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PLASTICITY OF SYMPATHETIC REFLEX ORGANIZATION FOLLOWING CROSS-UNION OF INAPPROPRIATE NERVES IN THE ADULT CAT

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SUMMARY

1. The present study has investigated the reflex organization of sympathetic neurones and its control of autonomic effector organs following nerve injury and repair. A well-defined population of vasoconstrictor neurones supplying blood vessels of the hairy skin was forced to innervate a territory that contained some appropriate, but mainly inappropriate autonomic effector organs. For this purpose the central stump of the cut sural nerve was sutured to the peripheral stump of the cut tibial nerve 11–12 months prior to the terminal experiment.

2. The activity of postganglionic sympathetic neurones was recorded from fine strands of the sural nerve proximal to the nerve lesion. Using a laser–Doppler device cutaneous blood flow was measured in the hairless skin of the hindpaw that was now reinnervated by the sural nerve. The results show a qualitative change of the reflex organization of sympathetic neurones following cross-union of these nerves.

3. Stimulation of arterial chemoreceptors by ventilating the animals with a hypoxic gas mixture (8% O_2 in N_2 for 3–8 min) increased the activity in twelve out of thirteen strands containing postganglionic sympathetic fibres. The increase of sympathetic activity contrasts with results from normal animals where systemic hypoxia causes a reflex decrease of activity in postganglionic fibres of the sural nerve.

4. Reflex changes of sympathetic activity were closely followed by corresponding changes of cutaneous blood flow. Systemic hypoxia produced vasoconstriction in operated animals in contrast to the vasodilatation observed in normal animals.

5. We conclude that the reflex organization of sympathetic neurones can change qualitatively following nerve lesion when sympathetic neurones regenerate and supply inappropriate target tissues. This long-lasting change reflects the plasticity of the autonomic nervous system and can produce a sustained abnormal control of reinnervated autonomic effector organs.

INTRODUCTION

The reflex organization of the sympathetic nervous system is target specific. The persistence of this specificity and consequently the adequate control of autonomic

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effector organs is of vital importance for maintaining an efficient autonomic control (Wallin, 1988). Separate sets of neurones supplying sympathetic effector organs in skin and skeletal muscles can be distinctly characterized by their functional response to a host of adequate natural stimuli in man and animals (Jänig, 1985). For example, stimulation of arterial chemoreceptors by systemic hypoxia results in a reflex inhibition of cutaneous vasoconstrictor neurones supplying hairy or hairless skin, and a parallel excitation of vasoconstrictor neurones innervating skeletal muscle (Jänig, 1985).

There is good evidence that the synaptic connectivity and the geometry of peripheral sympathetic neurones depends on a trophic support by autonomic effector organs throughout adult life (Matthews & Nelson, 1975; Purves, 1975; Purves & Lichtman, 1985; Purves, Snider & Voyvodic, 1988). Peripheral nerve lesions disrupt this relation. In the adult cat nerve transection causes a transient reversal of the reflex organization over a period of 1–2 months during which sympathetic neurones are excited by systemic hypoxia when regeneration is permanently prevented (Blumberg & Jänig, 1985). No reflex change occurs when severed sympathetic neurones are allowed to regenerate into their original innervation territory. However, when cutaneous vasoconstrictors are severed and forced to grow into skeletal muscle they acquire a reflex pattern that is appropriate for their new target (Blumberg & Jänig, 1985). This might suggest that the target tissue could in some way specify the functional organization of its new innervation in the adult animal, although the underlying mechanisms are so far not clear.

These situations, however, also differ from the condition created by the more prevalent injury of mixed nerves where axotomized postganglionic neurones have the opportunity to contact appropriate and inappropriate targets. We have asked whether this choice interferes with the reflex pattern of the regenerating postganglionic fibres and how the reinnervated autonomic effector organs will eventually be controlled. We have chosen the cross-union of purely cutaneous and mixed nerves as a model which replicates most features of such mixed nerve lesion by offering the variety of potential target organs while also allowing us to study the repair process of a well-defined type of sympathetic neurones. Thus, cutaneous vasoconstrictor neurones were severed and allowed to regenerate into a territory containing both skin and inappropriate target tissues such as skeletal muscle and other deep somatic tissues.

The results of the present study show that there is a qualitative change of the sympathetic reflex organization of most regenerating cutaneous vasoconstrictor neurones. The new reflex organization is permanent since it persisted long after a connection with the novel target had been established and produces an abnormal regulation of the autonomic effector organs.

METHODS

Cross-union of sural and tibial nerve

Adult cats of either sex were used in the present study. In five animals, weighing 2·7–4·8 kg at the time of the initial operation, the sural nerve, which innervates hairy skin, was transected, redirected and sutured to the peripheral stump of the cut tibial nerve which supplies both hairy and

hairless skin as well as deep somatic tissues, such as skeletal muscles, fascia, joint capsules and bones. These operations were performed under anaesthesia of methohexital (Brevimytal; 10–20 mg bolus as required, I.M.) following induction with ketamine (Ketanest; 15 mg kg^{-1}, I.M.) and diazepam (Valium; 0·2 mg kg^{-1}, I.M.). With antiseptic precautions the tibial nerve was exposed at the ankle and its proximal part mobilized. After sectioning, the central stump was ligated, the distal part retracted and placed on the surface of the lateral gastrocnemius muscle. Following transection of the sural nerve, its proximal stump was sutured with two or three epineural stitches (10–0, atraumatic) to the distal stump of the tibial nerve. The distance between nerve suture and central foot pad was 11–12·5 cm. The distal stump of the sural nerve was resected over a length of 1–2 cm. Finally, the incision was sutured in layers.

After the initial post-operative period, the animals did not display abnormal behaviour indicative for spontaneous pain and healing of the incision was uneventful in all animals. However, there was a transient and intermittent appearance of superficial wounds on the plantar skin of the feet in all animals which were insensitive to touch or pin-prick as judged by the absence of a flexion reflex. These lesions were not the result of autotomy, but probably caused by accidental lesions of the denervated tissue and some impairment of plantar flexion of the feet. The lesions ceased to occur once there was evidence that the sural nerve had regenerated. As expected there was severe wasting of small foot muscles. The operation interfered with stance or gait only slightly, and gross motor power of the limbs was well preserved allowing all animals to perform vertical jumps exceeding 1 m. Progress of nerve regeneration could be assessed by the flexion reflex elicited by pin-prick applied to the plantar surface of feet and distal hindlimb which is normally innervated by the tibial nerve and which was now progressively invaded by sural afferents.

Anaesthesia and animal maintenance in the acute and terminal experiments

The acute terminal experiments were carried out 337–361 days later. Acute experiments were conducted on the operated animals and seven unoperated control cats weighing 2·9–4·8 kg and 2·7–4·6 kg, respectively. Following induction with ketamine (15–20 mg kg^{-1}, I.M.) anaesthesia was maintained with α-D-glucochloralose (40–50 mg kg^{-1}, I.P.). Supplementary doses (5–10 mg kg^{-1}, I.V.) were given as required to maintain adequate anaesthesia as judged by the persistence of miotic pupils, assessed with frequent inspection, and by the absence of blood pressure and heart rate fluctuations except for the responses associated with non-noxious cardiovascular stimuli used in the present study. The trachea, superficial jugular vein and carotid artery were cannulated and the animals were immobilized throughout the experiments by repeated injection of pancuronium bromide (Pancuronium; 0·2 mg kg^{-1} per dose, I.V.). The cats were artificially ventilated and the end-expiratory CO_2 concentration adjusted at 3–4 % (v/v). Heart rate and systemic blood pressure were continuously monitored and mean arterial blood pressure always exceeded 80 mmHg. Body core temperature was measured intra-oesophageally and kept close to 38 °C by a heating plate. The terminal experiment lasted for 24–30 h at the end of which the animals were killed by intravenous injection of a saturated potassium chloride solution.

All experiments had been approved by the local animal care committee of the state administration and were conducted in accordance with German Federal Law.

Preparation

For recording of sympathetic activity the sural nerve and the site of the nerve suture were exposed and covered with warm paraffin oil in a pool made from skin flaps. The saphenous and common peroneal nerves were cut. Using a lateral approach, the lumbar sympathetic trunk was exposed retroperitoneally and isolated from the surrounding tissue by a plastic sheath between the lumbar paravertebral ganglia L4 and L5 (Fig. 1). The trunk was placed on a pair of platinum electrodes for electrical stimulation and the exposure covered with warm paraffin oil in the pool formed by the surrounding musculature. Electrical stimulation of the lumbar sympathetic trunk at this position results in the activation of the postganglionic neurones supplying the hindlimb by stimulation of the appropriate preganglionic neurones (McLachlan & Jänig, 1983).

Neurophysiological recording and stimulation procedures

Filaments containing single or few postganglionic axons were isolated from the sural nerve 2–3 cm proximal to the suture site (Fig. 1). Care was taken to leave at least 90 % of the sural nerve intact in order not to interfere with the regulation of the cutaneous blood flow through the plantar

skin by the remaining postganglionic neurones. Neural activity was recorded monopolarly with a platinum electrode with a reference electrode positioned nearby. Signals were amplified by a low-noise differential AC amplifier (input impedance 10 MΩ) and filtered with a variable bandwidth of 20–50 Hz to 1·2–1·5 kHz. Neural activity was displayed on an oscilloscope and stored on

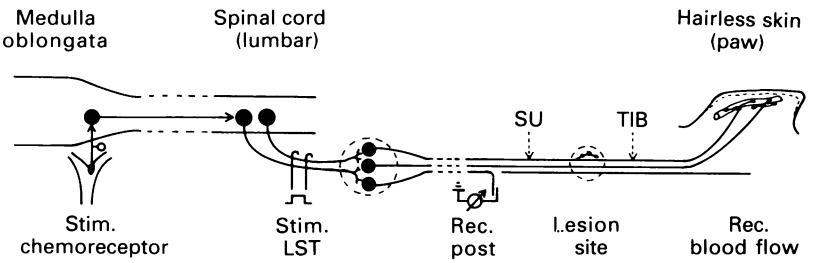

Fig. 1. Schematic drawing of the experimental set-up. The cross-union was performed between the proximal stump of the sural (SU) nerve and the distal stump of the tibial (TIB) nerve. Postganglionic fibres were recorded from the sural nerve proximal to the lesion site (rec. post). Blood flow through the hairless skin was recorded from the surface of the central pad of the hindpaw by a laser–Doppler flowmeter (rec. blood flow). The lumbar sympathetic trunk (LST) was stimulated electrically (stim. LST) and the arterial chemoreceptors were stimulated adequately (stim. chemoreceptor) by ventilating the animals with a hypoxic gas mixture of 8% O_2 in N_2.

magnetic tape for off-line analysis. After having passed a window discriminator neural activity was converted into unitary counts and read into a laboratory computer (Minc PDP 11) to construct peristimulus histograms.

The lumbar sympathetic trunk was stimulated with single supramaximal pulses of 0·2–0·5 ms duration at variable frequencies. Postganglionic axons were identified by electrical stimulation at a frequency of 0·1–0·2 Hz.

Measurement of cutaneous blood flow

Cutaneous blood flow through the dermis of the hairless skin of hindpaw was measured using the laser–Doppler technique (Periflux; Perimed) (Fig. 1). A time constant of 1·5 s and amplification factors of 1 or 3 were used. The laser–Doppler flow meter used does not give an absolute value for blood flow per volume of tissue. However, the relative change of flow is linearly related to a voltage reading [V^*] and to the number and velocity of circulating red blood cells under the probe whereby the complete absence of flow corresponds to a value of 0 V (see also Jänig & Lisney, 1989).

Manoeuvres affecting sympathetic activity and cutaneous blood flow

Blood flow and neural activity were measured during electrical stimulation of the lumbar sympathetic trunk and natural stimulation of arterial chemoreceptors. A reversible local anaesthetic block could be performed by topical application of a small cotton pledget soaked in 2% lidocaine (Xylocaine) onto the sural nerve. The lumbar sympathetic trunk was stimulated with trains of supramaximal pulses ranging between 1/64 and 25 Hz. Arterial chemoreceptors were stimulated by ventilating the animals with an hypoxic gas mixture of 8% O_2 in N_2 for a period of 2–8 min (Fig. 1; Gregor & Jänig, 1977). The hypoxic stimulus was terminated when a strong increase of arterial blood pressure and reflex bradycardia had been elicited. Peak increase or decrease of blood flow are given as relative changes to the resting blood flow which was measured over an interval of 2 min prior to each stimulus. The baseline of on-going sympathetic activity was averaged over the minute before the stimulation of arterial chemoreceptors. During hypoxia, the mean frequency of the sympathetic activity was calculated for the third minute of the hypoxic stimulus and values are given as the relative change of sympathetic activity compared to the baseline frequency. Both, the animal maintenance and the neurophysiological data analysis were identical to previous experiments from our laboratory (Blumberg & Jänig, 1985) and allow therefore a direct comparison with the results obtained in the present study.

RESULTS

Evidence for re-innervation of the hindpaw

The hindpaw of all five operated cats had been reinnervated by the sural nerve. Prior to the terminal experiments a flexion reflex could be elicited in non-anaesthetized animals by pin-prick applied on the plantar surface of the foot pad which is normally innervated by the tibial nerve and which had been invaded by sural afferents in the operated animals. The regeneration of primary afferent neurones into the new territory could also be demonstrated in anaesthetized cats by whole nerve recording of neural activity proximal to the nerve suture which was evoked by brushing or light mechanical deformation of both the hairy and non-hairy skin of the hindpaw. Sympathetic fibres had also functionally reinnervated cutaneous blood vessels of the paw pad. Electrical stimulation of the lumbar sympathetic trunk (LST) elicited in all animals strong vasoconstriction as measured by a decrease of voltage readings by the laser–Doppler meter (Fig. 2). Even at very low frequencies, which are below the range of the physiological discharge frequencies of most individual vasoconstrictor fibres (Jänig, 1985), a profound vasoconstriction was observed. Typically, the vasoconstriction elicited by single pulses fused at frequencies of 1/16–1/8 Hz and higher frequencies resulted in further absolute decrease of blood flow at the cost of a shorter duration (Fig. 2). This response is quantitatively remarkably similar to the observations made in normal control animals (Jänig & Koltzenburg, 1989) and suggests that many postganglionic fibres had regenerated and had achieved working contacts with blood vessels.

Further, vasoconstriction evoked by electrical LST stimulation was in all cases completely abolished by an impulse conduction block in the sural nerve which was performed by pledgets soaked in lidocaine and applied to the sural nerve proximal to the lesion site. This demonstrates that the vasoconstrictor neurones supplying the hindpaw travelled exclusively through the sural nerve at the mid-calf level. The local anaesthetic block produced an increase of blood flow ranging from 1·4 to 3·5 V corresponding to a change of 177 % (range 83–255 %) from the baseline (Fig. 3). The blood flow remained on the elevated level as long as the block was applied. In the presence of the block there were only slight spontaneous fluctuations of the cutaneous blood flow. After the cotton pledget was removed and the nerve rinsed with saline, blood flow decreased again to a pre-block level within 9–12 min. An increase of blood flow during nerve paralysis can also be observed in normal animals and corresponds to the blockade of the neurogenic component of vasomotor tone conveyed by on-going activity of sympathetic vasoconstrictor fibres. The blood flow increase during a nerve conduction block in operated animals therefore implies the presence of on-going activity of regenerated postganglionic fibres which maintain some degree of cutaneous vasoconstriction in the hindpaw.

Correlation of sympathetic activity and cutaneous blood flow

Although the synchronous excitation of sympathetic neurones produced vaso-constriction there was also evidence that the increase of the on-going asynchronous activity in sympathetic neurones led to a coincident reduction of blood flow. Brief

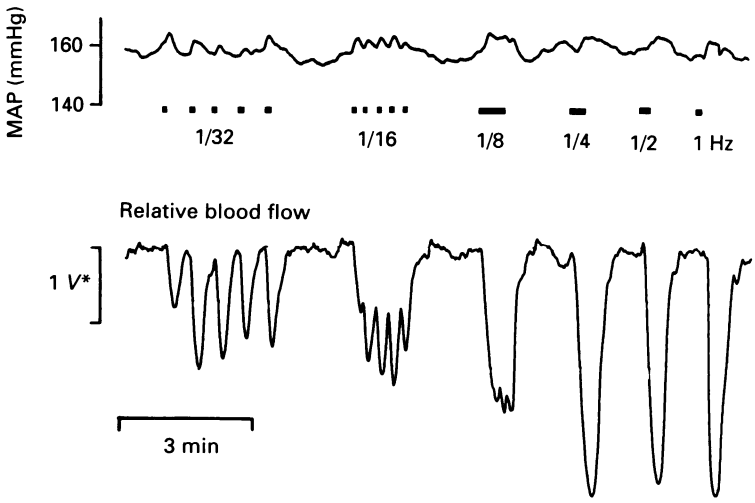

Fig. 2. Changes of relative blood flow through the hairless skin of the central pad of the cat hindpaw after electrical stimulation of the lumbar sympathetic trunk (LST) in an animal with nerve cross-union. The LST was stimulated between the paravertebral ganglia L4 and L5 with trains of five pulses (10 V, 0·2 ms) at intertrain intervals ranging from 32 to 1 s. The stimulus coincided with transient increases of the mean arterial blood pressure (MAP; upper trace).

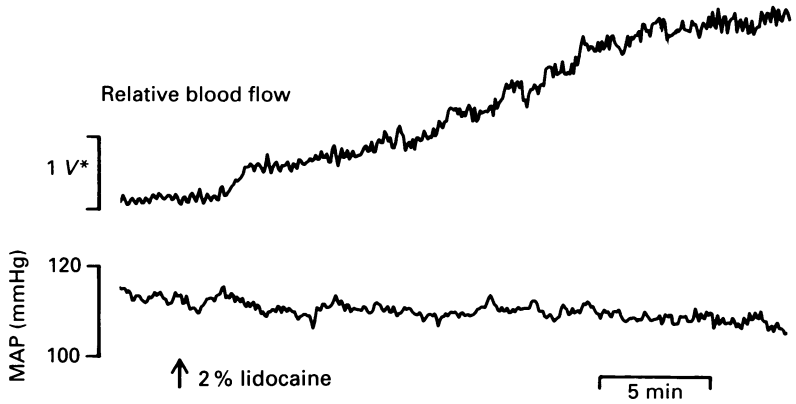

Fig. 3. Increase of blood flow after blockade of impulse conduction in the sural nerve by lidocaine (2% applied in soaked cotton pledget) in a cat with nerve cross-union. MAP, mean arterial blood pressure.

trains of supramaximal high-frequency stimulation of preganglionic axons in the lumbar sympathetic trunk (50 impulses at 25 Hz) of normal animals elicits a prolonged increase of asynchronous activity in postganglionic vasoconstrictor fibres that outlasts the initial stimulus by several minutes (Jänig, Krauspe & Wiedersatz, 1982; Blumberg & Jänig, 1983). This is caused by a non-nicotinic effect on the ganglionic transmission which precipitates a transient increase of the discharge frequency of postganglionic fibres (Jänig *et al.* 1982). This phenomenon was also

found in the operated animals of the present study and we have used it as a tool to transiently increase sympathetic activity and monitor parallel changes of blood flow. Generally, there was a good fit between the time course of increased postganglionic activity and the decrease of blood flow (Fig. 4). Thus, the recorded sample of

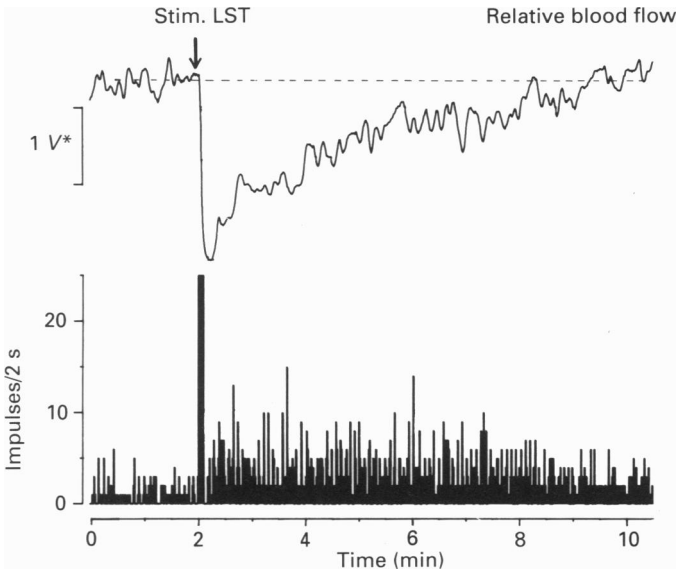

Fig. 4. Relation between blood flow changes (upper trace) and activity of postganglionic sympathetic neurones (lower trace). The preganglionic axons in the lumbar sympathetic trunk (LST) were stimulated for 2 s with fifty supramaximal pulses (5 V, 0·2 ms). This evoked a short-lasting high-frequency burst of the postganglionic fibres and a long-lasting increase of the on-going sympathetic activity that decreased slowly. There was an initial complete vasoconstriction that decreased subsequently in parallel to the decrease of the on-going neural activity.

postganglionic fibres were likely to represent the reaction of a large population of sympathetic efferents. Although there was a good general correlation between increase of sympathetic activity and vasoconstriction, small fast fluctuations of activity in postganglionic fibres were not necessarily followed by decreases of blood flow. Similar discrepancies are observed in normal animals and may be explained by summation and fusion of neuroeffector transmission and the asynchronous discharge pattern of cutaneous vasoconstrictor neurones. Therefore the discharge of sympathetic efferents which happened to be recorded may not be completely identical with that of neurones supplying the tissue under the probe of the laser–Doppler. Furthermore, blood flow is also influenced by non-neural local control mechanisms and some small fluctuations of the flow persist even when sympathetic activity to the target has been completely interrupted by a local anaesthetic block (Fig. 2). However, we cannot rule out the fact that some postganglionic axons from which we have recorded had failed to cross the lesion site.

Thus, the results indicated that a large proportion of sural sympathetic neurones had functionally reinnervated their new target and that there was generally a good

correlation between the on-going postganglionic activity and blood flow changes of the paw pad.

Stimulation of arterial chemoreceptors

Response of cutaneous blood flow

In normal control animals, there is a reflex increase of blood flow through the hairless skin when the arterial chemoreceptors are stimulated (Figs 5 and 6). This

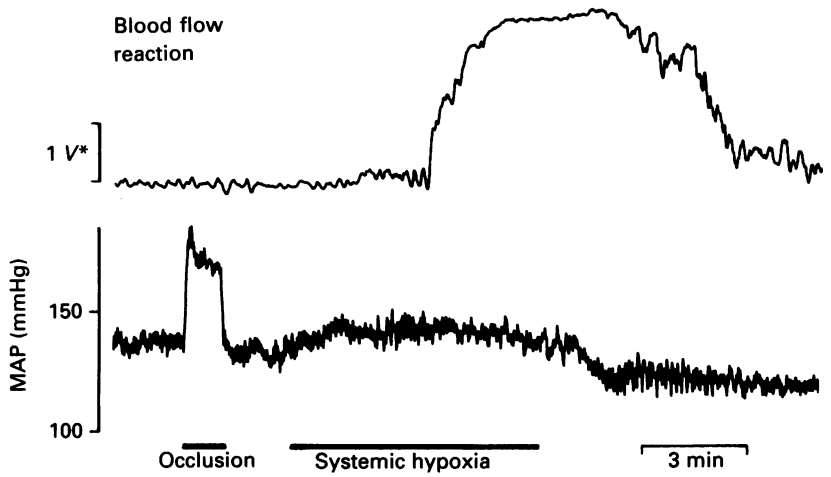

Fig. 5. Increase of systemic blood pressure provoked by bilateral occlusion of the carotid artery (unloading of arterial baroreceptors of the carotid sinus) has little effect on the blood flow through the hairless skin of the paw. However, a strong increase of the flow in the absence of a large coincident blood pressure increase was elicited by systemic hypoxia (8% O_2 in N_2).

increase is probably not passively mediated by the elevation of the arterial perfusion pressure, but is an active neural process caused by the withdrawal of on-going activity in vasoconstrictor neurones supplying the hindpaw (Jänig & Kümmel, 1981). The passive component contributing to blood flow fluctuations through the dermis is only small as evidenced by the different time course of the change in blood flow and mean arterial pressure (Fig. 5). Further, the strong increase of arterial pressure elicited by an unloading of the arterial baroreceptors in the carotid sinus during a short period of bilateral carotid occlusion results in negligible changes in cutaneous blood flow (Fig. 5). Moreover, relatively small increases of blood pressure accompany occasionally systemic hypoxia, but a strong vasodilatation can none the less be observed under these conditions (Fig. 6). This is in agreement with direct recordings from cutaneous vasoconstrictor neurones which have determined that arterial baroreceptors have little effect on the on-going activity in contrast to the strong inhibition evoked by arterial chemoreceptors (Jänig, 1985).

In contrast to the vasodilatation observed in normal animals, there was a marked decrease of cutaneous blood flow in operated animals during systemic hypoxia (Fig. 6). Vasoconstriction was observed in all operated animals. Like the increase of blood flow in the control animals the decrease in lesioned animals was delayed with respect

to changes of blood pressure. Figure 7 summarizes the changes seen in all control and experimental animals. The values give the maximal differences of the flow at the end of the hypoxic stimulus compared to the pre-hypoxia baseline. The laser–Doppler readings fell on average by 2·3 V corresponding to a mean reduction of 54 % (range

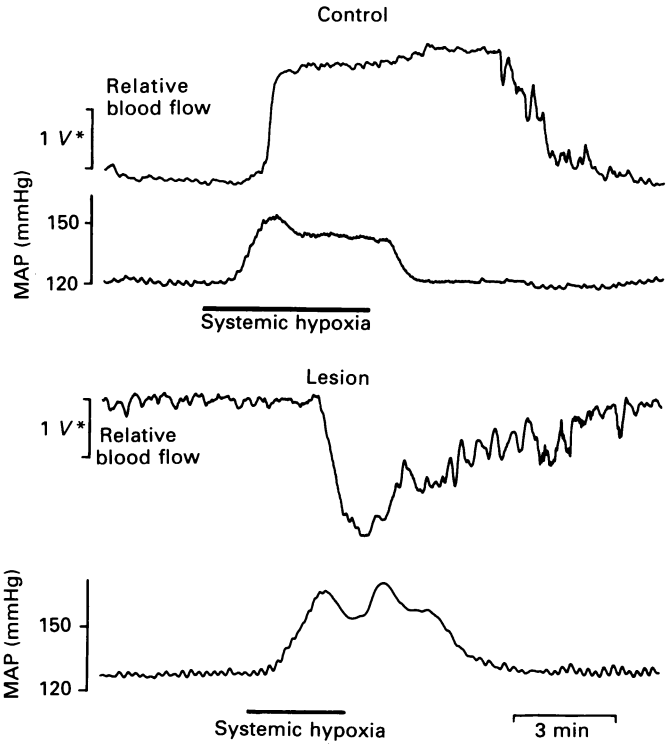

Fig. 6. Change of blood flow in the hairless skin of the central pad of the left hindpaw during stimulation of the arterial chemoreceptors by systemic hypoxia (8 % O_2 in N_2) in a control animal and in an animal with nerve cross-union.

11–77 %) from baseline level in lesioned animals. This may be an underestimate of the maximal difference, as in some animals blood flow increased or fell even further in normal and lesioned animals, respectively, when the stimulus had been terminated (see Fig. 5). This is expected, as there is a time lag before the animals have regained normal systemic oxygen saturation.

Response of postganglionic fibres

The reversal of the reflex control of cutaneous blood flow suggested that there was a qualitative change of neural activity in sympathetic neurones. In normal animals systemic hypoxia results in a reflex decrease of on-going activity of most (80–90 %) postganglionic fibres innervating the hairy and hairless skin (Jänig, 1985). However, in the experimental animals of the present series more than 90 % of the neurones were excited.

Strands containing postganglionic axons were isolated from the sural nerve and the filaments contained several postganglionic axons as evidenced by electrical

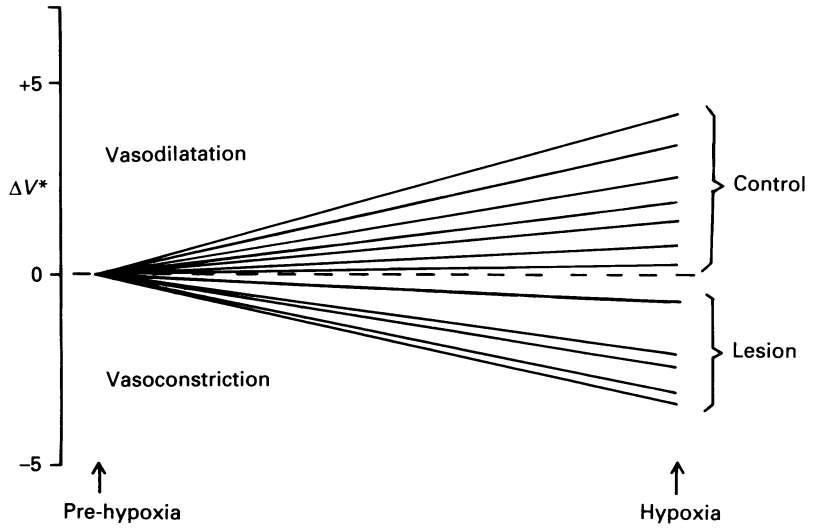

Fig. 7. Maximal changes of blood flow through the skin during stimulation of the arterial chemoreceptors by hypoxia at the end of the stimulus. Each line represents one experiment.

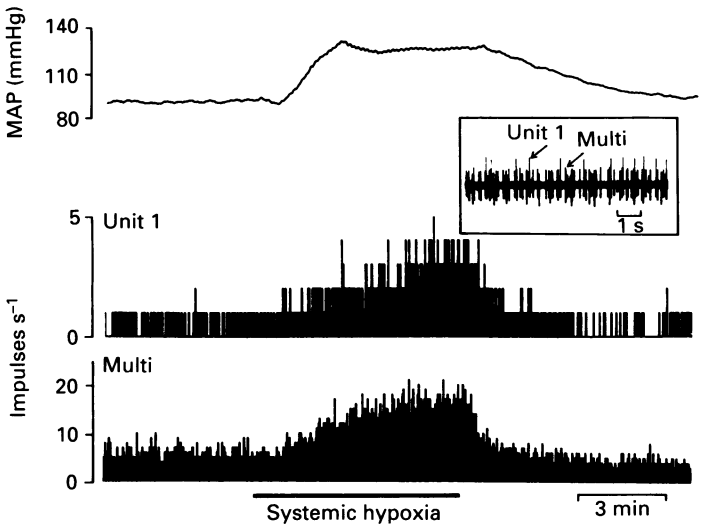

Fig. 8. Excitation of postganglionic fibres of the sural nerve by stimulation of arterial chemoreceptors. Inset shows the original record. Lower trace: histogram of multi-unit activity (multi in inset). Middle trace: activity of a single unit (unit 1 in inset). Upper trace: mean arterial blood pressure (MAP).

stimulation of the lumbar sympathetic trunk with single pulses. Recordings from thirteen filaments with postganglionic activity were analysed. In twelve strands activity increased during stimulation of arterial chemoreceptors whereas it decreased in one. Figure 8 illustrates a representative experiment. This filament contained several postganglionic fibres, but a single unit could be separately analysed from the

remaining multi-unit activity. Systemic hypoxia produced an increase of both single and multi-unit activity with a remarkably similar time course.

The quantitative analysis revealed that sympathetic neurones could on average increase their discharge frequency by a factor of 4·1 (range 1·5–13·6) during the

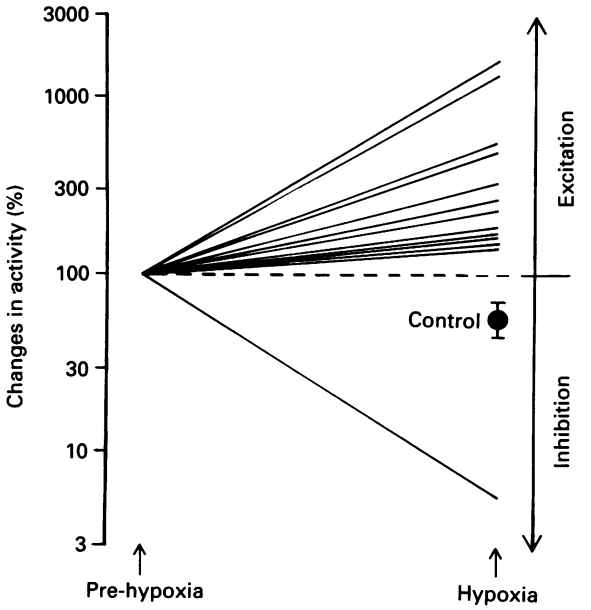

Fig. 9. Changes of the activity of postganglionic fibres projecting into the cross-unioned sural nerve during stimulation of arterial chemoreceptors by systemic hypoxia. The ordinate scale shows the relative changes of the postganglionic activity taking the pre-stimulus on-going activity as a baseline of 100%. Each line represents one filament. The dot and vertical bars give the mean and two standard deviations of the control data (reactions of postganglionic neurones recorded from fifty strands projecting to the hairy skin of the normal cat hindlimb). Data were taken from Blumberg & Jänig (1985). $n = 13$.

stimulus as compared to the pre-hypoxia baseline level. Using an identical data analysis the decrease of the activity in most cutaneous vasoconstrictor neurones innervating hairy skin has been previously determined in our laboratory and is given as mean ± 1 standard deviation (Blumberg & Jänig, 1985).

DISCUSSION

The results of the present study show a nearly complete qualitative reversal of the reflex organization of sympathetic neurones. The alteration of the chemoreceptor reflex observed in the vasoconstrictor neurones is well correlated with corresponding changes of the blood flow through the skin of the paw. This indicates that the aberrant discharges of the sympathetic efferents did not run idle, but entailed significant consequences for the control of the sympathetic effector organs.

In normal animals sympathetic neurones supplying hairless skin react very similarly to those innervating the hairy skin (Jänig & Kümmel, 1981; Jänig, 1985).

Thus, the sympathetic efferents which had regenerated to the foot pad displayed a reflex response that was also inappropriate for their new target. This is unexpected, because previous studies have shown that regenerating cutaneous vasoconstrictor neurones do not change appreciably when they grow back into skin (Blumberg & Jänig, 1985). However, the abnormal reflex response of the present study is not transient, but probably a permanent change, as it was measured in animals about a year after the cross-union of the sural to the tibial nerve. Supposing that regeneration occurred at a speed of 2–4 mm day^{-1} (Sunderland, 1978) the paw should have reinnervated within 3–4 months after the nerve suture.

There are principally two mechanisms that could have contributed to the reorganization of the sympathetic reflex function. They include the direct action on axotomized postganglionic neurones and more indirect sequelae mediated by the simultaneously severed sensory system. As shown by the elegant studies of Purves and colleagues (Purves & Lichtman, 1978; Purves, 1988; Purves, Snider & Voyvodic, 1988) the geometry of postganglionic neurones is regulated by long-term trophic interaction between targets and neurones throughout life influencing the axonal branching pattern in the periphery as well as the dendritic arborization in sympathetic ganglia. In particular, axotomy of postganglionic neurones causes retraction of preganglionic terminals, shrinkage of ganglionic dendrites and consequently a depression of synaptic potentials (Matthews & Nelson, 1975; Purves, 1975; Yawo, 1987). The dendritic arbors gradually expand and synaptic efficacy is reinstated when postganglionic fibres regenerate and successfully contact peripheral targets, but not when outgrowth of transected fibres is permanently prevented (Purves, 1975; Yawo, 1987). However, axotomized postganglionic fibres have little, if any ability to regenerate specifically to their appropriate target and there appears to be also no respecification of the preganglionic synaptic input, if postganglionic axons fail to contact their appropriate target tissue (Purves & Thompson, 1979). In aggregate these findings indicate that the qualitative reflex change observed in the present study are probably not the consequence of an inappropriate respecification of the preganglionic input within the sympathetic ganglia during regeneration.

It also seems unlikely that the reflex changes were the consequence of the selective survival of a small class of sympathetic neurones that is already excited by systemic hypoxia in non-lesioned animals. Firstly, this does not happen when cutaneous sympathetic efferents are permanently isolated from their original target (Blumberg & Jänig, 1985). Secondly, postganglionic fibres are not very discriminating, but readily contact target opportunities along whatever path they happen to follow (Langley, 1897; Purves & Thompson, 1979). Finally, the cat sural nerve contains only about one-third of the fibres than the tibial nerve and thus fewer postganglionic fibres have the opportunity to innervate a relatively vast territory (McLachlan & Jänig, 1983) and suturing a small nerve to a large stump is thought to favour regeneration and to minimize degeneration of unmyelinated fibres (Aldskogius, Arvidsson & Grant, 1985; Lisney, 1989).

Thus, factors that are beyond the direct effect on postganglionic fibres need consideration. For a number of reasons the reinnervation by primary afferent neurones was neither quantitatively nor qualitatively comparable to the normal innervation pattern. As evidenced by the whole nerve recordings primary afferent

fibres had successfully negotiated the lesion and had established working connections in the new territory. Yet, it is evident that the number of sural neurones innervating the tibial territory means a low density of afferents (McLachlan & Jänig, 1983). Moreover, after the cross-union the regenerating cutaneous primary afferents meet appropriate, but also inappropriate targets from which they can be readily activated (Lewin & McMahon, 1988; Banks & Barker, 1989). This means that there are mismatches between the peripheral receptor properties and the topography of their central connections (McMahon & Moore, 1988; Banks & Barker, 1989; Koerber, Seymour & Mendell, 1989) and this can include a change of the type and amount of neuropeptide stored by these fibres (McMahon & Gibson, 1987). Furthermore, some afferent neurones fail to cross the nerve suture altogether and form a local neuroma at the suture site (Häbler, Jänig & Koltzenburg, 1987) and it is well established that these aberrant sprouts acquire novel properties such as ectopic discharges or a sensitivity to endogenous catecholamines (Jänig, 1988; Jänig & Koltzenburg, 1990). Such changes will eventually lead to a distortion of central sensory maps extending from the spinal cord (Wall, 1987; Devor, 1988; Lewin & McMahon, 1989) up to the cortex (Merzenich, Recanzone, Jenkins, Allard & Nudo, 1988; Wall, 1988).

Importantly, a functional respecification of the spinal reflex organization can occur when nerves are severed and rerouted to an inappropriate target tissue and such heterosynaptic malleability has been demonstrated for the flexion reflex of the adult rat. In normal animals this reflex is briefly facilitated by electrical stimulation of a cutaneous nerve, but a more prominent and sustained facilitation can be elicited when the same conditioning stimulus is applied to a muscle nerve (Wall & Woolf, 1984). After forcing the skin nerve to innervate the muscle the facilitating potency of that nerve is then greatly enhanced and practically identical to the normal muscle input (McMahon & Wall, 1989). This raises the possibility that the reflex changes in the sympathetic system are of a similar kind and the consequence of perturbing the spinal reflex organization. It could mean that the descending pathways that transmit the afferent information of the chemoreceptor reflex impinge on an altered spinal circuitry that has not been adequately repaired after the nerve injury. This could also reconcile the results of the present study with previous findings that did not find reflex alterations when a severed cutaneous nerve was allowed to grow back to skin. In the present study an appreciable number of primary afferents in the nerve had to make contacts with inappropriate tissue which would not have happened, if the whole nerve had grown back to its original territory.

The reorganization of sympathetic reflex function that has been demonstrated in the present study bears some importance on the pathogenesis of trophic disturbances that follow nerve trauma (Jänig, 1990). The cutaneous vascular bed is particularly concerned with thermoregulation. Shunt and capacitance vessels are predominantly under neural control of cutaneous vasoconstrictor neurones and are less influenced by local metabolic or myogenic processes (Jänig, 1985, 1990). Under normal conditions the activity of this system is largely controlled by hypothalamine centres. Studies on some patients suffering from reflex sympathetic dystrophy demonstrate a severe deficit of thermoregulation in the affected limb although cutaneous blood vessels are innervated by sympathetic fibres (Blumberg, Griesser & Hornyak, 1990). This invites speculations that a chronically inadequate discharge of cutaneous

vasoconstrictor neurones and an abnormal state of the reflex organization precipitates tissue disturbances and may contribute to pathology that can follow nerve lesions.

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