTA, Katayama Chemical) and ethylene glycol bis-(l-aminoethyl)ether)-N,N,N',N'-tetraacetic acid (EGTA; Kanto Chemical, Tokyo). The former three agents were dissolved in distilled water as stock solutions and frozen. The stock solutions were thawed and diluted with 0.9% NaCl solution into appropriate concentrations before each experiment. All other reagents were of special grade.

Statistical analyses

Results are expressed as means ±S.E.M. Unless stated otherwise, "n" refers to the number of animals. Statistical
evaluation of the results was made by Student's *t*-test; the 0.05 level of probability was regarded as significant.

RESULTS

In the present experiment, changes in intraluminal pressure and longitudinal tension were recorded. These functional responses to electrical stimulation were, however, used merely for monitoring the effectiveness of electrical stimulation and were not analyzed in detail, because the vascular changes in contractile responses to electrical stimulation have been studied previously (6).

Reproducibility of the responses to repeated electrical stimulation

In the perfusion experiment, as shown in Fig. 2, electrical stimulation at a moderate frequency of 4 Hz with a 120-pulse train elicited a sharp increment in the intraluminal pressure and gradually returned to the basal level. When the intraluminal pressure was increased by electrical stimulation, the artery constricted and increased in longitudinal length, resulting in the concomitant decrease in longitudinal tension. In addition, the radioactivity of the perfusate collected during electrical stimulation increased consistently. Both the increases in intraluminal pressure and \(^{3}H\)-NA overflow in response to the first electrical stimulation (S1) were significantly larger than those to the subsequent electrical stimulation as shown in Fig. 2. However, when electrical stimulation was given with an interval of 16 min, the subsequent responses to repeated electrical stimulation (for about 10 times) following S1 seemed to be constant. Therefore, the electrical stimulation frequency-response relationship was examined using S2 or S3 and thereafter.

Nature of the responses to electrical stimulation

Both the increase in intraluminal pressure and that in radioactivity of the perfusate in response to electrical stimulation were almost completely abolished by \(10^{-6}\) M TTX, and these responses recovered after washout of TTX as shown in Fig. 3. The result indicates that the response to electrical stimulation primarily depends on the excitation of sympathetic nerves existing in the arterial preparation. Figure 4 also shows that both the increase in intraluminal pressure and that in tritium overflow induced by electrical stimulation depend on the \(Ca^{2+}\) concentration in the perfusate. These observations strengthen the notion that the responses to electrical stimulation reflect the activity of postganglionic sympathetic nerves existing in the arterial preparation. Thus, hereafter, electrical stimulation is referred to as transmural nerve stimulation (TNS).

![Fig. 2. A representative example showing reproducibility of the responses to repeated electrical stimulation at 4 Hz with a 120-pulse train in the decentralized artery. Electrical stimulation was given at 16-min intervals.](image-url)
Fig. 3. A representative example that shows $10^{-6}$ M tetrodotoxin (TTX) completely abolishes both the increase in intraluminal pressure and that in tritium overflow in response to electrical stimulation at 10 Hz with 120 pulses in the control artery.

**TNS frequency-response relationship in the control artery**

Figure 5 clearly shows that pressor responses to TNS depend on the TNS frequency. The threshold frequency for the pressor response was 0.2–0.5 Hz, and the maximum response was elicited at around 20 Hz. The bottom of Fig. 5 shows that the appearance of tritium overflow also increases frequency-dependently. In this panel, the heights of tritium overflow just before the stimulation with different frequencies were shown by the unshaded histogram and taken as the basal tritium overflow level. Then the portions of each column above this basal tritium overflow level, indicated as the shaded portion, was assumed to be the TNS-induced overflow.

**TNS frequency-response relationship in the decentralized artery**

As shown in Fig. 6, the decentralized artery responded to lower frequencies of TNS than the control artery (Fig. 5). The increase in intraluminal pressure was obvious even at 0.1 Hz and reached the maximum at 2 or 5 Hz. In addition, the tritium overflow was also prominent at a frequency as low as 0.1 or 0.2 Hz.

Although the tritium overflow induced by TNS appears to increase frequency-dependently as shown in Figs. 5 and 6, this was not the case. The pulses given at different frequencies were fixed as 120 pulses, except at 0.1 Hz where 60 pulses were given. Therefore, in the bottom portions of Figs. 5 and 6, each column which shows the data from perfusate collected for 2 min contained tritium overflow induced by different numbers of TNS pulses. TNS at 0.1 Hz with 60 pulses, for example, takes 10 min, and this period corresponds to 5 fraction collector columns. In contrast, TNS at or more than 1 Hz takes 2 min or less for 120 pulses, and the period corresponds to or is within one column. Therefore, it is not reasonable to simply compare the height of peak of tritium overflow among different frequencies. Thus, the TNS-induced tritium overflow per pulse was calculated as the total TNS-induced in-

![Graph showing pressure and radiolabeled molecule change](image-url)
Fig. 5. A representative example of the responses to various frequencies of electrical stimulation in the control artery. Please note the frequency-dependent increases in the intraluminal pressure and tritium overflow. Transmural nerve stimulation (TNS) was given by 120 pulses at 0.2–20 Hz and 60 pulses at 0.1 Hz. Increases in tritium overflow by TNS are shown by the shaded portions. Each column showing tritium overflow represents the radioactivity (d.p.m.) of the perfusate collected for 2 min.

Fig. 6. A representative example of the responses to various frequencies of electrical stimulation in the decentralized artery. Frequency-dependent changes in intraluminal pressure. In contrast, tritium overflow at higher frequencies did not differ. Please note that the decentralized artery responded to TNS at lower frequencies than the control. Each column showing tritium overflow represents the radioactivity (d.p.m.) of the perfusate collected for 2 min.
crease in tritium overflow divided by the number of stimulation pulses; i.e., 60 pulses at 0.1 Hz and 120 pulses at and more than 0.2 Hz. As shown in Fig. 7, although tritium overflow/pulse has a tendency to increase frequency-dependently at low frequencies in the control artery, there was no significant difference, suggesting that tritium overflow/pulse was similar among the different frequencies. On the other hand, in the decentralized artery, tritium overflow/pulse appears to decrease TNS frequency-dependently. Thus, tritium overflows from the decentralized artery induced by TNS at 0.1 and 0.2 Hz were significantly larger than those from the control artery.

3H-NA uptake and retention

The denervated artery expectedly took up a very small amount of 3H-NA both in the absence and presence of the neuronal uptake inhibitor cocaine as shown in Table 1. There was no difference in the 3H-NA uptake both in the absence and presence of cocaine between the control and decentralized arteries. On the basis of the difference in 3H-NA uptakes into the artery between in the absence and in the presence of cocaine, cocaine-sensitive uptake was calculated and taken to represent the capacity of neuronal uptake (8). They were 0.126±0.016 pmol/hr/mg tissue in the control and 0.139±0.027 pmol/hr/mg in the decentralized artery, with no difference between the two, indicating that decentralization failed to affect the endogenous catecholamine stores and the uptake and retention of 3H-NA.

Catecholamine contents in the artery and ganglion

Three catecholamines, dopamine, NA and adrenaline, endogenously contained in the artery and ganglion, were assayed by the radioenzymatic method. As shown in Table 2, the contents of adrenaline in the control, decentralized and denervated arteries were very small, and there was no significant difference among them. On the other hand, while the contents of both dopamine and NA were much less in the denervated artery than in the control and decentralized arteries, there was no significant difference in the contents of dopamine and NA between the control and decentralized arteries.

| Table 1. 3H-Noradrenaline uptake (pmol/hr/mg tissue) into the control, decentralized and denervated rabbit ear arteries in the absence or presence of 10⁻⁴ M cocaine |
|---------------------------------------------------|
| Control (n = 8) | Decentralized (n = 5) | Denervated (n = 3) |
| Cocaine (−) | Cocaine (+) | Cocaine (−) | Cocaine (+) | Cocaine (−) | Cocaine (+) |
| 0.170±0.022 | 0.038±0.004* | 0.171±0.028 | 0.032±0.002* | 0.031±0.003 | 0.029±0.004 |

Each value is the mean±S.E.M. for the number of arteries shown in parentheses. * The values in the presence of cocaine were significantly less than the values in the absence of cocaine (P<0.05).
The catecholamine contents in the SCG was much higher than in the artery on the basis of tissue wet weight, as demonstrated by comparing Tables 2 and 3. There was no significant difference in the contents of dopamine, NA and adrenaline between the control and denervated ganglia as seen in Table 3. Although the content of adrenaline in the ganglion was lowest among the three catecholamines as in the arterial tissue, the values per tissue wet weight were more than ten times as compared to those in the arteries. The dopamine/NA ratio in the ganglion (0.13–0.16) was much larger than that in the artery (0.01–0.02).

**Table 2.** Effects of chronic decentralization and denervation on the catecholamine contents (ng/g tissue wet weight) in the rabbit ear artery

| Artery            | n | Dopamine  | Noradrenaline | Adrenaline |
|-------------------|---|-----------|---------------|------------|
| Control           | 8 | 14.9 ± 2.5 | 1279 ± 389    | 3.5 ± 1.2  |
| Decentralized     | 5 | 13.7 ± 2.4 | 878 ± 166     | 3.3 ± 0.9  |
| Denervated        | 3 | 4.4 ± 2.0* | 30 ± 10**     | 2.4 ± 0.8  |

Each value is the mean ± S.E.M. for the number of arteries shown in the column n. Significantly different from the control, *P < 0.05 and **P < 0.01.

**Table 3.** Effects of chronic denervation (removal of the preganglionic fiber) on the catecholamine contents (ng/g tissue wet weight) in the rabbit superior cervical ganglion

| Ganglion        | n | Dopamine  | Noradrenaline | Adrenaline |
|-----------------|---|-----------|---------------|------------|
| Control         | 8 | 1.24 ± 0.31 | 10.37 ± 1.88 | 0.050 ± 0.009 |
| Denervated      | 5 | 1.41 ± 0.37 | 8.15 ± 1.51  | 0.065 ± 0.012 |

Each value is the mean ± S.E.M. for the number of ganglion shown in the column n. There was no significant difference between the control and denervated ganglion.

The catecholamine contents in the SCG was much higher than in the artery on the basis of tissue wet weight, as demonstrated by comparing Tables 2 and 3. There was no significant difference in the contents of dopamine, NA and adrenaline between the control and denervated ganglia as seen in Table 3. Although the content of adrenaline in the ganglion was lowest among the three catecholamines as in the arterial tissue, the values per tissue wet weight were more than ten times as compared to those in the arteries. The dopamine/NA ratio in the ganglion (0.13–0.16) was much larger than that in the artery (0.01–0.02).

DISCUSSION

Neuronal changes in the peripheral nervous system could be detected as alterations in impulse traffic frequency and/or transmitter release. The transmitter release can be measured only indirectly, and there are two main possibilities: measurement of transmitter overflow and measurement of postsynaptic (or postjunctional) response (11). Thus, by measuring the postjunctional response to TNS and that to exogenous NA, we have previously reported that chronic decentralization for 8 weeks produced postjunctional supersensitivity not only to exogenous NA and potassium (4, 5) but also to TNS in the rabbit ear artery (3). Based on the different time course of the development of supersensitivity to TNS from that to exogenous NA and potassium, we have suggested that the prejunctional change might be involved in the supersensitivity to TNS and that the prejunctional change develops more slowly than the postjunctional one (6).

When analyzed properly, overflow is closest to, and probably the best index of, transmitter release (11). Therefore, the present study was performed to confirm our above-described proposal that the transmitter release is increased in the chronically decentralized rabbit ear artery by measuring 3H-NA overflow evoked by TNS. Both increments in the intraluminal pressure and in the tritium overflow in response to electrical stimulation were almost completely abolished by TTX and were so sensitive to the external Ca concentration that lowering the concentration of Ca in the perfusing media remarkably diminished the responsiveness, and Ca-free media abolished these responses in both control and decentralized arteries. These and previous observations (4, 5) confirm that the response to electrical stimulation, under the present experimental conditions, is exclusively mediated by endogenous NA released from the intrinsic sympathetic nerve terminals within the adventitial-medial junction.

The present study clearly shows that neurotransmitter release from the chronically decentralized artery for 8 weeks by TNS at low frequencies is remarkably increased compared to the release from the control artery, while there was no difference in the increases in tritium overflow produced by TNS at higher frequencies between the control and decentralized arteries. Therefore, the increase in tritium overflow at low frequencies of TNS in the chronically decentralized artery seems to be specific. Thus, in contrast to the postjunctional supersensitivity of the decentralized artery, in which supersensitivity occurs as early as 1 week after decentralization (6), prejunctional supersensitivity to TNS appears more slowly. The influence of decentralization on the functional response to TNS over a wide range of frequencies has been analyzed previously in detail at various periods after decentralization (3, 6). The contractile response to TNS at low frequencies (0.1–1 Hz) was markedly potentiated by decentralization (6) as also clearly seen between the responses in the control artery (Fig. 5, upper tracings) and decentralized artery (Fig. 6, upper tracings) in the present study.

Catecholamine contents in the three ear artery groups, i.e., control, decentralized and denervated arteries, were assayed by the radioenzymatic method. There was no difference in the three catecholamines, dopamine, NA and adrenaline (Table 2). This is consistent with the previous observation that the density and distribution of adrenergic nerve terminals, demonstrated by catecholamine fluorescence, are similar in the decentralized and control car ear arteries (3). On the other hand, catecholamine contents in the denervated artery was significantly lower,
in agreement with the disappearance of postganglionic fibers in the denervated artery (5). Both the change in the intraluminal pressure and that in the tritium overflow in response to TNS were diminished almost completely in the denervated artery (data not shown), being consistent with the previous result of almost complete suppression of the TNS frequency-contraction response curve (5).

The catecholamine contents obtained in the present experiment seem to be slightly low but are within the range (1-2 µg/g wet weight of tissue) reported by a number of investigators who have employed the radioenzymatic method. For example, Head and Berkowitz (12) have reported that the contents of dopamine, NA and adrenaline in the control rabbit ear artery are 1.99, 0.025 and 0.004 µg/g tissue, respectively; and Araki et al. (13) reported that the content of NA in the control and denervated arteries are 1.675 and 0.054 µg/g tissue, respectively. In this context, Griffith et al. (14) have reported that there is a significant regional difference in the density of sympathetic innervation in the rabbit ear artery. Namely, the NA contents per g wet weight of tissue in the proximal and distal regions are 1.93 and 0.94 µg, respectively, when assayed by an electrochemical detector. Corresponding to this, they have observed that sympathetic nerve stimulation in vitro with frequencies up to 8 Hz elicits larger and faster contractions in the proximal ear artery, and the threshold frequency is less than in the distal region.

The catecholamine contents in the SCG were also assayed in the present experiment. There was no difference in the three catecholamines between the control and denervated SCG as shown in Table 3. The catecholamine contents in the SCG are remarkably higher, about ten times, than those in the ear artery on the per g wet weight of tissue basis. It should be noted that the ratios among dopamine : NA : adrenaline are significantly different between the SCG and the artery: It is 120 : 1000 : 4.8 in the former and 15 : 1000 : 3 in the latter. The present data on rabbit SCG seem to be consistent with those on rat SCG reported by Da Prada and Zürcher (10), i.e., 78 : 1000 : 1.6. Therefore, it seems likely that the deprivation of nerve traffic by preganglionic denervation does not produce any change in the catecholamine biosynthesis both in the SCG and postganglionic nerve terminals.

Rapid termination of the action of the adrenergic transmitter at the immediate receptor site is effected by a combination of simple diffusion and reuptake by the axonal terminals of most of the released NA. Thus, the loss of neuronal uptake of NA in the denervated tissue is the main reason for supersensitivity, and the uptake inhibition by cocaine also produces postsynaptic supersensitivity (1). There was, however, no difference in the neuronal uptake of 3H-NA between the control and chronically decentralized arteries, indicating that changes in transmitter uptake and retention may not be involved in the increased release of transmitter from the chronically decentralized artery.

Two possible mechanisms are proposed to underlie the denervation supersensitivity: one is a biochemical process and the other is electrophysiological alteration. The former has recently been reported by Babila and Klein (15) who showed that both the α and β subunits of Gs (stimulatory GTP binding regulatory protein) are increased by stimulus deprivation in the rat pineal gland. They have suggested that such increases may also play a central role in generating supersensitivity in other systems. The latter mechanism, electrophysiological alterations, has been observed in a number of effector smooth muscle cells after interruption of sympathetic innervation (2). It is proposed that partial depolarization is, at least in part, responsible for nonspecific postsynaptic supersensitivity in the guinea pig vas deferens (16), rat portal vein (17) and rabbit saphenous artery (18). This also seems to be the case in the denervated sympathetic ganglia, because Brown and Caulfield (19) found that the adrenergic cell bodies in the SCG of the rat were hyperpolarized through activation of α2-adrenoceptors as present in the adrenergic nerve terminals and because Sax and Westfall (20) observed that drug-induced blockade of ganglionic transmission produced a subsensitivity of the presynaptic α2-adrenoceptors and resulted in an increase in transmitter release from postganglionic nerve terminals in the rat and guinea pig vas deferens. Combining these observations, interruption of nerve impulse traffic may result in a loss or decrease in the function of the presynaptic feedback inhibition, i.e., subsensitivity of α2-adrenoceptors, resulting in depolarization of neurons and an increase in transmitter release (21). Regarding the signaling mechanisms of α2-adrenoceptors, in addition to hyperpolarization of neurons, inhibition of neuronal Ca2+ channels is also suggested to be involved (22). Therefore, it may be that changes in the intracellular Ca2+ mobilization, i.e., increased Ca2+ influx, is also responsible for the increase in transmitter release from denervated postganglionic nerve terminals.

A similar phenomenon has also been reported in the parasympathetic nervous system, i.e., a "paralytic" secretion occurred from the supersensitive decentralized glands. Garrett and Kyriacou (23) have suggested that acetylcholine released spontaneously from terminals of postganglionic parasympathetic nerves plays an essential role in the "paralytic" secretion that occurs from rabbit submandibular glands after preganglionic denervation. Therefore, interruption of nerve impulse traffic in the autonomic nervous system, both in the sympathetic and parasympathetic nerves, may cause not only postsynaptic-
al supersensitivity of the effector organ but also prejunctional supersensitivity of postganglionic nerve terminals to release a large amount of transmitter, which then may be involved in some pathophysiological states.

In conclusion, the results obtained in the present study indicate that the supersensitivity to TNS after chronic decentralization neither depends on deranged catecholamine uptake and storage mechanisms in adrenergic nerve terminals nor augmented transmitter biosynthesis, but depends upon the increased release of transmitter in response to low frequencies of TNS, although the precise underlying mechanism, such as the mechanism of frequency-dependent release of transmitter appearing in the chronically decentralized artery, remains to be resolved.

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