Sympathetically induced paradoxical increases of the cutaneous blood flow in chronically inflamed rats

（慢性炎症ラットの交感神経刺激によるパラドックスな皮膚血流増加反応）

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Abstract

In adjuvant arthritic (AA) rats, an abnormal responsiveness of nociceptors (C-fibre polymodal receptors) to sympathetic activities, *i.e.*, α_2-adrenoceptor mediated activation of C-fibre polymodal receptors (CPRs), has been observed [33]. The present investigations were undertaken to determine if a similar plastic change would occur in the cutaneous vascular system in the rat chronic inflammation model. The vascular responses were measured by a laser-Doppler flowmeter in the hindpaw skin of the AA rats after electrical stimulation of lumbar sympathetic trunk (sympathetic stimulation). In control non-arthritic rats, the sympathetic stimulation caused decrease in blood flow of the skin (SkBF) in all animals tested (n=7). On the other hand, the sympathetic stimulation in the AA rats caused both increase (n=15) as well as decrease (n=11) in SkBF. In contrast to the abnormal responsiveness of CPRs, the intra-arterial injection of noradrenaline caused the expected decrease in SkBF in all animals tested, and in no instances increase in SkBF were observed. To determine whether activation of nitric oxide (NO), which is known to be a potent endogenous vasodilatation substance, was involved in the vasodilating effect to sympathetic stimulation, an inhibitor of NO synthase, N^G-monomethyl-L-arginine (L-NMMA), applied systemically. L-NMMA significantly increased baseline blood pressure in the control and the AA rats, but it did not significantly alter the SkBF in the control or

the AA rats after the sympathetic stimulation, suggesting that NO is not a mediator in the vasoactive responses.

The results of current studies showed for the first time that electrical stimulation of the lumbar sympathetic trunk causes vasodilatation in the skin of the AA rats. This abnormal responsiveness of regional SkBF after sympathetic stimulation was not mediated by adrenergic or NO system.

1. Introduction

Reflex sympathetic dystrophy (RSD) and related disorders are characterized by autonomic, motor and sensory disturbances [6, 9, 36, 38]. Pain sensation in RSD can be aggravated by sympathomimetic conditions and can often be alleviated by sympathetic block [36]. Experiments carried out using a variety of animal models of chronic pain have shown that the sympathetic nervous activation could enhance nociceptive activity in certain pathological conditions [7, 10, 16, 28, 32]. Sympathetic nervous system is also known to contribute significantly to the generation and maintenance of chronic inflammatory states [19]. Vasomotor instability is another clinical feature of RSD and related disorders: clinically, the dystrophic limb is cool, pale or cyanotic and sweaty, the limb may also be warm, red, or suffused and dry [7]. Sudomotor changes tend to parallel vasomotor responses [9]. Warmth or cold sensations in RSD patients are well correlated with cutaneous blood flows [17]. It is well known that sympathetic stimulation generally produces vasoconstriction of cutaneous blood vessels in a normal condition. However, it has been reported that it could cause cutaneous vasodilatation in pathological condition [21].

Our previous observation in adjuvant-induced arthritic rats showed that sympathetic stimulation as well as close arterial injection of noradrenaline evoked discharges in units of cutaneous C-fibre polymodal receptors (CPRs). These abnormal excitation of CPRs which are plastic changes under chronic inflammatory states are blocked by α_2-adrenoceptor antagonists [33].

The present investigations were undertaken to determine if similar plastic changes, *i.e.*, abnormal responsiveness of nociceptors to sympathetic activities in the AA rats, would occur in the cutaneous vascular system under similar experimental conditions using a laser-Doppler flowmetory.

2. Materials and Methods

Preparation of adjuvant arthritis animals

Male Lewis rats weighing 200-260 g were used. Adjuvant arthritis was induced by an inoculation of *Mycobacterium butyricum* (Difco Laboratories) suspended in paraffin oil, and the solution (0.6 mg/0.1ml) was injected intradermally at the distal third of the tail. After the inoculation, the animals were housed in cages with free access to food and water until the day of the experiment. The arthritic score (AS) was used as an index of the severity of the adjuvant polyarthritis and was determined by observation of redness,

swelling and deformity of the tested right hindpaw [3, 22]. AS=1 was with only slight redness, AS=2 was with redness and swelling, AS=3 was with redness, swelling and deformity, and AS=4 was with deformity only. AS=0 was with no redness, and animals with AS=0 were excluded from the present experiment. Animals with AS=1-4 were used in the experiment as adjuvant arthritic (AA) rats.

Experimental procedures

Animals were initially anesthetized with urethane and α-chloralose (500 and 100 mg/kg *i.p.*, respectively) and received supplemental anesthesia (half of initial dose, *i.v.*) as needed. A minimum of 10 min elapsed when the supplement was applied before proceeding with the experiment. The animals breathed room air spontaneously through a tracheal cannula. The right carotid artery was cannulated to measure mean arterial pressure (MAP). The left jugular vein and a branch of the right femoral artery were cannulated to allow drug administration, and heart rates (HR) were continuously measured. Rectal temperature was maintained at 37.5±0.5 °C using a heating pad and a lamp throughout the surgery and experiment. Skin temperature at the location of blood flow measurement and environmental temperature over the experimental table were continuously monitored throughout the experiment.

The lumbar sympathetic trunk (LST) on the right side was sectioned between L3 and L4 ganglia by using a retroperitoneal approach under a binocular microscope, since blood

flow at the tested hindpaw is innervated by these segments and below [2]. The nerves were kept in mineral oil and the peripheral end of the LST was placed on a bipolar platinum stimulating electrode. In pilot studies on normal animals, electrical stimulation of the peripheral cut end of the LST with 5 rectangular pulses of 0.2 ms duration, 10 V amplitude and 1 Hz was adequate to produce maximum blood flow changes in the right hindpaw skin (Fig. 1). The effects of the electrical stimulation of the LST (sympathetic stimulation) were tested at intervals greater than 10 min. We also examined the effect of noradrenaline (NA) (400 ng/kg, Sankyo Co., Ltd., Tokyo, Japan) on SkBF. NA was infused slowly for 30 s into a branch of the right femoral artery supplying the tested hindpaw skin. The effects of N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of nitric oxide synthase (Sigma Chemical Co., St. Louis, MO, USA) were examined. L-NMMA (10 mg/kg) was administered intravenously. These drugs were dissolved in saline and administered in volumes not exceeding 0.2 ml per injection.

The relative changes in local blood flow of the skin (SkBF) at the right hindpaw was measured by a laser-Doppler flowmeter (ALF 21, Advance Co., Ltd., Tokyo) attached to the central pad of the hindpaw. Electric signals from the laser-Doppler flowmeter [30], MAP and HR were simultaneously recorded and fed into the computer through a DIGIDATA 1200 (Axon Instruments, Inc., Foster City, CA, USA) using a data analysis program (AXOTAPE, Version 2.0) at sampling intervals of 40 or 100 ms. The values were expressed as arbitrary units in SkBF, mmHg in MAP, and pulses per min in HR in the text

and Figures 1, 2, 5 and 8. An apparent vascular conductance (VC) was calculated as the ratio of SkBF to MAP.

The baseline values in SkBF, MAP, VC or HR were average values sampled with 40- or 100-ms bin during pre-sympathetic stimulation or pre-administration of NA or L-NMMA period of 60 s. Change in SkBF to sympathetic stimulation with more than twice the standard deviation of the baseline value was used as the criterion of the response. To analyze vascular changes quantitatively, the following values were measured, the onset latency of the response: the duration from the beginning of sympathetic stimulation until the initiation of the response; the entire duration of the response: the duration from the initiation until the completion of the response; the peak change (ΔPEAK): the difference between the baseline value and either the maximum or the minimum values after sympathetic stimulation or drug injection; the mean change (ΔMEAN): the difference between the baseline value and the average value sampled with the 40- or 100-ms bin during the entire duration of the response after sympathetic stimulation, during 3 min-period after the injection of L-NMMA, and during 30 s-period after the injection of NA. In case of NA injection, responses in SkBF during the first 30 s from the beginning to the end of the injection were discarded in order to eliminate the disturbance on SkBF recordings produced by the close arterial injection.

Results are expressed as the mean±SEM. The statistical significance was determined as follows: by Dunnett's multiple comparison tests for comparison of ΔPEAK and

ΔMEAN in SkBF, MAP and VC, the time factors of the responses in SkBF, and the baseline values in HR and MAP; by two-tailed Mann-Whitney U test for comparisons of parameters in pre-stimulus conditions and ΔMEAN in SkBF, MAP and VC after the injection of L-NMMA; by analysis of variance (ANOVA) followed by Fisher's test for comparison of the responses in VC from the baseline values, and ΔMEAN in SkBF, MAP and VC after the injection of NA or L-NMMA. Results termed significant if $p<0.05$.

3. Results

The electrical stimulation of lumbar sympathetic trunk (sympathetic stimulation) decreased SkBF in all normal rats, while it increased or decreased SkBF in the AA rats. Animals were classified into three groups as the control (CTL: n=7) rats, the AA rats with increased SkBF (iAA: n=15), and the AA rats with decreased SkBF (dAA, n=11). Representative examples of the CTL, iAA and dAA rats are shown in Figure 2. For further analysis of the data, above-mentioned numbers in each group are used in Figures 3,4,5,6 and 7 with average results.

Pre-stimulus conditions of test animals prior to sympathetic stimulation among these three groups are shown in Table 1. These parameters, *i.e.*, HR, MAP, SkBF, skin temperature, were monitored for more than 30 min to ensure stability of the animal

conditions before measurements were taken. HR in the iAA rats was significantly ($p<0.05$) higher than that in the CTL rats. HR in the dAA rats and MAP in both AA groups tended to be higher than those in the CTL rats, although statistically not significant. The baseline SkBF in the dAA rats tended to be lower compared to the other groups (Table 1 and Fig. 2). Accordingly, the dAA rats had the lowest baseline VC.

Sympathetic stimulation significantly ($p<0.01$) decreased SkBF in the CTL and the dAA rats, and it significantly ($p<0.01$) increased SkBF in the iAA rats compared to the baseline. The magnitudes of the SkBF responses to sympathetic stimulation in the three groups were examined using two criteria, the ΔPEAK (A in Fig. 3) and the ΔMEAN (B in Fig. 3) in SkBF changes. In the iAA rats, the increased response in SkBF was clearly shown in both the ΔPEAK and the ΔMEAN in SkBF. The ΔPEAK and ΔMEAN in the dAA rats were not significantly different from those in the CTL rats.

To compare the time factors of responses in SkBF in the three groups (Fig. 4), the onset latency (A), the duration from the initiation to the maximum or the minimum value of responses in SkBF (B) and the entire duration of responses in SkBF (C) were analyzed. In the iAA rats, all three parameters tended to be shorter than those in the other groups.

To clarify whether the effects by the stimulation of peripheral cut end of LST are caused due to stimulation of purely peripheral components or are including any components reflexively elicited by coincidental activation of afferents, we examined changes in HR and MAP. Figure 5 shows HR and MAP tracings that are computed by

averaging the total cases in each of the three groups, when the 1st sympathetic stimulation was tested. The baseline values in HR in the AA groups were significantly ($p<0.05$) higher than that in the CTL rats. Slight and inconsistent increases of MAP after sympathetic stimulation were seen. The sympathetic stimulation, therefore, did not cause any substantial systemic changes on the circulation, but caused local changes in SkBF. To compensate for passive changes in SkBF caused by systemic arterial pressure, VC, *i.e.*, SkBF values divided by MAP values, were calculated and averaged tracings for the three groups are shown in Figure 6. The baseline values in VC were not different between the iAA and the CTL rats, but that for the dAA rats was significantly ($p<0.05$) lower, compared to that in the iAA rats. Sympathetic stimulation significantly ($p<0.01$) decreased VC in the CTL and the dAA rats, and it significantly ($p<0.01$) increased VC in the iAA rats compared to the baseline values. The ΔMEAN in VC of all three groups were summarized in Figure 7. ΔMEAN in the dAA rats was not significantly different from that in the CTL rats. In contrast to the CTL and the dAA groups, the average VC change in the iAA rats was a positive value, confirming that change is in SkBF by sympathetic stimulation in the iAA rats is not passively caused by changes in MAP but depends on local vasodilatation in the skin.

The effects to NA (400 ng/kg *i.a.*) on SkBF and MAP were tested in five CTL, six iAA and four dAA rats. The baseline values in SkBF, MAP and VC were not significantly different among the three groups as are also shown in pre-stimulus condition (Table 1 & 2), although the baseline value in MAP in the dAA rats in this series of the experiment was

significantly higher than that of the CTL rats. The effect of NA injection on SkBF lasted up to 120 s in all cases. In most cases, it showed initial rises in SkBF within 10-20 s immediately after the close arterial injection of NA followed by sudden declines. These initial rises in SkBF were also reproduced by saline injections indicating the rises were artifact caused by the injection. The maximal decreases of the responses occurred at approximately 30 s after the injection. The responses subsequently gradually returned to the baseline level. The SkBF, MAP and VC in the three groups during the second 30 s after the injection of NA were significantly changed from the baseline values, although the SkBF in the dAA rats was not statistically significant ($p<0.06$). The changes in SkBF, MAP and VC among the three groups were not significant (Table 2).

To determine whether activation of nitric oxide was involved in the vasodilating effect to sympathetic stimulation in the iAA rats, the effects of L-NMMA (10 mg/kg) on SkBF, MAP and VC were tested in six iAA and five CTL rats. The baseline values in SkBF, MAP and VC before the injection of L-NMMA were not significantly different between the two groups (Fig. 8 and Table 3). The MAP markedly increased immediately after the injection of L-NMMA and reached the maximum value within 3 min in all animals tested (Fig, 8). ΔMEAN in MAP during the initial 3-min period were 40.6±1.5 mmHg in the CTL rats and 24.2±2.5 mmHg in the iAA rats, both changes were significant ($p<0.01$). ΔMEAN in MAP in the iAA rats during the initial 3 min-period was significantly less than that in the CTL rats (Table 3). In contrast, L-NMMA did not cause substantial changes in SkBF. Changes in SkBF to L-NMMA were minimal, although

either slight increases or decreases in SkBF occurred in early phases after the injection of L-NMMA in both groups of the rats. Consequently, L-NMMA decreased VC in the initial 3 min-period (Fig. 8). The SkBF, MAP and VC during the initial 3 min-period were averaged and summarized (Fig. 8 and Table 3). Once MAP reached the maximum value, it slowly declined to the pre-injection level. At 10 min after the injection, MAP in the CTL rats decayed from it's peak by about 50 % but was still significantly ($p<0.01$) higher than the baseline values. The rise in MAP lasted for 33.0±5.7 min. In the iAA rat at 10 min after the injection, MAP decayed from it's peak by about 20 % but was still ($p<0.05$) higher than the baseline values, although in one case it had already returned to the pre-injection level within 5 min. The MAP in this group decayed from the peak by about 50 % at 30 min after the injection. The rise in the iAA rats lasted for 38.0±12.0 min. In contrast to the marked changes in MAP, the effects of L-NMMA on regional SkBF during the 30 min period after the injection were not significant in both groups, although SkBF in the iAA rats tended to increase for 30 min (Table 3.). Therefore, the changes in VC to the L-NMMA injection were mainly due to increases in MAP. L-NMMA significantly ($p<0.01$) decreased VC in the CTL rats for 10 min after the injection (Table 3 and Fig. 9). At 30 min after the injection, the baseline values had still not returned to pre-injection levels in the CTL rats. In contrast, the baseline changes in VC in the iAA rats were less marked.

In regard to the response to sympathetic stimulation in the CTL rats, the magnitude of response in VC tended to decrease 10 min after the injection when the baseline value was the lowest (Fib. 9 and Table 3.) At 30 min after the injection, the responses were

returning to pre-injection levels as well as the baseline values (Fig. 9). L-NMMA had minimal effects on the responses in VC to the sympathetic stimulation after the injection in all iAA rats (Fig. 9). In the iAA rats, ΔMEAN of responses in SkBF and VC (values not presented) to sympathetic stimulation before, 10 min after and 30 min after the injection of L-NMMA were not significantly different.

4. Discussion

It is well known that sympathetic nerve stimulation generally produces vasoconstriction of the skin as we observed in all normal rats in the present experiment. However, unexpectedly, electrical stimulation of lumbar sympathetic trunk caused cutaneous vasodilating effects in about half of the AA rats.

We have shown previously that sympathetic stimulation as well as close arterial injection of NA evoked discharges in 35-40 % of units of cutaneous C-fibre polymodal receptor (CPRs) in the AA rats while these stimuli were totally ineffective in normal rats [33]. Similar findings of the abnormal responses in CPRs were seen in neuropathic pain models, *e.g.*, partial nerve cut model in rabbits [32] and streptozotocin-induced diabetic neuropathy model in rats [35]. These abnormal changes in CPRs appear to be responsible for sympathetically dependent pain states as seen in RSD and related disorders. The

abnormal increased responses in SkBF to sympathetic stimulation observed in the same condition as the experiment on CPRs in the AA rats may also represent an autonomic dysfunction in RSD and related disorders.

The paradoxical vasodilating effects to sympathetic stimulation were observed in a partial population of the AA rats. Previous studies [32, 33, 35] also showed that sympathetic stimulation induced excitation in some population of the CPR units. Adjuvant-induced inflammation in our experiment was a generalized chronic disease characterized primarily by arthritis at both the ankles and feet, accompanied by several localized lesions in the skin and mucous membrane scattered over the whole body as is also reported by Pearson et al [26. 27]. Therefore, the vasodilatation responses in the AA might be induced at vascular beds in only some regional parts of the skin.

The abnormal excitation of CPRs by sympathetic stimulation is mimicked by intra-arterial injection of NA and both responses are blocked by α_2-adrenoceptor antagonists [33]. In contrast, the injection of NA, however, induced similar vasoconstrictive effects in all three groups of rats. There was no statistical difference in SkBF, MAP and VC after the injection of NA among the three groups (Table 2). This indicates that the responsiveness to adrenergic agents in the AA rats was not different from that in the other two groups. Preliminary data in our laboratory showed that neither of α_2-, α_1-adrenoceptors nor muscarinic antagonists blocked the increased responses in SkBF to sympathetic stimulation

(unpublished data). The mechanisms underlying the vasodilatation responses, therefore, appear to be different from those in the abnormal responses of CPRs.

Possible mechanisms implicated in this paradoxical vasodilatation are as follows: (1) an abnormal sympathetic reflex induced by coincidental activation of afferents, (2) an antidromic stimulation of peptidergic afferents contained in the sympathetic trunk, and (3) abnormal responsiveness of sympathetic efferents either at the preterminal or vascular sites.

The first question is whether the paradoxical vasodilatation is due to abnormal changes in central vasomotor reflexive circuits in the AA rats. Studies have provided evidence for vasodilatation in the skin induced in the states of increased sympathetic activities such as, at an arousal state, in a cold environment, under mental stress, by painful intraneural electrical stimulation, and by deep breaths [1, 16, 25]. In the present study pre-stimulus HR and MAP in the AA groups were higher than those in the CTL rats, suggesting that tonic sympathetic activities were increased also in the AA groups. However, stimulation of the peripheral cut end of LST did not affect HR in all animals tested, although it slightly changed MAP (Fig. 5). This indicates the vasodilatation response is a purely peripheral phenomenon. Furthermore, the onset latencies of vasodilatation responses in the AA rats were short (Fig. 4). These observations, therefore, exclude that the vasodilating effects appear to be caused by an central abnormal sympathetic reflex.

The second possible mechanism is an antidromic stimulation of peptidergic afferents contained in the sympathetic trunk. Earlier studies [8, 29, 40] showed vasodilatation under noradrenergic neuron blocking agents may represent existence of this mechanism. Vasoactive neuropeptides released in peripheral sites are involved in antidromic vasodilatation responses to peripheral nerve stimulation [5, 12, 31, 37]. In these studies, time-courses of the vasodilating effect were longer, lasting more than 6 min, and the onset latencies were also long varying from 5 s [37] to more than 15 s [5, 31]. In the present study, however the entire duration of vasodilating effect was 18.2±1.56 s and the onset latency was 2.5±0.20 s. It is, therefore, less likely that the increased responses in SkBF are induced by vasoactive neuropeptides. In addition it has recently been reported that rats with acute carrageenan-induced inflammation of joints showed markedly reduced vasoconstriction responses to peripheral nerve stimulation, but enhanced vasodilatation responses to substance P and calcitonin gene-related peptide [18]. On the other hand, in inflamed joints of rats, induced by intra-articular injection of Freund's adjuvant, virtually no response of the blood flow to either peripheral nerve stimulation or substance P application appeared at one week after the injection [23]. In the present study, overt clinical signs of arthritis did not appear until the second week post-inoculation, reached plateau in the third week followed by remission and exacerbation, and lasted up to 40 weeks. The longer courses in the inflammatory states may differ from the states that showed variety of responses in blood flow to either peripheral nerve stimulation or

peptides in the acute inflammation. To reach the definite conclusion, systemic studies using antagonists for various types of vasoactive peptides are needed.

Thirdly, the vasodilatation responses in the AA rats may be due to either abnormality at the terminal of the sympathetic postganglionic neuron (SPGN) or abnormality of the responsiveness in the peripheral vascular system. We examined if abnormal changes in adrenergic or nitric oxide system at vascular beds are involved in this paradoxical responses. As mentioned above, in contrast to our previous experiments on CPRs, involvement of adrenergic abnormality in the cutaneous vasodilatation appear to be less likely. It is well known that NO is released from the vascular endothelium by the action of several endothelium dependent vasodilators, including acetylcholine, substance P, bradykinin, histamine, etc. [11, 15, 24]. Numerous studies using NO synthase inhibitors have provided evidence for the relaxing properties of NO in blood vessels [13, 39]. In the present experiment, L-NMMA systemically applied markedly increased the baseline MAP in all animals tested, L-NMMA, however, did not affect the vasodilatation responses to sympathetic stimulation in the AA rats. This indicates that NO system play active roles in regulation of vascular tones in both the CTL and the AA rats. However, the NO system was not involved in producing the cutaneous vasodilatation responses to sympathetic stimulation in the AA rats. Since it was presumed that hyperalgesic states in chronically inflamed rats were dependent on abnormality in SPGN [19, 20], an involvement of the abnormality at the terminal of SPGN in the vasodilatation responses to sympathetic stimulation remains to be clarified in the future experiment.

In conclusion, electrical stimulation of lumbar sympathetic trunk decreased regional blood flow of the skin in all normal rats. Unexpectedly, vasodilatation responses to the sympathetic stimulation were observed in approximately half of adjuvant-induced arthritic . The vasodilatation responses were apparently a purely peripheral phenomenon and that they were not mediated by adrenergic mechanism or NO system. Taken together with our previous findings on abnormal responses of cutaneous nociceptors, the paradoxical vasodilatation responses to sympathetic stimulation observed in the present experiment using the same animal model may represent an autonomic dysfunctions occurring in RSD and related disorders.

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Table 1. Pre-stimulus conditions in the three groups of animals.

	HR (pulses/min)	MAP (mmHg)	SkBF	VC	ST (°C)	RT (°C)	EnvT (°C)	Wts (g)	Anes-1stSS (min)	AS	Adj-Wks (weeks)
CTL	315.7±13.5	83.5±6.1	152.4±22.9	1.8±0.2	32.9±1.9	37.5±0.2	25.3±0.6	310±25	239.3±34.1	na	na
iAA	363.1±14.3 *	86.6±6.9	156.8±22.0	1.8±0.2	35.1±0.6	37.5±0.1	24.3±0.5	293±20	222.0±17.3	2.6±0.3	6.3±2.5
dAA	357.9±16.4	94.7±7.1	120.3±24.1	1.3±0.2	31.7±1.1	37.6±0.1	24.0±0.6	299±18	210.5±14.3	3.0±0.4	10.2±2.9

HR: heart rates, MAP: mean arterial pressure, SkBF: blood flow of the skin, VC: vascular conductance, ST: skin temperature at the SkBF-measured spot, RT: rectal temperature, EnvT: environmental temperature, Wts: weights of the animals at the time of experiment, Anes-1 st SS: duration from the initiation of general anesthesia until the first SS, AS: arthritic scores on the day of experiment, Adj-Wks: duration from the day of inoculation of M.butyricum until the day of experiment, CTL: control rats, n=7, iAA: adjuvant arthritic (AA) rats with increased SkBF response to the electrical stimulation of the lumbar sympathetic trunk (SS), n=15, dAA: AA rats with decreased SkBF response to the SS, n=11, Data of SkBF, MAP and HR were average values sampled with the 40-ms bin during the pre-stimulus 60 s. Data of RT, ST and EnvT were values measured during the pre-stimulus 60 s. Data are mean±SEM. *, statistical significance (p=0.03) from the control value by Mann-Whitney U test. na, not applicable.

Table 2. Effects of noradrenaline on mean arterial pressure (MAP), blood flows in the skin (SkBF) and apparent vascular conductance (VC).

	MAP (mmHg)		SkBF		VC	
	PRE	POST	PRE	POST	PRE	POST
CTL	74.77±6.47	91.25±6.45**	162.55±31.30	116.21±17.17*	2.13±0.30	1.25±0.12**
iAA	83.69±6.23	91.16±6.65**	202.88±32.87	155.39±27.78**	1.81±0.33	1.26±0.26**
dAA	105.44±10.03†	118.23±11.60*	140.75±36.30	116.02±34.69	1.35±0.30	1.03±0.30**

CTL: control rats, n=5, iAA: adjuvant arthritic rats with increased SkBF response to the electrical stimulation of the lumbar sympathetic trunk (sympathetic stimulation), n=6, dAA: adjuvant arthritic rats with increased SkBF response to sympathetic stimulation, n=4, PRE: the baseline values before the injection of noradrenaline (NA), POST: the values during the second 30 s after the NA injection. Data are mean±SEM. *, statistical significance from the baseline value by ANOVA followed by Fisher's test (*, $p<0.05$; **,$p<0.01$). †, statistical significance ($p<0.05$) from the control value by Mann-Whitney U test .

Table 3. Effects of an inhibitor of nitric oxide synthase on mean arterial pressure (MAP), blood flows in the skin (SkBF) and apparent vascular conductance (VC).

	MAP (mmHg)		SkBF		VC	
	CTL	iAA	CTL	iAA	CTL	iAA
PRE	88.43±8.93	91.62±2.69	181.1±20.7	188.1±47.2	2.1±0.3	2.1±0.5
0-3 min	129.05±10.45**	115.86±5.22** †	190.0±26.8	197.2±47.2	1.5±0.2**	1.7±0.4
10 min	109.50±12.03**	110.44±6.60*	142.8±11.4	195.8±55.6	1.3±0.1**	1.7±0.4
30 min	92.57±6.58	103.33±8.43	153.2±8.4	193.9±46.7	1.7±0.2	1.9±0.5

CTL: control rats, n=5, iAA: adjuvant arthritic rats with increased SkBF response to the electrical stimulation of the lumbar sympathetic trunk, n=6, PRE: the baseline values before the injection of an inhibitor of nitric oxide synthase, L-NMMA, 0-3 min: average values during 3 min immediately after the L-NMMA injection, 10 min: the baseline values at 10 min after the L-NMMA injection, 30 min: the baseline values at 30 min after the L-NMMA injection. Data are mean±SEM. *, statistical significance from the baseline value by ANOVA followed by Fisher's test (*, $p<0.05$; **,$p<0.01$). †, statistical significance ($p<0.05$) from the control value by Mann-Whitney U test.

LEGEND OF FIGURES

Figure 1. Blood flows in the hindpaw skin of a normal rat after electrical stimulation of lumbar sympathetic trunk.

Blood flows of the skin (SkBF, middle traces) were measured by a laser-Doppler flowmeter. The responses in SkBF and mean arterial pressure (MAP, lower traces) to electrical stimulation of single and five pulses (0.2 ms duration, 10 V amplitude) with one and 10 Hz frequency are shown. ES, electrical stimulation of the lumbar sympathetic trunk (upper traces).

Figure 2. Representative examples of blood flows of the skin (SkBF, upper traces) and mean arterial pressure (MAP, lower traces) after electrical stimulation in three groups.

The electrical stimulation of lumbar sympathetic trunk (SS, arrows) with five pulses of 0.2 ms, 10 V and one Hz was used throughout this experiment. Examples of SkBF and MAP changes to the SS in a control (CTL, left) rat with decreased response in SkBF, and those in adjuvant arthritic (AA) rats with increased (iAA, middle) and decreased (dAA, right) responses are shown. Scales of SkBF and MAP on the ordinate of the CTL also apply for the iAA and the dAA.

Figure 3. Average responses of blood flows of the skin after the electrical stimulation of lumbar sympathetic trunk in the three groups.

A, ΔPEAK in SkBF change. B, ΔMEAN in SkBF change. Mean±SEM in the CTL (left column), the iAA (middle column) and the dAA (right column) rats are shown in this and subsequent Figures. *, $p<0.01$, by Dunnett's multiple comparison test. ns, statistically not significant.

Figure 4. Changes in time factors of the responses in blood flows of the skin (SkBF) after the electrical stimulation of lumbar sympathetic trunk.

Average onset latencies of the SkBF responses (A), average duration from the onset to maximum or minimum values of the responses in SkBF (B), and average duration of the entire responses in SkBF (C) are shown.

Figure 5. Tracings of average time-courses in heart rates (HR) and mean arterial pressure (MAP) after the electrical stimulation of lumbar sympathetic trunk (SS) in the three groups.

Average HR (upper traces) and MAP (lower traces) changes are shown. Scales of HR and MAP on the ordinate apply for all three groups. The baseline values

in HR in the AA groups were significantly higher than that in the CTL rats ($p<0.05$, by Dunnett's multiple comparison test). Arrows indicate SS.

Figure 6. Average values in apparent vascular conductance (VC) after the electrical stimulation of lumbar sympathetic trunk (SS).

The VC was calculated as the ratio of SkBF to MAP. Tracings of VC averaged in the three groups are shown. The baseline value in VC was significantly different between the AA groups ($p<0.05$, by Dunnett's multiple comparison test). The SS significantly decreased VC in the CTL and the dAA rats, but it significantly increased VC in the iAA rats compared to the baseline values ($p<0.01$, by ANOVA followed by Fisher's test). Scale of VC on the ordinate apply for all three groups. Arrows indicate SS.

Figure 7. The difference in apparent vascular conductance (VC) between pre-stimulus baseline values and average values sampled during the entire duration of the responses (ΔMEAN) in the three groups.

The ΔMEAN in VC in the iAA rats was significantly different but not that in the dAA compared to that in the CTL. *, $p<0.01$, by Dunnett's multiple comparison test. ns, not statistically significant.

Figure 8. Effects of an inhibitor of nitric oxide synthase, L-NMMA on apparent vascular conductance (VC), mean arterial pressure (MAP) and blood flow of the skin (SkBF).

Average VC (upper traces), MAP (middle traces) and SkBF (lower traces) in the CTL rats (n=5) and the iAA rats (n=6) were shown. Arrows indicate the injection of L-NMMA. Note that in this figure time scale is 30 s indicated by the horizontal bar. Scales of VC, MAP and SkBF on the ordinate apply for both groups.

Figure 9. Effects of an inhibitor of nitric oxide synthase, L-NMMA on apparent vascular conductance (VC) to the electrical stimulation of lumbar sympathetic trunk (SS).

Average VC to SS before (PRE), 10 min after (10 min) and 30 min after (30 min) the injection of L-NMMA in the CTL rats (upper traces, n=5) and the iAA rats (lower traces, n=6) are shown. Arrows indicate SS. Scales of VC on the ordinate apply for PRE, 10 min and 30 min.

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Figure 1.

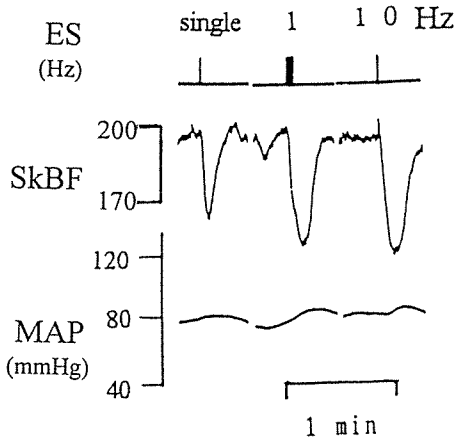
ES
(Hz)
single
1
10 Hz
200
SkBF
170
120
MAP
(mmHg)
80
40
1 min

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Figure 2.

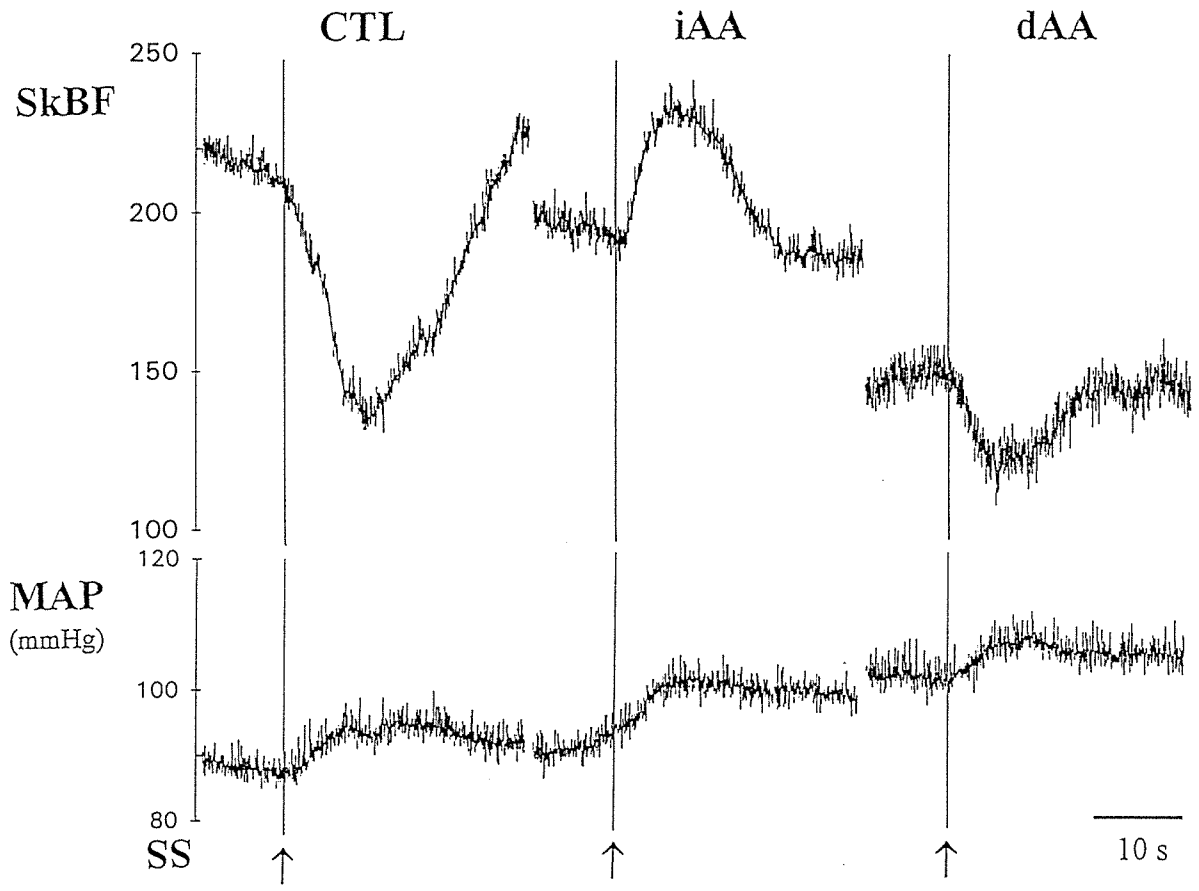
CTL
iAA
dAA
SkBF
250
200
150
100
MAP
(mmHg)
120
100
80
SS
10 s

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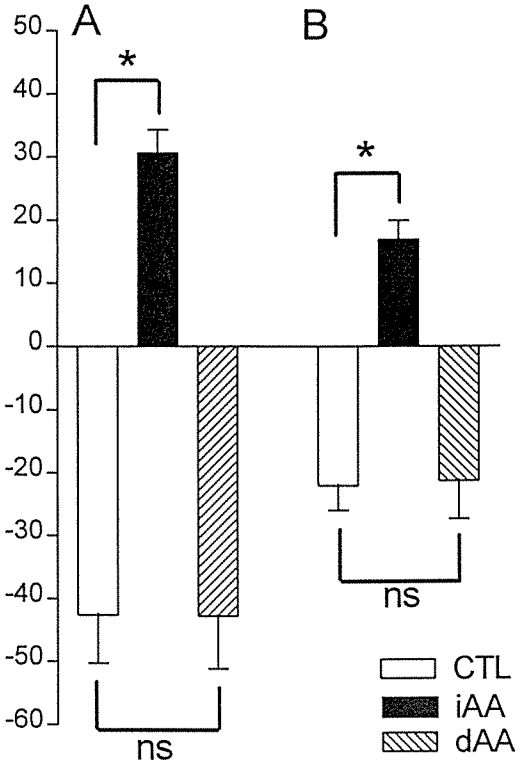
Figure 3.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40
30
20
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-40
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ns
CTL
iAA
dAA

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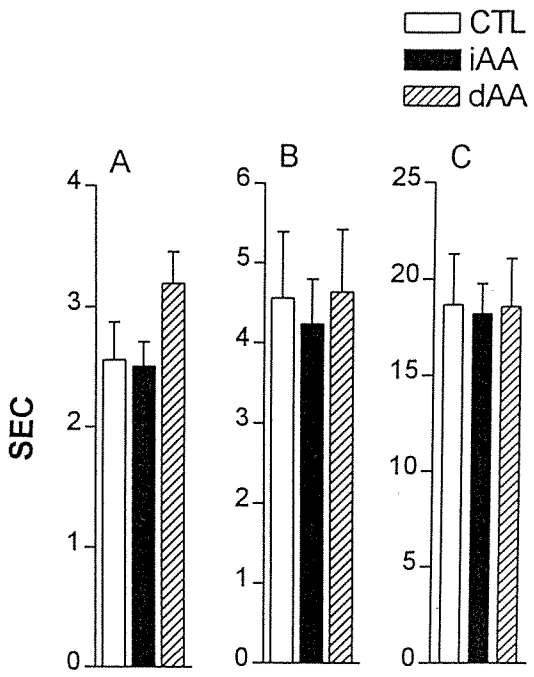
Figure 4.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dAA
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SEC
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3
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4
3
2
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20
15
10
5
0

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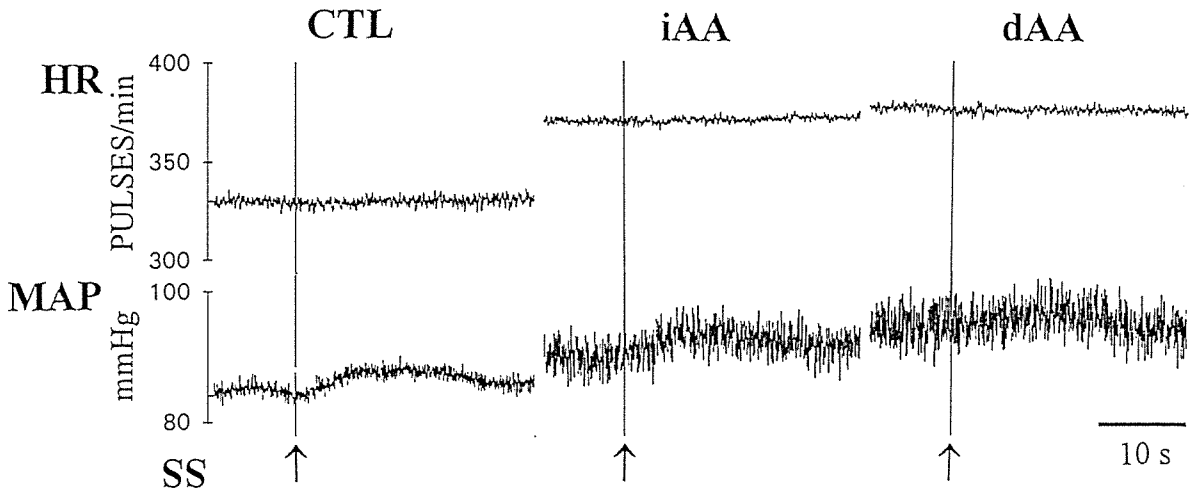
Figure 5.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iAA
dAA
HR
PULSES/min
400
350
300
MAP
mmHg
100
80
SS
10 s

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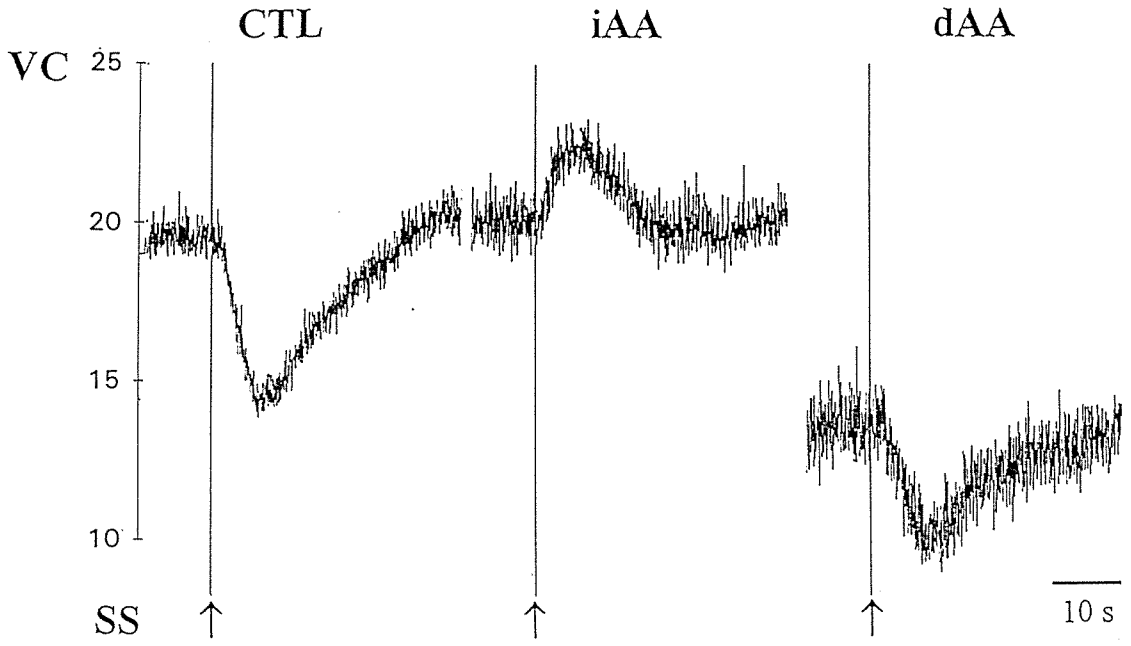
Figure 6.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iAA
dAA
VC
25
20
15
10
SS
10 s

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Figure 5.

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Figure 5.

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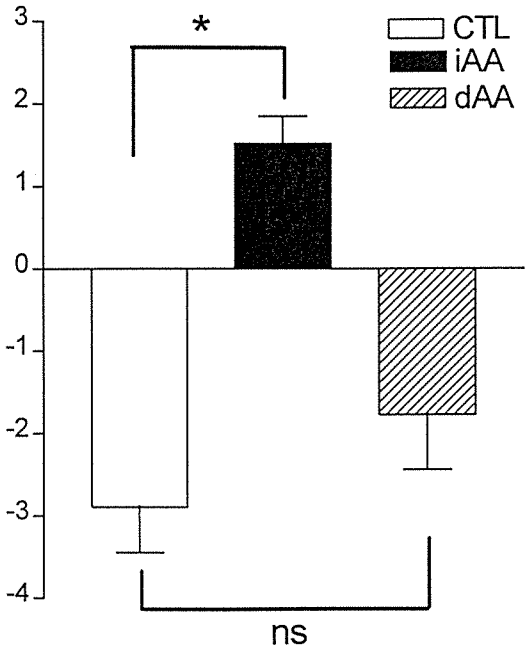
Figure 7.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-1
-2
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*
CTL
iAA
dAA
ns

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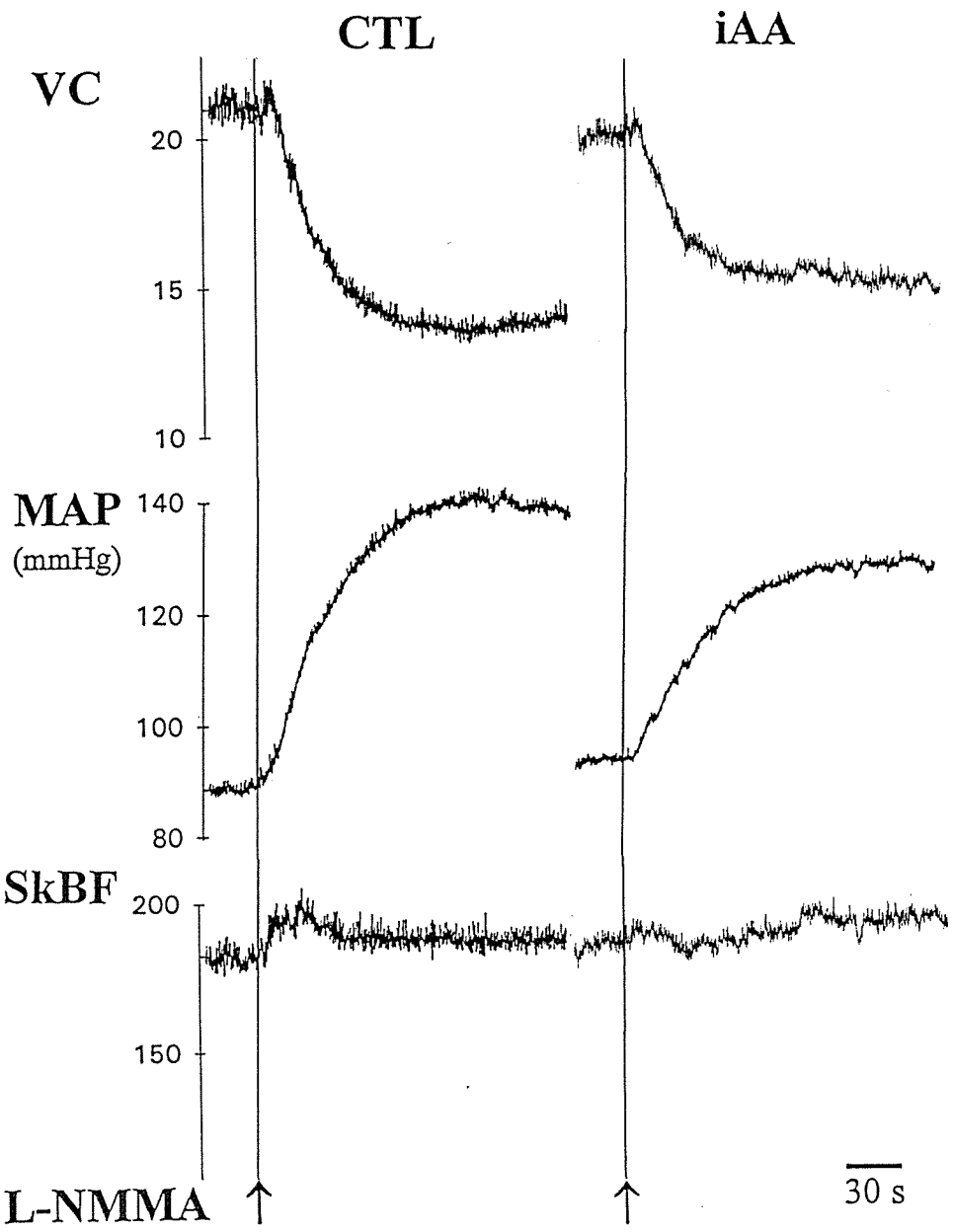
Figure 8.


CTL
iAA
VC
20
15
10
MAP
(mmHg)
140
120
100
80
SkBF
200
150
L-NMMA
30 s

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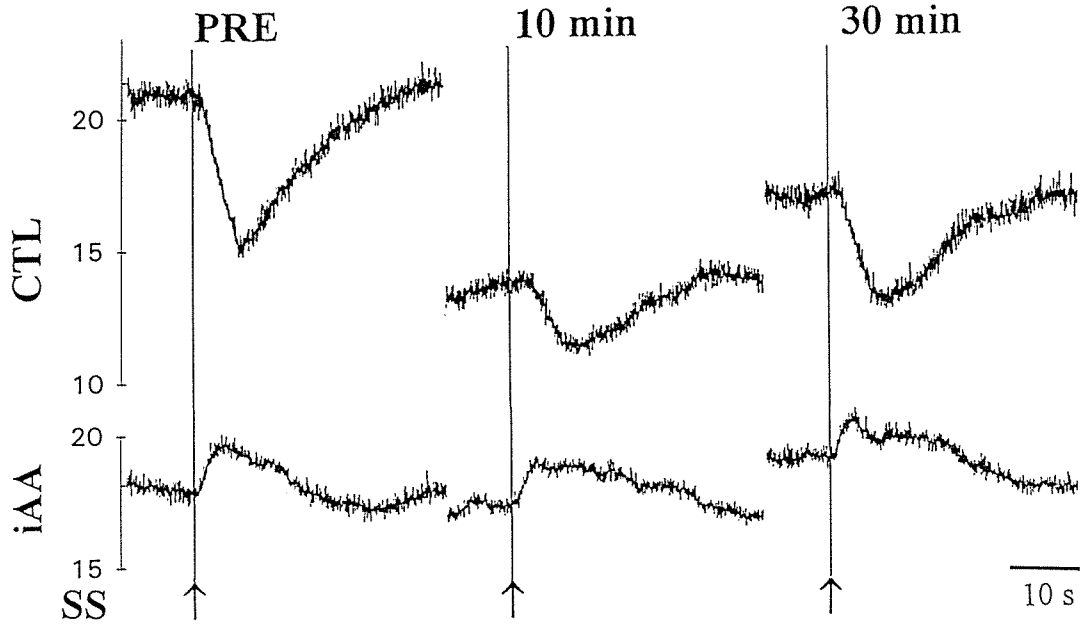
Shigeyuki Suzuki and Takao Kumazawa

Figure 9.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30 min
CTL
20
15
10
iAA
20
15
SS
10 s