Neuronal regulation of cochlear blood flow in the guinea-pig

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1. Previous studies have shown that electrical stimulation (ES) of the guinea-pig cochlea causes a neurally mediated increase in cochlear blood flow (CBF). It is known that the centrifugal neuronal input to the cochlea comes through the perivascular sympathetic plexus from the cervical sympathetic chain and along the vestibular nerve (VN) from the periolivary area of the brainstem. Both of these neuronal systems are distributed topographically in the cochlea.

2. In order to study the neural origins of ES-evoked CBF increase, laser Doppler flowmetry was used to test the following hypotheses. (a) The response is regional, that is, limited to the area of the cochlea stimulated. To test this we performed differential ES of the cochlear turns. CBF was measured from either the third or the first turn. (b) The response is mediated via autonomic receptors within the cochlea. To study this, we applied atropine, succinylcholine and idazoxan locally to the cochlea. (c) The response is influenced by neuronal input via the sympathetic cervical chain (SC) and components of the VN. We stimulated and sectioned the SC, and sectioned the VN, to test this hypothesis.

3. We observed that the CBF response was topographically restricted to the stimulated region. Locally applied muscarinic or nicotinic antagonists (atropine and succinylcholine respectively) did not affect the response. However, local idazoxan (an α_2-blocker) eliminated the response. Locally applied adrenaline and SC stimulation modified the dynamic range of the response. SC sectioning enhanced the responsiveness of the cochlear vasculature to ES. The VN section caused a temporary decrease in CBF and elimination of the ES-evoked CBF response.

4. We conclude that the release of dilating agents is topographical with respect to ES current flow, the ES-evoked CBF increase is peripherally mediated via α_2-receptors, and the response is influenced by input via the SC. The elimination of the response by VN sectioning proximal to the brainstem indicated that fibres of the VN mediate the CBF increase during direct cochlear ES. The data suggest that these fibres may be the efferent limb of a neural loop involved with the regulation of CBF. Such a system could provide a mechanism for the rapid increase in CBF with organ stress.

Electrical stimulation (ES) of the guinea-pig cochlea causes an increase in cochlear blood flow (CBF) as measured by the laser Doppler flowmeter (LDF) or intravital microscopy (LaRouere, Sillman, Nuttall & Miller, 1989; Sillman, LaRouere, Masta, Miller & Nuttall, 1989*a*; Sillman, Masta, LaRouere, Nuttall & Miller, 1989*b*). Increased CBF, induced by ES, has also been reported in humans (Miller, Bredberg, Grénman, Suonpää, Lindström & Didier, 1991). The evoked response appears to be neurally mediated, and it is maximized by an appropriate combination of frequency and intensity of ES. The roles of evoked metabolic activity, oxygen-free radical formation, and heat in this response were examined in previous studies (Sillman *et al.* 1989*b*). No evidence was obtained to support

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a significant role for any of these factors. Only pharmacological blockade of all autonomic, afferent and efferent fibres in the cochlea by tetrodotoxin applied to the round window (RW) eliminated the response (Sillman *et al.* 1989*b*), indicating that the response is neurally mediated.

The influence of the autonomic nervous system in controlling CBF has long been a matter of controversy. Sympathetic input to the cochlea has been suggested as an explanation for a number of physiological observations as well as for cochlear pathology (Hultcrantz & Angelborg, 1978; Miller, Hultcrantz & Short, 1986; Miller & Dengerink, 1988). Yet, until recently, physiological evidence to support a significant role of the sympathetic system in the control of CBF has been scant. Using the microsphere method, Hultcrantz & Angelborg (1978) reported that ES of the superior cervical ganglion in the sympathetic cervical chain (SC) caused a 25% decrease in CBF. Recently, this has been confirmed by Ren, Laurikainen, Quirk, Miller & Nuttall (1993) who demonstrated a 15% decrease in ipsilateral CBF with ES of the superior cervical ganglion, and by Laurikainen, Kim, Didier, Miller, Nuttall & Ren (1993) who reported a 10–15% decrease in bilateral CBF with ES of the stellate ganglion. An adrenergic basis for the evoked decrease in CBF with stellate ganglion stimulation was demonstrated by elimination of the response with a locally applied α-blocker. Neuroanatomic investigations of the cochlea (Spoendlin & Lichtensteiger, 1966; Spoendlin, 1981), demonstrating sympathetic perivascular and blood vessel-independent nerve fibres originating from the cervical chain, provide an adequate anatomical basis for each of the physiological observations. Moreover, Brechtelsbauer, Prazma, Garret, Carrasco & Pillsbury (1990) have confirmed the perivascular catecholaminergic innervation of the cochlea. They also found a wide distribution of these nerve endings along supplying vessels in all cochlear turns.

The *increase* in CBF with direct ES of the cochlea is paradoxical and may be based on several possible mechanisms that have yet to be assessed. (1) ES may cause direct depolarization of vascular smooth muscle, leading to vasodilation. (2) ES may release a local or systemic vasoactive factor (e.g. peptides, metabolic products, etc.) affecting vascular smooth muscle directly or indirectly. (3) ES may cause vasodilation via autonomic stimulation, involving local or centrally originating neural control, e.g. sympathetic inhibitory feedback control. (For a review of such a mechanism, see Vanhoutte, Verbeuren & Webb, 1981.)

Regarding the first point, transmural ES of non-cochlear blood vessels has been used extensively since this method was described (Patterson, 1965). Most isolated blood vessels contract when stimulated electrically (Vanhoutte *et al.* 1981). However, ES of certain blood vessels *in vitro* can cause relaxation of chemically precontracted smooth muscle cells (Kalsner, 1974; Lee, Su & Bevan, 1976; Duckles & Silverman, 1980). Thus, it is theoretically possible that ES may cause direct smooth-muscle relaxation, depending on the innervating fibre system and stimulation conditions. Rooke, Cohen, Verbeuren & Vanhoutte (1982) demonstrated that the relaxation of the chemically contracted dog coronary artery with ES was dependent on the intensity, frequency and duration of the stimulus. Silverman & Jenden (1978) showed by *in vitro* experiments that 0·01–1 kHz stimulation frequency activates the intramural nerve fibres with no obvious effects on the muscle cells. Sillman *et al.* (1989*a*) showed that the ES-evoked increase in CBF occurred with frequencies of 0·01–10 kHz, with the optimal response occurring at 0·5 kHz. Furthermore, the effect of ES on CBF was eliminated by locally applied procaine and tetrodotoxin. Tetrodotoxin is known to specifically affect Na^+ channels, inhibiting the initial fast component of the action potential; it does not have an effect on motor endplates, smooth muscle membrane or cell soma (Lombard, Burke, Contney, Willems & Slekiel, 1982). Thus, the ES effect probably depends upon nerve fibres and propagated action potentials. A direct relaxing effect of ES on smooth muscle fibres in CBF response appears to be unlikely.

Regarding the second mechanism, locally released peptides and/or systemic hormones could evoke an increase in CBF. Calcitonin gene-related peptide (Hillerdal & Andersson, 1991), substance P (McLaren *et al.* 1993), and systemic and topical administration of sodium nitroprusside (by *in vivo* conversion to nitric oxide (also known as endothelial-derived relaxing factor); Ohlsén, Didier, Baldwin, Miller, Nuttall & Hultcrantz, 1991*b*) are reported as increasing CBF after systemic and topical administrations (Ohlsén *et al.* 1991*b*). However, since the ES-evoked CBF response can be blocked with procaine and tetrodotoxin, the vasodilator responsible must be released by neurons and as a result of ES-evoked neural activity.

Regarding the third possibility, autonomic neurons described within the cochlear nerve and vestibular nerve (VN) could provide the anatomical basis for an ES-evoked CBF increase (Spoendlin & Lichtensteiger, 1966; Ross, 1969). Using plethysmography, Suga & Snow (1969) found that atropine sulphate (which blocks the muscarinic receptors when administered I.V. in guinea-pig), slightly decreased CBF while systemic blood pressure increased. Sillman *et al.* (1989*b*) administered atropine and gallamine (which blocks the nicotinic receptors) by systemic injection prior to stimulation without effect on the ES-evoked CBF response. However, Smith, Brown, Toman & Goodman (1947) showed that neuromuscular blocking agents (quaternary amines) are virtually devoid of central effects following the I.V. administration even of excessive doses because of the inability of these drugs to penetrate the

blood–brain barrier. Access by such blocking agents to the cochlear vascular receptors might be similarly inhibited. Thus, a more valid test of the effects of the anticholinergic agents on the ES-evoked CBF response would be performed by local infusions of the agents into the cochlea or applications of these drugs on the RW, rather than by systemic administration.

These considerations led us to design a study examining the cholinergic and adrenergic blockade of ES-evoked CBF change. Since the autonomic fibres to the cochlea and the blood vessels within the cochlea demonstrate topographical organization (Spoendlin & Lichtensteiger, 1966; Axelsson & Vertes, 1978; Brechtelsbauer *et al.* 1991), we examined the regional nature of the ES-evoked CBF response. In addition, the role of the centrifugal efferent nerve fibres going to the cochlea was studied by sectioning the SC and VN.

We found that the increase was topographically related to the stimulated area of the cochlea, that the ES-evoked CBF increase was locally mediated via α-adrenergic receptors (cholinergic (muscarinic or nicotinic) receptors within the cochlea did not influence this phenomenon), and that both the sympathetic fibres from the SC and the fibres passing along the VN affected this response. These findings lead us to propose an autonomic adrenergic model to explain our observations.

Part of this work was presented at the 14th Midwinter Meeting of the Association for Research in Otolaryngology held in 1992 at St Petersburg, FL, USA (Laurikainen *et al.* 1992).

METHODS

Animals and surgical procedure

Twenty-three pigmented guinea-pigs of either sex, weighing 250–350 g, were used. All animals had normal Preyer reflex and were free from middle ear infection. The study was consistent with NIH guidelines for humane treatment of animals and was reviewed and approved by the University of Michigan Committee on Use and Care of Animals. The animals were anaesthetized with pentobarbitone sodium (0·6 mg kg^{-1} I.P.) and Innovar-Vet, a mixture of fentanyl (0·9 mg kg^{-1}) and droperidol (75·0 mg kg^{-1}) given I.M., with supplemental fentanyl–droperidol doses (0·45 mg kg^{-1} h^{-1} fentanyl–32·5 mg kg^{-1} h^{-1} droperidol I.M.) to maintain an appropriate level of anaesthesia. Each animal was wrapped in a heating pad with a feedback system to maintain normal core temperature (38 ± 1 °C).

Following tracheotomy, the carotid artery was cannulated for mean systemic blood pressure (BP) measurement. The head was fixed in a head restraint containing a feedback-regulated heating element to compensate for heat loss in the otic capsule due to middle ear exposure. The bulla was exposed via a ventral approach. An opening, of sufficient size to permit visualization of the entire otic capsule, was created. Care was taken to maintain an intact ear drum and middle ear ossicular chain. The mucosa covering the cochlea was removed from the apex and also from areas over the third,

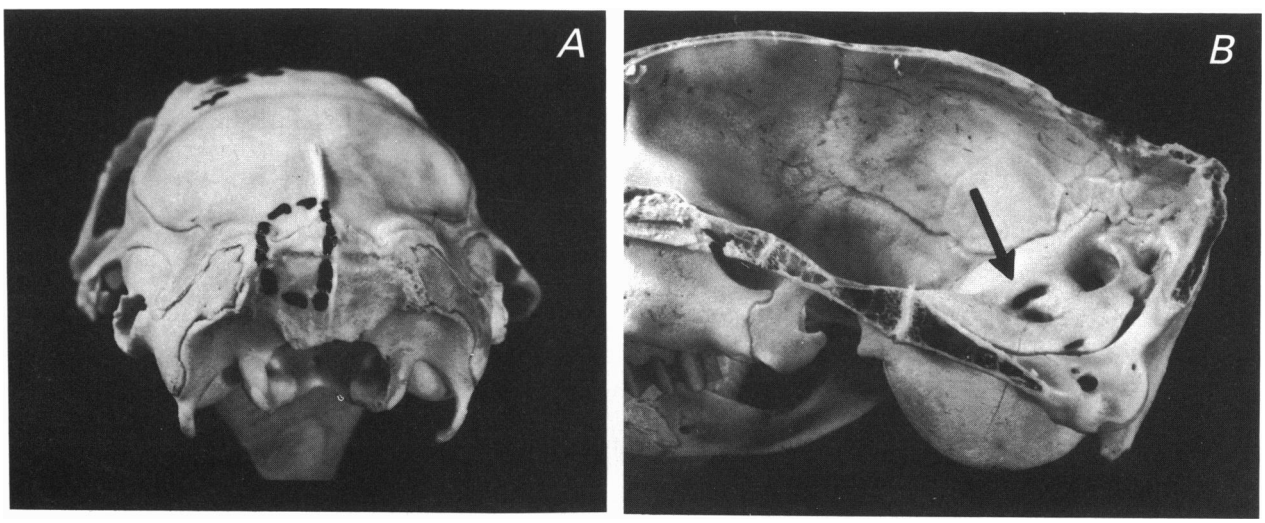

Figure 1. Views of the guinea-pig skull showing the surgical approach and location of the vestibular nerve root

A, part of the parieto-occipital region of the skull (shown by dashed lines) was removed by drilling. The skull opening extended to the external occipital crest (medially), transversal sinus (anteriorly) and the sulcus of the temporal bone (laterally). *B*, a guinea-pig skull transected midline to show the orifices of the vestibular, facial and cochlear nerves. These can be seen through the surgical approach when the brainstem is gently compressed medially.

second and basal turns by gentle wiping with a cotton pledget. Petroleum jelly was used between the laser probe and the bone to improve the optical coupling by excluding blood or fluid accumulation from under the probe.

Cochlear blood flow recording

A TSI laser Doppler flowmeter (VasaMedics Corp., St Paul, MN, USA) was used to measure CBF. The probe diameter was 0·8 mm. This method has been described thoroughly elsewhere (Miller & Nuttall, 1990). The measurements were made over the basal and third turn by placing the LDF probe perpendicular to the stria vascularis. The CBF, BP and a measure of cochlear vascular conductivity (C= CBF/BP) were recorded on a strip chart recorder (model 7404A, Hewlett Packard, CA, USA). Since the LDF output is in arbitrary units, in this study the results of all measurements are given as percentage changes (ΔCBF, ΔBP and ΔC) from the average of the 15 min baseline (ABL) preceding each stimulation period, with ABL set at 100%.

Electrical stimulation

ES was provided by a charge-balanced, sinusoidal, capacity-coupled constant-current stimulator. A stimulus frequency of 500 Hz was used throughout the study and the intensity varied from 10 to 300 μA. The ball-tipped (0·2 mm diameter) electrodes (5 T Teflon-coated platinum–iridium wires) were placed on the RW, through a small fenestra in the bone over the membranous labyrinth of the second turn, and at the apex of the cochlea. Thus, by variously pairing the electrodes, bipolar stimulation of 2–3 min duration was delivered to the sections of cochlea between the second turn and RW, second turn and apex, or apex and RW. The intensity required to cause a 5% increase above the ABL level was defined as the threshold level.

Superior cervical ganglion stimulations were provided with a capacity-coupled constant current stimulator at 6 pulses s^{-1}, a 20 ms pulse width, and at 0·5 mA. A custom-designed bipolar cuff electrode (3 T Teflon-coated platinum–iridium wire) with 1 mm interelectrode separation was used for these stimulations.

Vestibular nerve section

The VN was exposed by removing the parieto-occipital bone from the left side of the skull (Fig. 1*A*). The facial nerve, VN and cochlear nerve run separately into the lateral wall through foramina which are separated by a bony ridge of approximately 1 mm (Fig. 1*B*). This anatomical feature makes the sectioning of the VN possible, without affecting the cochlear nerve. However, to ensure that the cochlear artery remained intact in each animal, the responsiveness of CBF to a systemic BP change induced by a subcutaneous injection of adrenaline (40 i.u.) was tested. Furthermore, local responsiveness of the cochlea was tested with a RW application of 2 μl of 1·5 mM hydralazine, a vasodilating agent known to cause an increase in CBF when applied locally (Ohlsén *et al.* 1991*b*). To further ensure that the labyrinthine artery and cochlear nerve had not been damaged, cochlear sensitivity was evaluated before and after VN sectioning by measuring the compound action potential (CAP) thresholds at 2, 4, 8, 16 and 24 kHz, using a silver wire electrode on the RW and a reference electrode in the neck muscles. Threshold was defined as the sound intensity to elicit a 1·0 μV N_1 (the first negative deflection of the CAP waveform) response from the computerized average of 256 responses (0·3–3 kHz bandpass). The stimulus had a rise/fall time of 1 ms and a duration of 15 ms.

Study protocol

Three different procedures were used as summarized in Table 1.

Protocol I. To test if ES of the cochlea causes a topographical CBF response, the apical or basal portions of the cochlea were selectively stimulated whilst recording the CBF from a cochlear area between, or to the side of, the electrodes. Five animals were used for this part of the study. The stimulations were repeated several times in each preparation while LDF recordings were made over the first or the third cochlear turns.

Protocol II. The effect of altered presynaptic sympathetic input on the ES-evoked CBF response was determined by two manipulations. Increased sympathetic activity (given simultaneously with ES) was provided by electrical stimulation of the superior cervical ganglion. Decreased sympathetic input occurred when the SC was surgically sectioned at the superior cervical level. Three animals were used for both of these tests.

A second approach was taken to alter the state of 'sympathetic activity'. Adrenaline (10 mg ml^{-1}) was topically applied to the RW, and the ES-evoked CBF response was measured 20 min after the application of the drug when the α-agonist effect on CBF had stabilized. Three animals were used for this portion of the study.

To pharmacologically block the adrenergic α_2-receptors, idazoxan (2 μl, 2·0 mM) was applied to the RW in four guinea-pigs.

Table 1. Outline of the study protocols

Protocol I	Protocol II	Protocol III
Topical responsiveness	Pharmacological and neural manipulations	The possible role of neurons in VN
Examined the response of CBF to the ES given in discrete cochlear areas	Examined: (1) the interaction of superior cervical sympathetic activity and direct cochlear ES; (2) the effect of adrenergic (adrenaline and idazoxan) agents on the ES-evoked CBF response	Examined the effect of VN sectioning on the ES-evoked CBF increase
Five guinea-pigs	(1) Three guinea-pigs; (2) seven guinea-pigs, three for transection of the sympathetic chain and four for applications of drugs to the round window	Eight guinea-pigs

ES, electrical stimulation; CBF, cochlear blood flow; VN, vestibular nerve.

Protocol III. To study the influence of cochlear centrifugal fibres on the ES-evoked CBF response, the VN was sectioned in eight animals. Cochlear ES at 100 μA intensity was repeated 2–3 times to obtain a stable response before sectioning. The stimulations were then repeated at 5 and 10 min after VN sectioning. CAP thresholds were also determined before and 30 min after the VN section. Prior to the termination of each experiment, the effect of locally applied hydralazine on CBF was tested to demonstrate vascular reactivity.

RESULTS

Baseline recordings of BP and CBF were made for 15 min prior to any stimulation period. To ensure the responsiveness of the preparation, observations were made with ES prior to collecting experimental data. Only data from preparations that were physiologically stable, with BP > 40 mmHg, and a threshold for the ES-evoked CBF increase < 75 μA were included in this study. The overall range of current to evoke a threshold response in these experiments was 25–75 μA. The stimulus intensity selected for most experiments was 100 μA. This stimulus intensity was at a suprathreshold level, but well within the linear portion of the dynamic range of evoked responsiveness. Furthermore, the effect of the stimulation on BP was slight at this level, causing < 5% increase in BP. In contrast, CBF increased by 10–100% with ES. It is clear that this response resulted directly from the ES and was not secondary to a systemic BP change.

Figure 2 shows the mean effect of ES of the cochlea on CBF in five animals. Stimulations at 100 μA for 2 min were delivered between the electrodes placed on the apex and the second turn area. The CBF recording was made over the third turn. The CBF increase followed stimulation onset with a latency of 8 ± 3 s. The increased CBF was maintained throughout the 2 min stimulation period. Linear regression lines were fitted to the rising (onset) and falling (recovery) portions of the CBF response. The slope of the regression line for the recovery was shallower than that for the onset period (regression slopes 0·98 and −0·44 respectively).

The regionality of electrically evoked cochlear blood flow increase

Figure 3 presents the mean results of stimulating the apical and basal areas of the cochlea on regional CBF change. The LDF recordings were made from the first or third turn of the cochlea. When the laser probe was placed on the third turn and this section of cochlea was stimulated (electrodes on the apex and second turn; Fig. 3*A*), the mean increase in CBF was clearly larger than the change recorded simultaneously from the basal turn section (62 *vs.* 28%). When the laser probe was placed on

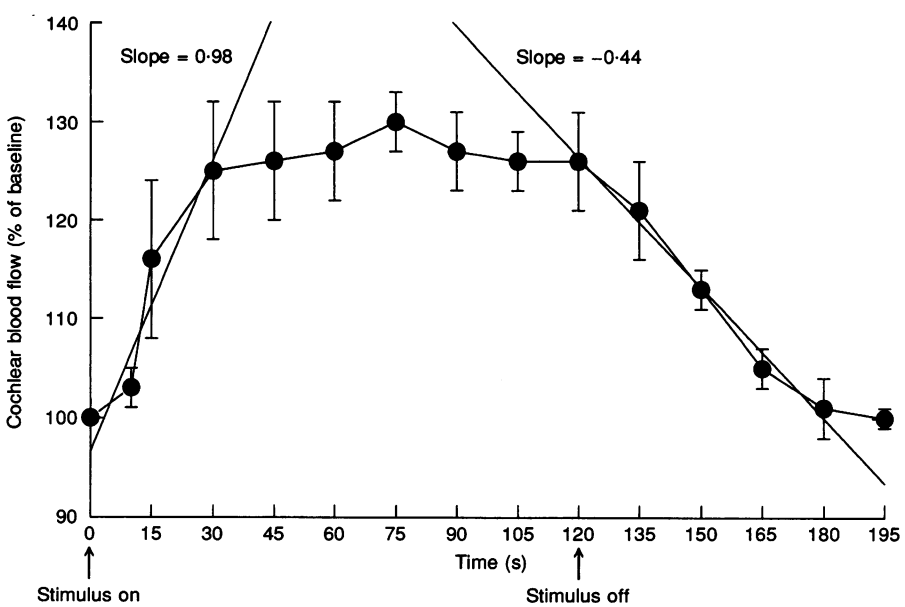

Figure 2. The cochlear blood flow (CBF) response to bipolar electrical stimulation of the guinea-pig cochlea
A 120 s stimulus (indicated by arrows) was given at an intensity of 100 μA. Stimuli were constant-current capacity-coupled sinusoids at 500 Hz. Data points are shown as means ± S.D. from five animals. Electrodes were placed on the apex and second turn and the laser Doppler recording site was over the third turn of the cochlea. Linear regression lines were fitted to the first 30 s following stimulus onset and for 60 s following removal of the stimulus. CBF onset response is faster than the offset.

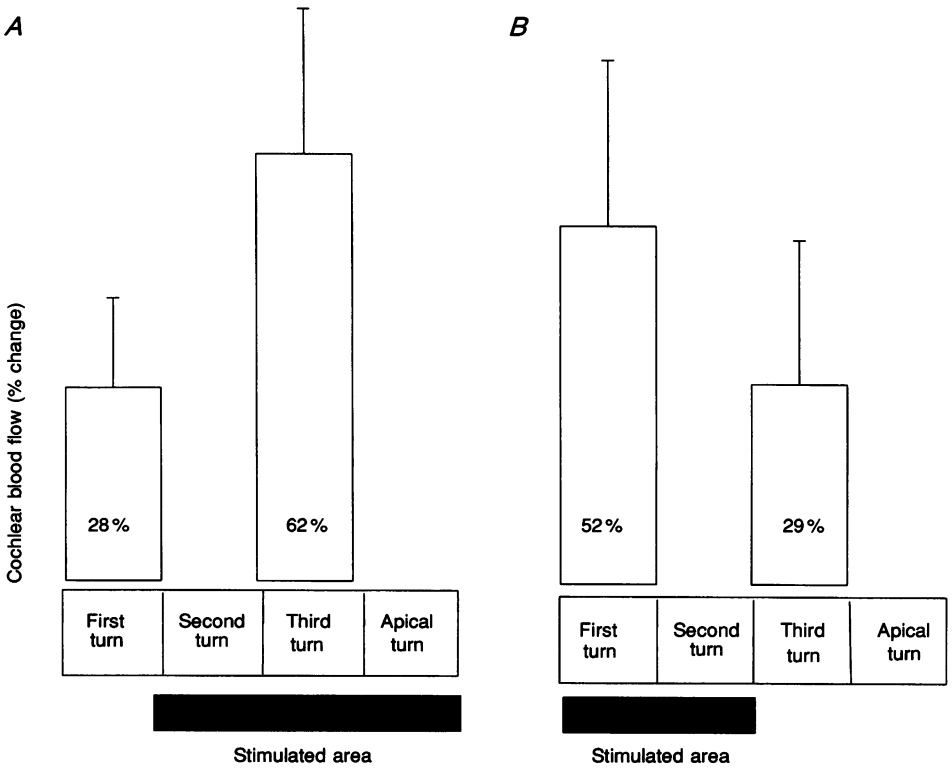

Figure 3. The laser Doppler flowmetry measured increases in CBF at two sites of the guinea-pig cochlea when 100 μA electrical stimulation (500 Hz) was delivered
The electrodes were placed on the apex and second turn (*A*), or the round window and second turn (*B*). Vertical bars are ± 1 S.D. from the mean. The stimulated area of the cochlea is indicated diagramatically by the thick black bars below the graphs. The height of the vertical bars indicates the mean CBF change (given as %) and the vertical lines show 1 S.D. These graphs show that the CBF change is greatest in the region of the cochlea stimulated by the electrode pairs. $n = 5$.

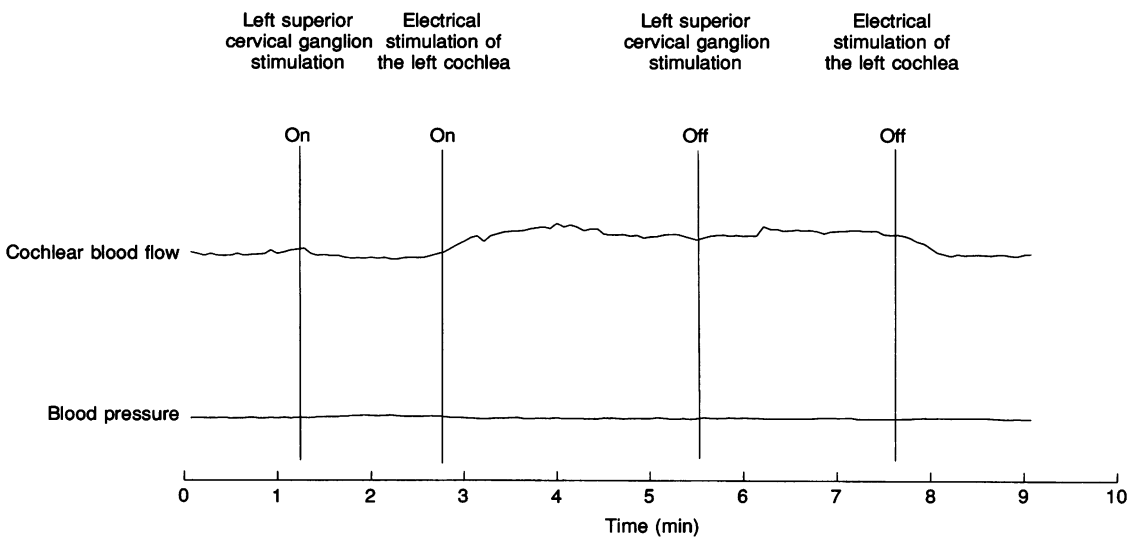

Figure 4. Effects of simultaneous stimulation of superior cervical ganglion and cochlea on CBF and blood pressure
Electrical stimulation of the ipsilateral superior cervical ganglion was at 0·5 mA intensity, 6 pulses s^{-1} and direct electrical stimulation of the cochlea was at 100 μA, 500 Hz.

the first turn and this region was stimulated (electrodes on the RW and second turn; Fig. 3*B*), a 52% increase in CBF was observed. The increase in CBF recorded from the third turn during this stimulation was 29%. Overall, the difference in ES-evoked CBF response between the stimulated and non-stimulated field was statistically significant (Student's paired two-tailed t test; $P < 0{\cdot}05$; $n = 5$). These findings indicate that the ES-evoked CBF increase is largely restricted to the stimulated area.

The effect of sympathetic input

To assess the autonomic influence on ES-evoked CBF changes, the sympathetic input was increased by ES of the superior cervical ganglion (SCG; $n = 3$) and reduced by sectioning of the SCG ($n = 3$). The effect of ES of the ipsilateral SCG on the ES-evoked CBF response is demonstrated in Fig. 4. This trace from one experiment shows the opposite and interacting effects of ES of the SCG and cochlea on CBF. Across examined animals, ES of the SCG caused a 9% decrease in CBF. Cochlear ES alone evoked a 32% increase in CBF. Simultaneous ES of the cochlea and SCG evoked a 47% CBF increase, 32% above the pre-ES baseline. At termination of the SCG ES, CBF remained stable; after termination of the cochlear ES, CBF returned to the pre-SCG ES baseline level.

In three additional guinea-pigs, the cochlear vessels were chemically preconstricted with topically administered adrenaline, as reported by Ohlsén, Baldwin, Nuttall & Miller (1991*a*). Within 20 min of the application of 2 μl of adrenaline (10 mg ml^{-1}) to the RW, there was a 15–20% decrease in CBF, establishing a new baseline. In these animals, regional ES-evoked CBF increases were also relatively enhanced: prior to the drug application, ES evoked a 14% elevation of CBF above ABL; after drug application, ES evoked a 40% elevation above the new *lower* baseline. However, when compared to the pre-drug baseline level, this increase was the same as the pre-drug response (14%). Thus, the effect of adrenaline application on the ES-evoked CBF response was qualitatively the same as that of SCG stimulation.

Sectioning of the SCG did not affect the resting CBF but caused an enhancement of the CBF response to ES. More importantly, following this partial sympathectomy (there are additional sympathetic fibres from the stellate ganglion), the threshold for the responses to ES was consistently reduced from 25–75 μA to less than 10 μA (not illustrated). Thus, partial elimination of the sympathetic input to the cochlea by sectioning the SCG enhanced the sensitivity of the ES-evoked CBF response.

Participating autonomic receptors within the cochlea

Figure 5 shows the effect of idazoxan (an α_2-blocker), applied to the RW, on the ES-evoked CBF response. Blocking the sympathetic activity by local drug applications, as has been shown earlier but using drugs which are mixed α_1, α_2 blockers (Ohlsén *et al.* 1991*a*; Laurikainen, Kim, Didier, Miller, Nuttall & Ren, 1993).

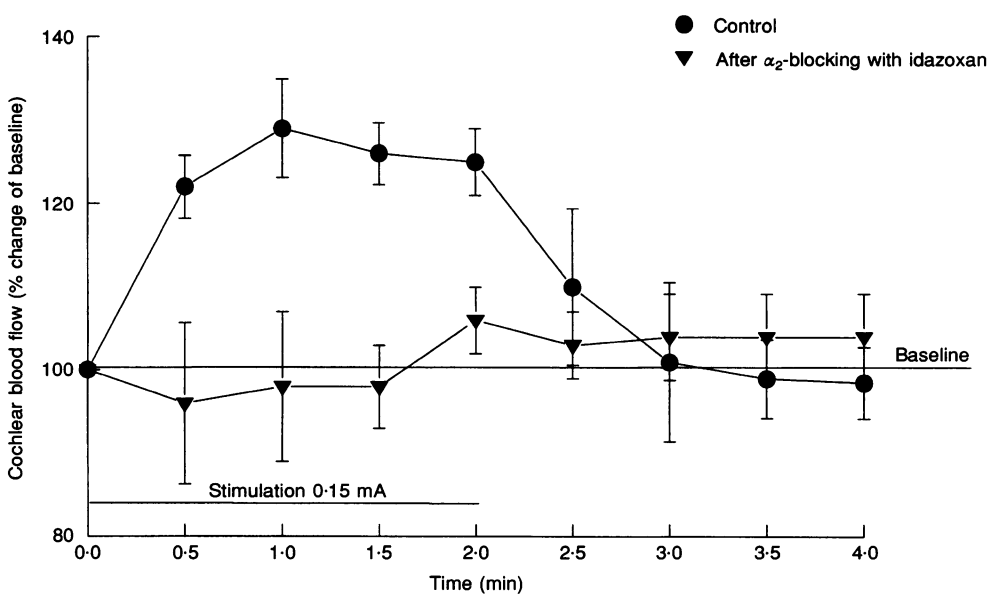

Figure 5. The effect of a round window application of 2 μl of saline containing 2 mM idazoxan (an α_2-blocker) on the electrically evoked (150 μA) cochlear blood flow increase
Electrodes were located on the round window and the apex. The laser Doppler recording site was over the second cochlear turn. Idazoxan abolished the electrically evoked CBF increase. Means ± S.D., 4 guinea-pigs.

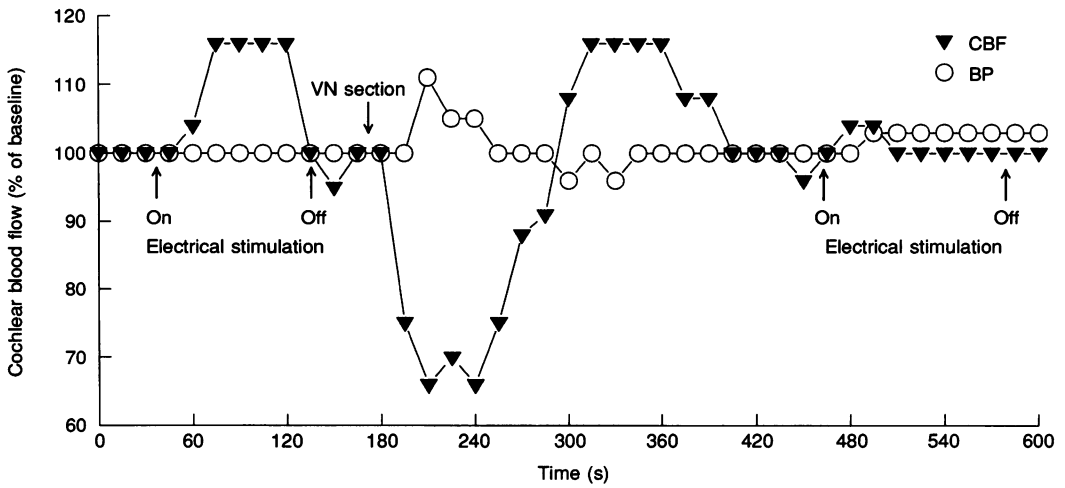

Figure 6. The effect of vestibular nerve sectioning on electrically evoked cochlear blood flow increase and mean systemic blood pressure in one representive animal
Vestibular nerve transection causes a transient decrease/increase in CBF. The electrically evoked CBF response seen in the first (control) stimulation is abolished in the second stimulation.

Idazoxan application itself did not affect the CBF resting level. The ES response was absent, in comparison with that observed prior to the drug application, 20 min after idazoxan application (Student's paired two-tailed t test; $P < 0{\cdot}001$, $n = 4$). In these experiments, the CBF responsiveness to ES started to recover 1·5–2 h after the drug application (not illustrated).

The effect of vestibular nerve section

Figure 6 shows, for one representative animal (of a total of eight), the effect of VN sectioning on the CBF resting levels and ES-evoked responses. Sectioning of the VN resulted in an immediate 35% decrease in CBF, which then slowly recovered, overshot, and returned to baseline over a 5 min period. Following CBF recovery, no CBF responses were observed for ES intensities from 100 μA to 1 mA after VN sectioning. There was concern that this result may be due to: (1) damage to the cochlear artery during the surgical procedure; and/or (2) the possibility that the autoregulatory capacity of the cochlear vasculature was exceeded due to damage to the supplying vessels. Therefore, the local responsiveness of the cochlear circulation was tested. In five preparations, application of hydralazine to the RW caused a 30–40% increase in CBF during the 10–15 min period after drug application, as previously reported (Ohlsén *et al.* 1991*a*), indicating that: (1) the blood supply into the cochlea was functional; and (2) local responsiveness to direct vascular smooth muscle relaxant remained. Finally, the CAP, measured for tone burst sounds at 2, 4, 8, 16 and 24 kHz 30 min after VN sectioning and following the recovery of CBF, showed no decrease when compared to measurements made prior to sectioning.

DISCUSSION

Our results clarify several features of the ES-evoked CBF response: (1) it is topographically related to the site of ES, that is, the primary response is generated in the stimulated region of the cochlea; (2) adrenergic receptors within the cochlea are involved; (3) SCG stimulation and adrenaline (applied locally to the RW) affect the ES-evoked CBF response similarly; (4) VN sectioning causes an immediate but temporary decrease in resting CBF and abolishes the ES-evoked CBF increase during the entire study period (up to 2 h). This last effect is probably not caused by altered CBF due to damage to the cochlear artery since, following VN sectioning, the CBF responded to systemic BP changes and locally applied vasoactive drugs, and demonstrated unchanged sensitivity of sensory function in comparison to that observed prior to the VN section.

In preliminary studies we confirmed the lack of effect of systemically administered cholinergic (nicotinic and muscarinic) antagonists on the ES-evoked CBF increase as shown previously in the guinea-pig (Sillman *et al.* 1989*b*). With regard to the general principle of a cholinergic receptor mediation, we note that a cholinergic response usually exhibits a fast on–off function due to the quick release of acetylcholine in the nerve endings and fast (200–300 ms) breakdown of the transmitter by acetylcholinesterase (Colquhoun, 1979). Thus, the slow initiation (8 ± 3 s latency) and recovery (over 1 min) of the ES-evoked CBF increase found in our study (Fig. 2) would argue for a non-cholinergic transmission in this response.

Sillman *et al.* (1989*b*) have proved that the ES-evoked increase in CBF is due to the stimulation of neuronal elements. One model to account for the locally restricted response would be the locally stimulated release of vaso-

dilative substances. The main problem of this model, in relation to our data, is that the VN sectioning abolishes the ES response. One might expect stimulation of the distal portion of the sectioned VN fibre to remain, at least for a short time, following the sectioning. However, the threshold may be significantly higher due to cathodal blockage and different membrane excitability characteristics of the small efferents compared to the larger myelinated cochlear afferents. We were not able to examine higher levels of stimulation due to the evoked systemic BP changes.

Several studies have shown that, in certain organs, stimulation of either preganglionic efferent fibres or the afferent limb of an autonomic nervous system reflex loop results in an inhibitory effect in the target organ (Porszasz, Such & Porszasz-Gibiszer, 1972; Such, Porszasz & Gibiszer, 1972; Flock & Russell, 1976; Szurszewski & Weems, 1976). Thus, there is the possibility that the ES-evoked CBF increase is not a direct dilating response of cochlear vessels to the ES of incoming nerve fibres. Specifically, Koss & Kawarai (1991) have reported that the stimulation of *preganglionic* sympathetic nerve fibres of the rat forelimb causes an increase in blood flow to the digits when measured by LDF. This increase was abolished by a systemic α_2-antagonist but not by an α_1-antagonist. These observations resemble our findings in the guinea-pig cochlea.

Sympathetic fibres enter the cochlea along the cochlear artery (Spoendlin & Lichtensteiger, 1966), and other autonomic centrifugal innervation into the cochlea is described as being mediated via olivo-cochlear fibres. These fibres enter the cochlea with the VN (Spoendlin & Lichtensteiger, 1966; Ross, 1969). Ross (1969) also describes autonomic fibres within the cochlear, VN and

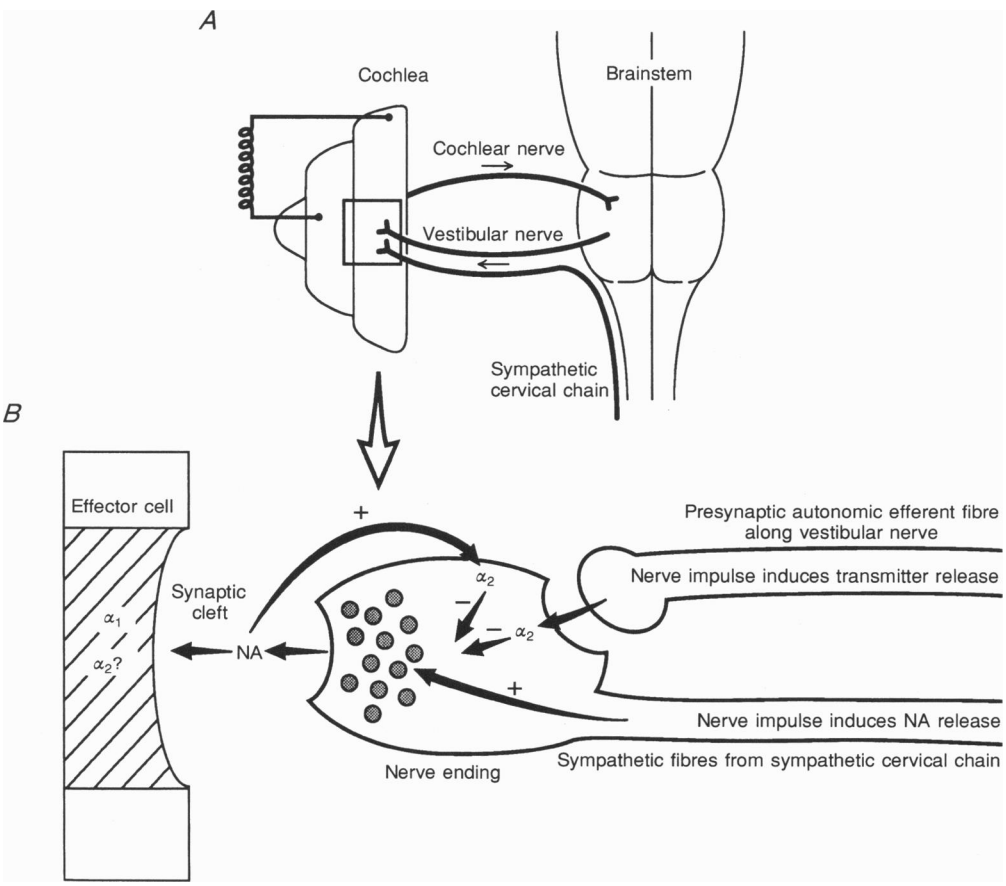

Figure 7. A schematic model for autonomic control of regional blood flow regulation in the cochlea
A, to account for the data of this study the model includes a neural loop involving afferent fibres in the cochlear nerve and efferent autonomic fibres in the vestibular nerve. One origin of the efferent fibres could be the cervical sympathetic chain. *B*, a model of the synaptic control of CBF tonic sympathetic input (the release of noradrenaline, NA) via the sympathetic cervical chain could be modulated by presynaptic efferent autonomic fibres running along the vestibular nerve. The 'effector cells', the smooth muscle of the cochlear vessels, have α_1- and possibly α_2-receptors. The modulating nerve fibres are hypothesized to release an α_2-agonist.

intermediate nerves. Recently, Laurikainen, Ren, Miller, Quirk & Nuttall (1991) have reported that bilateral stellate ganglion blockade causes a brief CBF increase in guinea-pig. The nature and form of this increase is consistent with release from sympathetic tone, as reported for other organs (Gero & Gerovà, 1978). Together these reports provide anatomical and functional possibilities that permit a second model to explain our observations. In this model, the ES-evoked CBF increase is a result of the excitation of an inhibitory reflex rather than resulting from direct stimulation of the cochlea and incoming nerve fibres. This inhibitory-type reflex could be mediated by somatic afferent fibres in the cochlear nerve and autonomic efferent fibres in the VN. Such a model is schematically presented in Fig. 7*A*. Our data showing elimination of the ES response by VN sectioning can be interpreted as blocking the efferent limb of the reflex activated by ES of afferent cochlear fibres.

Autonomic sympathetic innervation via the SCG and stellate ganglia plays a role in the regulation of CBF via local α-receptors (Ohlsén, Baldwin, Nuttall & Miller, 1991*a*; Laurikainen *et al.* 1993). Thus, our findings that cervical sympathectomy enhances cochlear vasculature responses to ES and that an α_2-blocker, idazoxan, inhibits it, support the role of α_2-receptors in the ES-evoked CBF. Figure 7*B* schematically presents a general model of local modulation of adrenergic neuroeffector interaction in the blood vessel wall in other organs (Vanhoutte *et al.* 1981). On the basis of our data we suggest that this model is incomplete in the cochlear vascular system. The effect of ES on preconstricted vessels treated with the locally applied α-agonist, adrenaline, or SCG stimulation are not explained by this general model. These observations argue for a more complex, non-linear postsynaptic system, which may be triggered by the activation of a neuronal loop (Fig. 7*A*). However, based on the current data, it is not possible to speculate how this co-modulated system might function, and more work is needed to resolve this problem.

The magnitude of observed vascular responses also argues for a more complex interaction between the neuronal function and the CBF changes. ES of moderate intensity may increase CBF by more than 100% of ABL (Sillman *et al.* 1989*a*; Miller *et al.* 1991). An inhibition of on-going sympathetic tone alone would seem inadequate to account for changes of this magnitude. Other vasoactive mechanisms (peptidergic, humoral, myogenic and/or metabolic), more potent than inhibition in the adrenergic system, may contribute to this response. A metabolic–adrenergic interaction is also possible (see Meininger & Faber, 1989, for arterioles in skeletal muscle), and changes in regional blood flow of longer than 30 s (as was often the case in the current work) can involve metabolic mechanisms which are capable of contributing to the autoregulatory response of the system in general (for review, see Johnson, 1986). Thus, the extracochlear fibre systems (via the SC and VN) may regulate smooth muscle tone via α-adrenoreceptors and this may be modulated by other local vasoactive systems within the cochlea. This kind of complex co-operation of neural functions and local factors has also been proposed for the regulation of cerebral circulation (Bevan & Bevan, 1993).

In conclusion, our results expand the functional role for the previously described autonomic nerve fibres within the cochlear and vestibular nerves. The data are consistent with the formation of a neural loop which participates in local regulation of CBF. The previous anatomical observations on wide distribution of these fibres over all cochlear turns is in accordance with our observations of the topographical increase of CBF due to direct cochlear ES. Thus, it is interesting to speculate that this neural loop may be activated by sound in the inner ear and that this loop may provide a relatively rapidly responding system to increase CBF as required by the metabolically driven system. Such a reflex may then supplement local metabolic factors that increase CBF in the active system.

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